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Some Cyclization Reactions of 6-Mercaptopurine with Expected Biological Activity

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The reaction of 6-mercaptopurine **1** with aromatic ketones **2a-h** using the acidified acetic acid method afforded the S-acetyl derivatives **3a-h** in good yields. While, the cyclized product **4i** was obtained directly upon reaction of **1** with 9-acetyl anthracene. Compounds **3a-h** were cyclized directly to the corresponding 1,4-thiazino[2,3,4-bc]purine derivatives (**4a-h**). In the same manner the reaction of **1** with aliphatic, aromatic, alicyclic ketones, α-tetralone and 1-methyl-4-piperidone gave compounds **6**, **7**, **8**, **9a,b**, **11** and **12**. The cyclized compounds **10a,b** were obtained by cyclization of **9a,b**. The synthesized compounds were screened for their *in vitro* antitumor activity at National Cancer Institute.

Key Words: Cyclization reactions, 6-Mercaptopurine, Biological activity.

INTRODUCTION

The development of curative antitumor drugs has been only partially successful. In the research for new effective chemotherapeutic agents a wide variety of new drugs with completely different chemical structures has been prepared and tested. These drugs can be classified into alkylating agents, nucleic acid, intercalating agents, topoisomerases inhibitors. DNA-binding agents demonstrated to be one of the most effective classes of anticancer drug, whose mechanism of action involves binding either in the major or minor grooves, or intercalation between base pairs of double-stranded DNA. Generally, agents with DNA-intercalative properties are characterized by the presence of a planar chromophore, a tri- or tetracyclic ring system, to which flexible basic side chains may be bound to improve DNA binding properties[1-3].

On the basis of these findings, in carrying out a wide program to prepare new heteropolycyclic compounds containing the purine nucleus that might exhibit anti-proliferative activity[4], in the last few years, the synthesis of numerous molecules which contain the purine nucleus fused several heterocyclic systems such as purinoquinazoline[5], purinopyridopyrimidine[6], 2,4-benzodiazepinopurine and 2,3,5-triazocinopurine derivatives[7] were reported.

EXPERIMENTAL

Melting points were recorded on a Gallen Kamp Melting apparatus and are uncorrected. Infrared spectra were obtained on Nexus 470-670. ^{1}H NMR spectra and ^{13}C NMR run on JEOL-400 MHz in DMSO-d_6. The mass spectra were recorded on Ms-S 988 operating at 70 V. microanalysis were performed by using Perkin-Elmer 2400 CHN analyzer. The newly synthesized compounds were screened *in vitro* antitumour activity at Cairo University, National Cancer Institute, Cancer Biology Department, Pharmacology Unit.

General procedure

Synthesis of thioacetyl derivatives of purine 3a-h and 4i: A mixture of 6-mercaptopurine (**1**) (10 mmol) and acetato derivatives **2a-h** (10 mmol) was refluxed in acetic acid (20 mL) containing a few drops of conc. H_2SO_4 for 2-3 h. The reaction mixture was cooled and neutralized with NH_4OH solution. The resulting precipitate was collected by filtration, washed with water several times and dried under vacuum. The crude product was crystallized from the proper solvent.

Synthesis of 4-aryl-1,4-thiazino[2,3,4-bc]purines (4a-h) and (10a,b): A mixture of compounds **3a-h** or **5** or **9a,b** (10 mL) was refluxed in acetic acid (20 mL) containing a few drops of conc. H_2SO_4 for 2 h. Then acetic anhydride (7 mL) was added to the reaction mixture and refluxing was continued further for 2 h. The reaction mixture was cooled and worked up as described above.

Formation of compounds, 9a,b and the cyclized compounds 6, 7, 8, 11 and 12: A mixture of **1** (10 mL) and aliphatic or aromatic and/or alicyclic ketones (10 mL) was refluxed in acetic acid (20 mL) containing a few drops of conc. H_2SO_4 for 2-3 h. The reaction mixture was cooled and neutralized with NH_4OH (10 mL) and the resulting solid product was crystallized from the proper solvent.

Compound **6** was chromatographed over 300 g of silica gel (using chloroform as eluent) to give 0.4 g of **6** sutable for X-ray anlysis. The physical and spectral data of the synthesized compounds are given in Tables 1 and 2.

