Department of Chemistry, Faculty of Education, Ain Shams University, Roxy, Cairo, Egypt

Synthesis of heterobicyclic nitrogen systems bearing the 1,2,4-triazine moiety as anti-HIV and anticancer drugs, part III

Z. EL-GENDY, J. M. MORSY, H. A. ALLIMONY, W. R. ABDEL-MONEM ALI and R. M. ABDEL-RAHMAN

Some new fused heterobicyclic nitrogen systems such as 1,2,4-triazino[3,4-*b*] [1,3,4] thiadiazolones/thiadiazinones **4**–**15** and the related compounds **16**–**21** have been synthesized from treatment of 4-amino-3-mercapto-6-substituted-1,2,4-triazin-5-ones **1** with bifunctional oxygen and halogen compounds via heterocyclization reactions. Structures of the products have been deduced from their elemental analysis and spectral data. Significant anti-HIV and anticancer activities were observed in vitro for some members of the series, compounds **1e**, **4e**, **4f**, **5**, **6** and **16** showing a significant activity in Leukemia, Lung, Breast and CNS anticancer evaluation.

1. Introduction

1,2,4-Triazines possess important biological [1–3], pharmacological [4–7] and medicinal activities [8, 9]. All 4-amino-3-thioxo-1,2-4-triazin-5-ones are used as herbicides [10–12]. The use of herbicides is very effective, and many years of experience gained in this field show that herbicides appreciably lower the cost of controlling weeds and facilitate an increase in the yields of agricultural crops. On the basis of these observations, the present work deals with the synthesis of fused 1,2,4-triazino[3,4-*b*][1,3,4]thiadiazolones/thiadiazinones with respect to their biocidal effects as anti-HIV and anticancer drugs to establish a correlation between structure and activity.