Biological studies

Antitumor activity (*in vitro*): Ehrlich A sites carcinoma cells (EAC) is drawn from mice bearing, in sterile test tube, where 2.5×10^5 tumour cell/mL were suspended in phosphate buffer saline. Three different concentration for each compound (25, 50, 100 μg/mL) were prepared. Add 2.5×10^5 tumour cell for each test tube at 37 ºC 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[8] and examined under microscope. The dead cells stained blue and live cell not stained then carried out to calculate the percentage of non-viable cells. Compounds producing more than 70 % non-viable cell are considered active[9].

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

TABLE-1
PHYSICAL CHARACTERIZATION DATA OF THE SYNTHESIZED COMPOUNDS

Compd.	m.p. (ºC)	Yield (%) / Solvent	m.f. (m.w.)	Elemental analysis (%): Calcd. (Found)			
				C	H	N	S
3a	180	50 (EtOH)	$C_{13}H_{10}N_4OS$ (270)	57.77 (57.70)	3.70 (3.40)	20.74 (20.70)	11.85 (11.50)
3b	205	55 (EtOH)	$C_{14}H_9N_5OS$ (295)	56.94 (56.80)	3.05 (3.00)	23.72 (23.68)	10.84 (10.55)
3c	230	60 (EtOH)	$C_{14}H_{12}N_4O_2S$ (300)	56.00 (56.33)	4.00 (4.11)	18.66 (18.23)	10.66 (10.80)
3d	220	75 (EtOH)	$C_{11}H_8N_4OS_2$ (276)	47.82 (47.55)	2.89 (2.90)	20.28 (20.50)	23.18 (23.00)
3e	260	70 (EtOH)	$C_{11}H_8N_4O_2S$ (260)	50.76 (50.90)	3.07 (3.38)	21.53 (21.27)	12.30 (12.22)
3f	300	60 (EtOH)	$C_{11}H_9N_5OS$ (259)	50.96 (50.98)	3.47 (3.50)	27.02 (27.00)	12.35 (12.30)
3g	240	65 (EtOH)	$C_{12}H_9N_5OS$ (271)	53.13 (53.00)	3.32 (3.52)	25.83 (25.99)	11.80 (11.60)
3h	235	75 (EtOH)	$C_{19}H_{14}N_4OS$ (346)	65.89 (66.00)	4.04 (3.80)	16.18 (16.00)	9.24 (9.54)
4a	240	55 (EtOH)	$C_{13}H_8N_4S$ (252)	61.90 (62.00)	3.17 (3.88)	22.22 (22.70)	12.69 (12.80)
4b	320	50 (AcOH)	$C_{14}H_7N_5S$ (277)	60.64 (60.84)	2.52 (2.32)	25.27 (25.55)	11.55 (11.78)
4c	>360	65 (AcOH)	$C_{14}H_{10}N_4OS$ (282)	59.57 (59.50)	3.54 (3.65)	19.85 (19.52)	11.34 (11.00)
4d	>360	70 (AcOH)	$C_{11}H_6N_4S_2$ (258)	51.16 (51.87)	2.32 (2.68)	21.70 (21.30)	24.80 (24.45)
4e	>360	70 (AcOH)	$C_{11}H_6N_4OS$ (242)	54.54 (54.81)	2.47 (2.50)	23.14 (23.58)	13.22 (13.00)
4f	>360	75 (AcOH)	$C_{11}H_7N_5S$ (241)	54.77 (54.80)	2.90 (3.00)	29.04 (28.92)	13.27 (13.68)
4g	>360	60 (Dioxane)	$C_{12}H_7N_5S$ (253)	56.91 (56.60)	2.76 (2.24)	27.66 (27.80)	12.64 (12.89)
4h	>360	70 (Dioxane)	$C_{19}H_{12}N_4S$ (328)	69.51 (69.80)	3.65 (3.72)	17.07 (17.00)	9.75 (10.02)
4i	230	60 (EtOH)	$C_{21}H_{12}N_4S$ (352)	71.59 (71.12)	3.40 (3.13)	15.90 (16.00)	9.09 (9.00)
6	>360	55 (AcOH)	$C_8H_6N_4S$ (190)	50.52 (50.72)	3.15 (3.30)	29.47 (29.50)	16.84 (16.70)
7	210	65 (EtOH)	$C_{10}H_8N_4OS$ (232)	51.72 (51.80)	3.44 (3.20)	24.13 (24.00)	13.79 (14.00)
8	275	75 (EtOH)	$C_{15}H_{10}N_4OS$ (294)	61.22 (61.00)	3.40 (3.70)	19.04 (19.60)	10.88 (11.00)
9a	270	85 (EtOH)	$C_{10}H_{10}N_4OS$ (234)	51.28 (51.00)	4.27 (4.40)	23.93 (23.60)	13.67 (13.88)
9b	250	80 (EtOH)	$C_{13}H_{16}N_4OS$ (276)	56.52 (56.87)	5.79 (5.65)	20.28 (20.55)	11.59 (11.86)
10a	>360	70 (Dioxane)	$C_{10}H_8N_4S$ (216)	55.55 (55.80)	3.70 (3.51)	25.92 (25.80)	14.81 (14.90)
10b	>360	65 (Dioxane)	$C_{13}H_{14}N_4S$ (258)	60.46 (60.66)	5.42 (5.50)	21.70 (21.43)	12.40 (12.68)
11	240	70 (EtOH)	$C_{15}H_{10}N_4S$ (278)	64.74 (64.28)	3.59 (3.44)	20.14 (20.00)	11.51 (11.30)
12	255	70 (EtOH)	$C_{11}H_{11}N_5S$ (245)	53.87 (53.53)	4.48 (4.20)	28.57 (28.38)	13.06 (13.00)

TABLE-2
SPECTRAL DATA OF THE SYNTHESIZED COMPOUNDS

Compd.	IR (ν_{max}, cm^{-1})	^1H NMR δ (ppm)/^{13}C NMR, δ (ppm)/ms
3a	3145 (NH), 3046 (CH-Ar), 2800 (CH aliph.) 1660 (CO)	4.88 (s, 2H, CH_2), 7.22-7.58 (m, 5H, Ar–H), 8.23 (s, 1H, CH-pyrimidine), 8.60 (s, 1H, CH–imidazol), 13.96 (S, 1H, NH).
3b	3140 (NH), 3055 (CH-Ar), 2800 (CH aliph.) 2222 (CN), 1665 (CO)	4.80 (s, 2H, CH_2), 7.48-7.68 (m, 4H, Ar–H), 8.20 (s, 1H, CH-pyrimidine), 8.92 (s, 1H, CH–imidazol), 13.90 (s, 1H, NH-).
3c	3145 (NH), 3065 (CH-Ar), 2900 (CH aliph.) 1665 (CO)	3.88 (s, 3H, OCH_3), 4.50 (s, 2H, CH_2), 7.25-7.80 (m, 4H, Ar-H), 8.50 (s, 1H, CH-pyrimidine), 9.00 (s, 1H, CH–imidazol), 13.81 (s, 1H, NH).
3d	3140 (NH), 3050 (CH-Ar), 2850 (CH aliph.) 1670 (CO)	4.65 (s, 2H, CH_2), 7.00, 7.32 (2d, 2H, thiophene, J = 5.80 Hz), 7.90, 8.40 (2s, 2H, Ar-H and CH-pyrimidine), 9.22 (s, 1H, CH–imidazol), 13.80 (s, 1H, NH).
3e	3148 (NH), 3040 (CH-Ar), 2900 (CH aliph.) 1660 (CO)	4.50 (s, 2H, CH_2), 7.12, 7.48 (2d, 2H, furan, J= 5.22 Hz), 7.72, 8.44 (2s, 2H, Ar-H and CH-pyrimidine), 9.00 (s, 1H, CH–imidazol), 13.68 (s, 1H, NH).
3f	3142 (NH), 3050 (CH-Ar), 2920 (CH aliph.) 1680 (CO)	4.32 (s, 2H, CH_2), 7.25-7.66 (m, 3H, Ar-H) 8.52 (s, 2H, CH-pyrimidine), 9.12 (s, 1H, CH–imidazol), 13.80 (s, 1H, NH).
3g	3150 (NH), 3040 (CH-Ar), 2880 (CH aliph.) 1680 (CO)	4.40 (s, 2H, CH_2), 7.44-7.86 (m, 4H, Ar-H) 8.20 (s, 1H, CH-pyrimidine), 9.00 (s, 1H, CH–imidazol), 13.88 (s, 1H, NH).
3h	3142 (NH), 3080 (CH-Ar), 2920 (CH aliph.) 1685 (CO)	4.50 (s, 2H, CH_2), 7.22-7.80 (m, 8H, Ar-H) 8.00 (s, 1H, CH-pyrimidine), 9.88 (s, 1H, CH–imidazol), 14.00 (s, 1H, NH).
4a	(3080) (CH-Ar)	7.44-7.85 (m, 6H, Ar-H and CH-thiazine), 8.02 (s, 1H, pyrimidine), 9.22 (s, 1H, CH-imidazole); ^{13}C: 131.13, 131.98, 132.18, 134.00, 134.22, 135.11, 137.23; ms: m/z = 252 (8.11%).
4b	3090 (CH-Ar), 2202 (CN)	7.33-7.88 (m, 5H, Ar-H and CH-thiazine), 8.20 (s, 1H, CH–pyrimidine), 9.00 (s, 1H, CH-imidazole) ms: m/z = 277 (12.38%).
4c	3090 (CH-Ar), 2800 (CH aliph.)	3.30 (s, 3H, CH_3), 7.50-7.82 (m, 5H, Ar-H and CH-thiazine), 8.00 (s, 1H, CH–pyrimidine), 9.28 (s, 1H, CH-imidazole); ms: m/z = 282 (0.08%).
4d	3010 (CH-Ar),	7.00-7.86 (m, 4H, Ar-H and CH-thiazine), 8.20 (S, 1H, pyrimidine), 9.50 (s, 1H, CH-imidazole)
4e	3000 (CH-Ar)	7.18-7.98 (m, 4H, Ar-H and CH-thiazine), 8.62 (s, 1H, CH-pyrimidine), 9.28 (s, 1H, CH-imidazole)
4f	3142 (NH), 3055 (CH-Ar)	7.00-7.99 (m, 4H, Ar-H and CH-thiazine), 8.22 (s, 1H, CH-pyrimidine), 10.00 (s, 1H, CH-imidazole) 13.00 (s, 1H NH); Ms: m/z = 241 (18.00%)
4g	3090 (CH-Ar)	7.38-7.82 (m, 5H, Ar-H and CH-thiazine), 8.50 (s, 1H, CH-pyrimidine), 9.00 (s, 1H, CH-imidazole)
4h	3080 (CH-Ar)	7.11-7.86 (m, 10H, Ar-H and CH-thiazine), 8.00 (s, 1H, CH-pyrimidine), 9.12 (s, 1H, CH-imidazole) ms: m/z = 328 (3.11%)
4i	3082 (CH-Ar)	7.44-7.80 (m, 10H, Ar-H and CH-thiazine), 8.50 (s, 1H, CH-pyrimidine), 9.00 (s, 1H, CH-imidazole) ms: m/z = 352 (13.70%)

6	3065 (CH-Ar) 2900 (CH-aliph.)	2.20 (s, 3H, CH_3), 7.88, 8.00 (2s, 2H, CH-pyrimidine and CH-thiazine), 9.35 (s, 1H, CH-imidazole), ^{13}C: 14.70 (CH_3), 133.19, 135.00, 137.88 ms:m/z = 190 (7.82%)
7	3068 (CH-Ar) 2882 (CH-aliph.), 1680 (CO), 1590 (C=C)	2.20 (s, 3H, CH_3), 3.30 (s, 3H, $COCH_3$) 8.22, 9.00 (2s, 2H, CH-pyrimidine and CH-imidazole), ms:m/z = 232 (6.12%)
8	3080 (CH-Ar) 2900 (CH-aliph.), 1700 (CO), 1610 (C=C)	2.20 (s, 3H, CH_3), 7.22-8.18 (m, 6H, Ar-H and CH-pyrimidine), 9.11 (s, 1H, CH-imidazole),
***9a**	3400 (NH), 3040 (CH-Ar) 2900-2700 (CH-aliph.), 1710 (CO)	1.80 (m, 2H, CH_2), 2.10 (m, 2H, CH_2), 2.25 (m, 2H, $COCH_2$), 3.30 (t, 1H, S-CH_3, J = 8.11 Hz), 8.00 (s, 1H, CH-pyrimidine), 8.82 (s, 1H, CH-imidazole) 13.00 (br, 1H, NH); ^{13}C: 44.19, (CH_2) 47.23 (CH_2), 138.00, 139.98; ms: m/z = 234 (2.08 %)
***9b**	3384 (NH), 3055 (CH-Ar), 2900-2800 (CH–aliph.) 1695 (CO)	1.40-2.50 (m, 10H, cyclooctanyl-H) 2.40 (t, 2H, CH_2O, J = 10.11Hz), 4.80 (t, 1H, SCH, J=9.00Hz), 8.00 (s, 1H, CH-pyrimidine), 8.80 (s, 1H, CH-imidazole), 12.66 (br, 1H, NH))
***10a**	3030 (CH-Ar) 2888-2770 (CH-aliph.), 1600 (C=C)	2.45 (m, 2H, CH_2), 2.90 (m, 2H, CH_2), 3.40 (m, 2H, CH_2), 7.90 (S, 1H, CH-pyrimidine), 8.82 (S, 1H, CH-imidazole); ms:m/z = 216 (5.52%)
***10b**	3090 (CH-Ar) 2880-2700 (CH-aliph.), 1560 (C=C)	1.20-2.40 (m, 12H, cyclooctanyl-H) 8.20 (s, 1H, CH-pyrimidine), 9.00 (s, 1H, CH-imidazole) ms:m/z = 258 (22.11%)
11	3088 (CH-Ar), 2980 (CH-aliph.)	1.19 (t, 2H, CH_2, J = 10.11 Hz), 2.20 (m, 2H, CH_2), 7.22-8.12 (m, 5H, Ar-H and CH pyrimidine), 9.22 (s, 1H, CH-imidazole); ms:m/z = 278 (15.80%)
12	3070 (CH-Ar), 2900 (CH-aliph.), 1580 (C=C)	1.90 (t, 2H, CH_2, J = 11.00 Hz), 2.60-2.88 (m, 2H, CH_2), 3.00 (s, 2H,CH_2) 8.82 (s, 1H CH-pyrimidine), 9.40 (s, 1H, CH-imidazole); ms:m/z = 245 (4.44%)

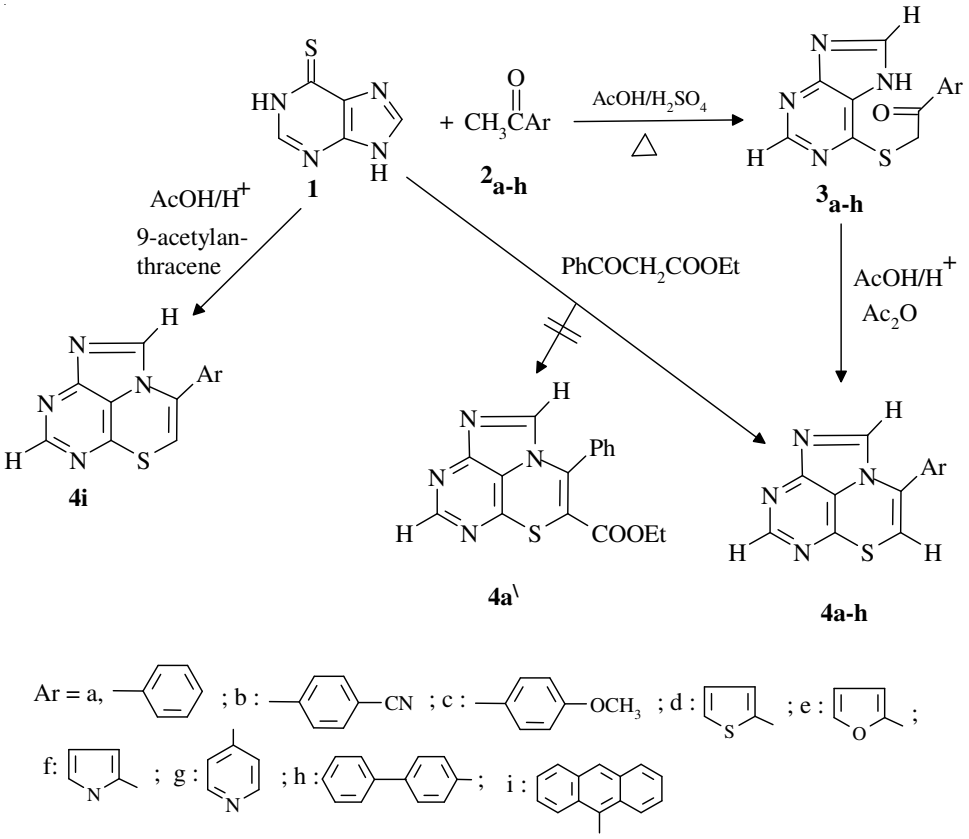
***9a,b** ***10a,b**: ^{1}H NMR in ($CDCl_3$).

Antitumor screening (*in vitro* anti-HPG2 testing): Potential cytotoxicity of the compounds was tested using the method of Skehan *et al.*[9]. Cells were plated in 96 multiwell plate (10 cells/well) for 24 h before treatment with the compound to allow attachment of cell to the wall of the plate. Different concentrations of the compounds under test (0, 10, 25, 50 and 100 μg/mL) were added to the cell, mono-layer triplicate wells were prepared for each individual dose. Mono layer cells were incubated with the compounds for 48 h at 37 °C in an atmosphere of 5 % CO_2. After 48 h, cells were fixed, washed and stained with sulfo-rhodamine-β-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. The relation between surviving fraction and drug concentration was plotted to obtain the survival curve of tested cell, the response parameter calculated was IC_{50} value.

RESULTS AND DISCUSSION

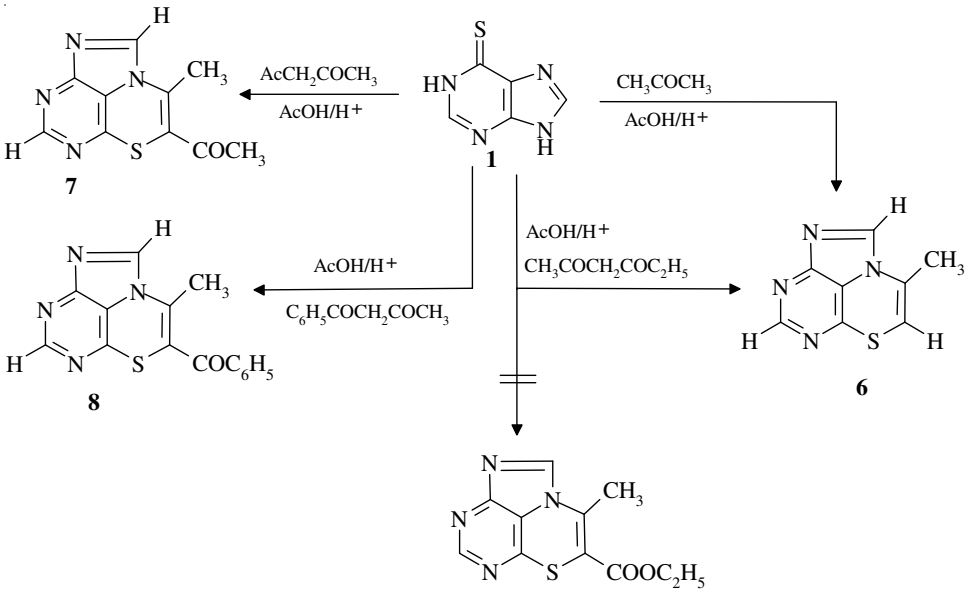
In continuation of our studies of heterocyclic condensed derivatives with, the aim of evaluation their pharmacological activity such as analgesic, antibacterial[10],

antitumor[11], antineoplastic agent[12] and radioprotective activity[13]. In present studies, the interaction of 6-mercaptopurine **1** with aromatic ketones **2a-h** in boiling acetic acid, containing a few drops of conc. H_2SO_4, for 2 h[14] afforded the 6-S-acetyl derivatives (**3a-h**) in good yields, is reported. The cyclized product **4i** was obtained directly upon reaction of **1** with 9-acetyl anthracene. Compound **3a-h** were cyclized directly to the corresponding 4-aryl-1,4-thiazino[2,3,4-bc]purines (**4a-h**) on refluxing the reaction mixture for 2 h in the presence of acetic anhydride as cyclizing agents. Interestingly, interaction of **1** with ethyl benzoylacetate under the same reaction conditions gave **4a** instead of the compound **4′a**. The formation of **4a** may be explained as a result of **4a** ester hydrolysis followed by decarboxylation of the expected product. This was confirmed by TLC, IR, m.p. and m.m.p. (**Scheme-I**).



Scheme-I

Similarly, reaction of **1** with ketone such as acetone, acetyl acetone and/or benzoyl acetone using the acidified acetic acid method gave the cyclized products **6**, **7** and **8** directly. Also, compound **6** was obtained when ethyl acetoacetate reacted with **1** under the same reaction conditions. This was confirmed by TLC, IR, m.p. and m.m.p. (**Scheme-II**).



Scheme-II

The structure of thiazino[3,4-c:2,3-b]purine derivative was confirmed by a single crystal X-ray analysis of **6** (Fig. 1)[15]. The X-ray structure shows a fused triheterocycle with an almost planar shape obeying the necessary aromatic form.

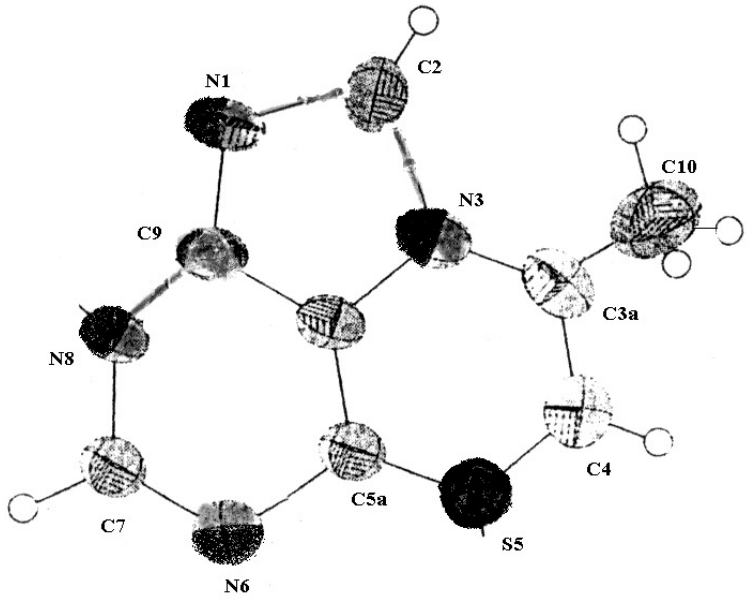
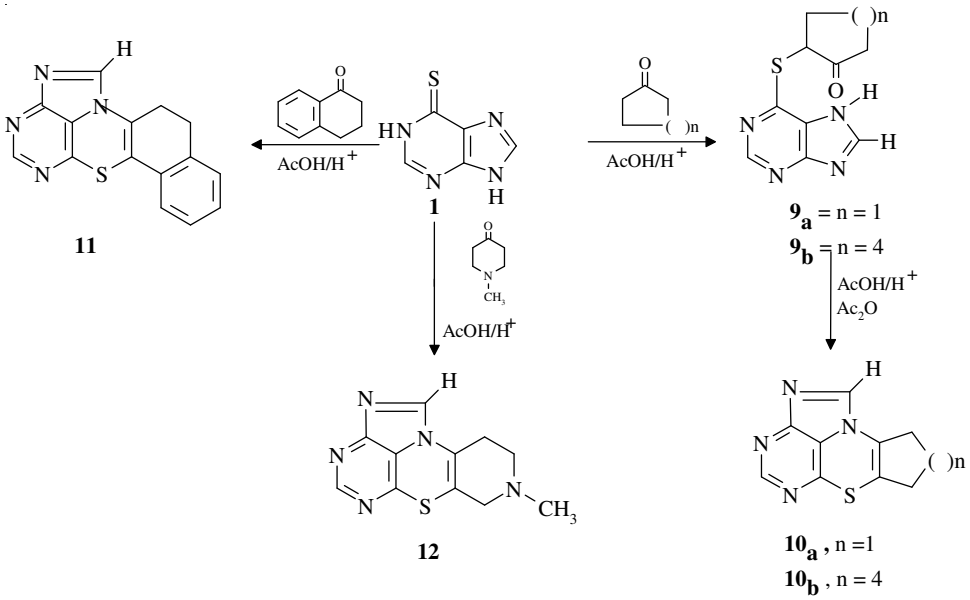


Fig. 1. ORTEP representation of compound **6** with crystallographic numbering scheme

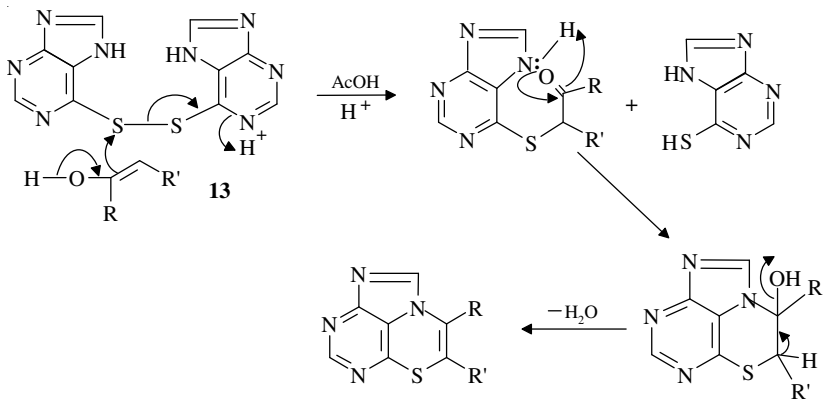
Alicyclic ketones like cyclopentanone and cyclooctanone were allowed to react with **1** in the same condition ($AcOH/H_2SO_4$)[14], afforded the 2-(6-purinylthio)-cycloalkanone **9a,b**. compounds **9a,b** were cyclized[14], to the corresponding **10a,b** either by addition of acetic anhydride to the reaction mixture as the cyclizing agents. The cyclized products 1,2,3,4-tetrahydronaphthalene[2,3-b]-1,4-thiazino[3,4-c:2,3-b]-purine (**11**) and N-methyl-1,2,4-trihydropyrido[2,3-c]1,4-thiazino[3,4-c:2,3-b]purine (**12**) were formed by reaction of compound **1** with α-tetralone and/or 1-methyl-4-piperidone in the same condition ($AcOH-H_2SO_4$) (**Scheme-III**).



Scheme-III

In previous work[16] the mechanism of the formation of the cyclized compounds can, therefore, be explained by the nucleophilic attack of α-aryl/alkyl-α-hydroxy methylene carboxylate [formed by esterification of enol form] on the dimeric disulfide **13**[19] to give the carbonium ion intermediate following by oxygen-acetyl bond fission to give open compounds or interamolecular cyclization to yield the cyclized compounds directly (**Scheme-IV**).

Biological results: Compound **3a-h**, **5** and **9a,b** did not show any activity or toxicity at different concentration, but cyclized compounds **4a,d**, **6, 7, 8**, **11** and **12** showed cytotoxicity at different concentration. While compounds **4b,c,e-i** and **10a,b** did not show any activity at different concentration, probably because of a solubility problem in the used culture media (Table-3). Thus the 1,4-thiazino[2,3,4-bc]purine heterocyclic system proved to be the most active antitumor agent, in addition the pattern of substitution on those heterocyclic ring favoured the antitumor activity[18]. Compound **13** showed the highest activity among the tested compounds and was able to reduce activity of cells by 100 %. This results imply that the (S–S) link is an



Scheme-IV

TABLE-3
in vitro CYTOTOXIC ACTIVITY OF SYNTHESIZED COMPOUNDS

Compd.	Non-viable cells (%) concentration (μg/mL)		
	100 (%)	50 (%)	25 (%)
3a-h	-ve	-ve	-ve
4a	100	90	90
4b,c,e-i	-ve	-ve	-ve
4d	100	100	95
5	-ve	-ve	-ve
6	100	90	90
7	100	90	85
8	100	90	80
9a,b	-ve	-ve	-ve
10a,b	-ve	-ve	-ve
11	100	100	90
12	100	95	90
13	100	100	95
Doxorubicin [Ref. 18]	100	55	26

essential pharmacophoric site. These heterocycles could be considered as useful templates for future development and further derivatization or modification to obtain more potent and selective antitumor agents. Compounds **4a,d, 6, 7, 8, 11, 12** and **13** were able to reduced magnitude of actively of (EAC) to 100 % and were examined against liver carcinoma cell line (HEPG2) (Table-4).

Compounds **4a,d, 6, 7, 8, 11, 12** and **13** were more effective than the positive control (doxorubicin) towards HEPG2 cells. On the basis of the structure of tested compounds, it is concluded that the structure activity relationships provide the idea of geometry, size and shape of the compound as their substituents. These heterocyclic could be considered as useful templates for further development and derivatizations or modification to obtain more and potent and selective antitumour agent.

TABLE-4
in vitro ANTI-HEPG2* TESTING RESULTS

Comp.	**IC_{50} (μg/mL)	Comp.	**IC_{50} (μg/mL)
4a	27.33	**11**	9.16
4d	12.50	**12**	38.11
6	24.21	**13**	12.82
7	8.18	Doxorubicin***	43.6
8	25.00		

*Liver carcinoma cell line; **Concentration of compound which cause inhibition of cell growth, ***Positive control.

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