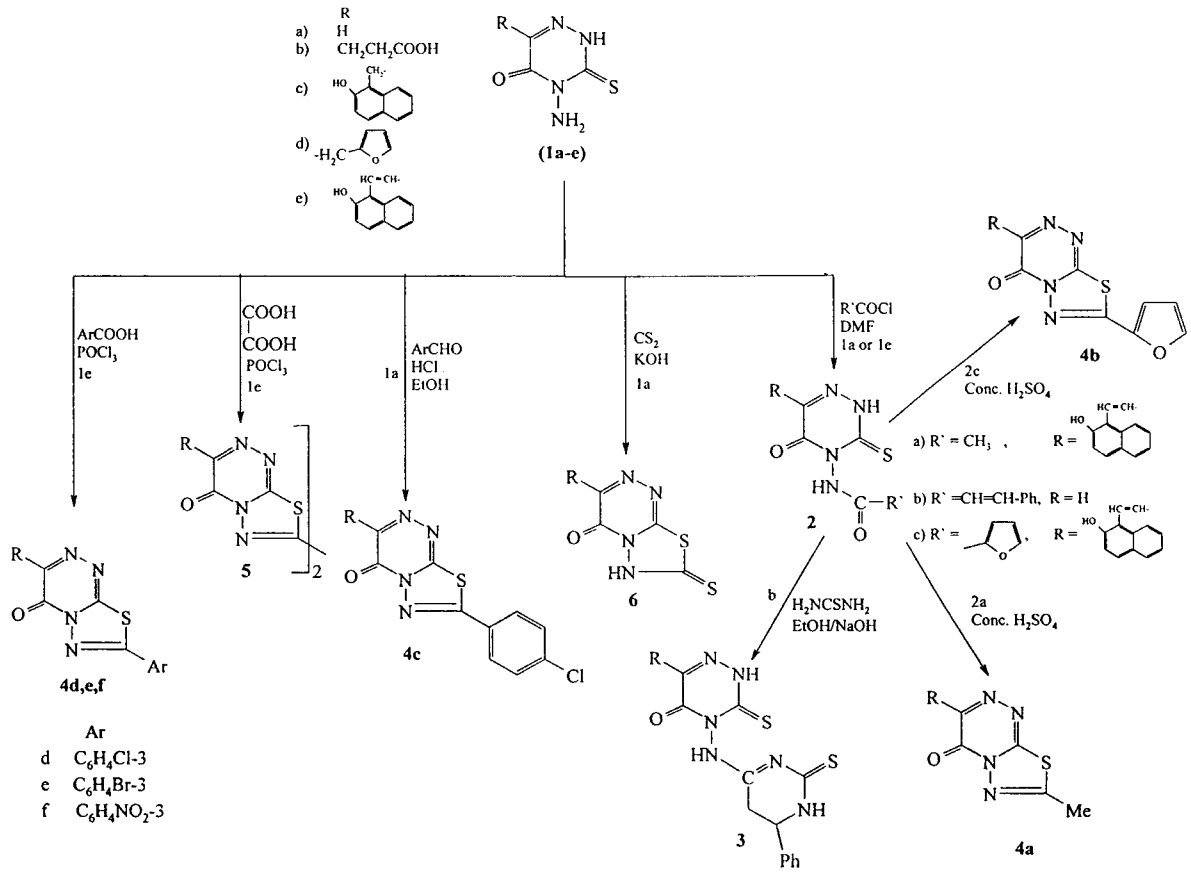
2. Investigations and results

2.1. Chemistry

4-Amino-3-mercapto-6-substituted-1,2,4-triazin(2*H*)ones **1a**–**e** have been obtained by refluxing thiocarbohydrazide [13] with α,β-bioxygen compounds [14] in the presence of abs. EtOH containing a few drops of conc. H_2SO_4. The structure of **1e** was characterized by UV, IR, ^{1}H NMR and MS.

Acylation and aroylation of compound **1** using acid chlorides in DMF yielded the N^4-acyl/aroylamino-3-mercapto-6-substituted-1,2,4-triazin-5-ones **2a**–**c**. The structures of **2** were deduced from treatment of **2b** with thiourea in

Scheme 1



EtOH–NaOH or stirring **2a** and **2c** with conc. H_2SO_4 [15] to give 6-[3-thioxo-1,2,4-triazin-5(2*H*)one-4-yl]amino-2-thioxo-4-phenyl-hexahydropyrimidine (**3**); 3-(2-hydroxynaphthylethenyl)-7-methyl-1,2,4-triazino[3,4-*b*][1,3,4]thiadiazol-4-one (**4a**) and 3-(2-hydroxynaphthylethenyl)-7-(furyl-2-yl)-1,2,4-triazino[3,4-*b*][1,3,4]thia-diazol-4-one (**4b**), respectively (Scheme 1).

The synthesis of heterocylic systems containing a thiadiazole moiety has gained much attention because of their rapid effect in various diseases [16]. Thus, a facile route to synthesis of fused 1,2,4-triazino[3,4-*b*]thiadiazolone was deduced from that of 4-amino-3-mercapto-1,2,4-triazinone (**1**).

Thus, condensation of **1a** with *p*-chlorobenzaldehyde in EtOH–HCl [17] yielded triazinothiadiazolone **4c**, while refluxing compound **1** with aromatic acids in the presence of $POCl_3$ [16] gave 7-aryl-3-(2′-hydroxynaphthylethenyl)-1,2,4-triazino[3,4-*b*][1,3,4]thiadiazol-4-ones **4d**–**f**. On the other hand, refluxing compound **1** with $POCl_3$ only [16] led to the direct formation of 7,7′-bis [3-(2′-hydroxynaphthylmethyl)-1,2,4-triazino[3,4-*b*][1,3,4]thiadiazol-4-one] (**5**). Also, boiling compound **1a** with CS_2 in aq. KOH resulted in 4-oxo-1,2,4-triazino [3,4-*b*][1,3,4]-thiadiazol-7-(6*H*) thione (**6**) (Scheme 1).

As a part of a research program to explore the potential biological properties of the 1,2,4-triazino[3,4-*b*][1,3,4]-thiadiazinone systems, we now present a novel procedure for the preparation of these starting material from 4-amino-3-mercapto-1,2,4-triazin-5-ones (**1**). Thus, reaction of compound **1e** with chloroacetaldehyde dimethylacetal in DMF yielded 8*H*-3-(2′-hydroxynaphthylethenyl)-1,2,4-triazino[3,4-*b*][1,3,4]thiadiazin-4-one (**7a**). Similarly, cyclo-condensation of compound **1e** with phenacyl bromide in DMF yielded 1,2,4-triazino[3,4-*b*][1,3,4]thiadiazin-4-one (**7b**) (Scheme 2).

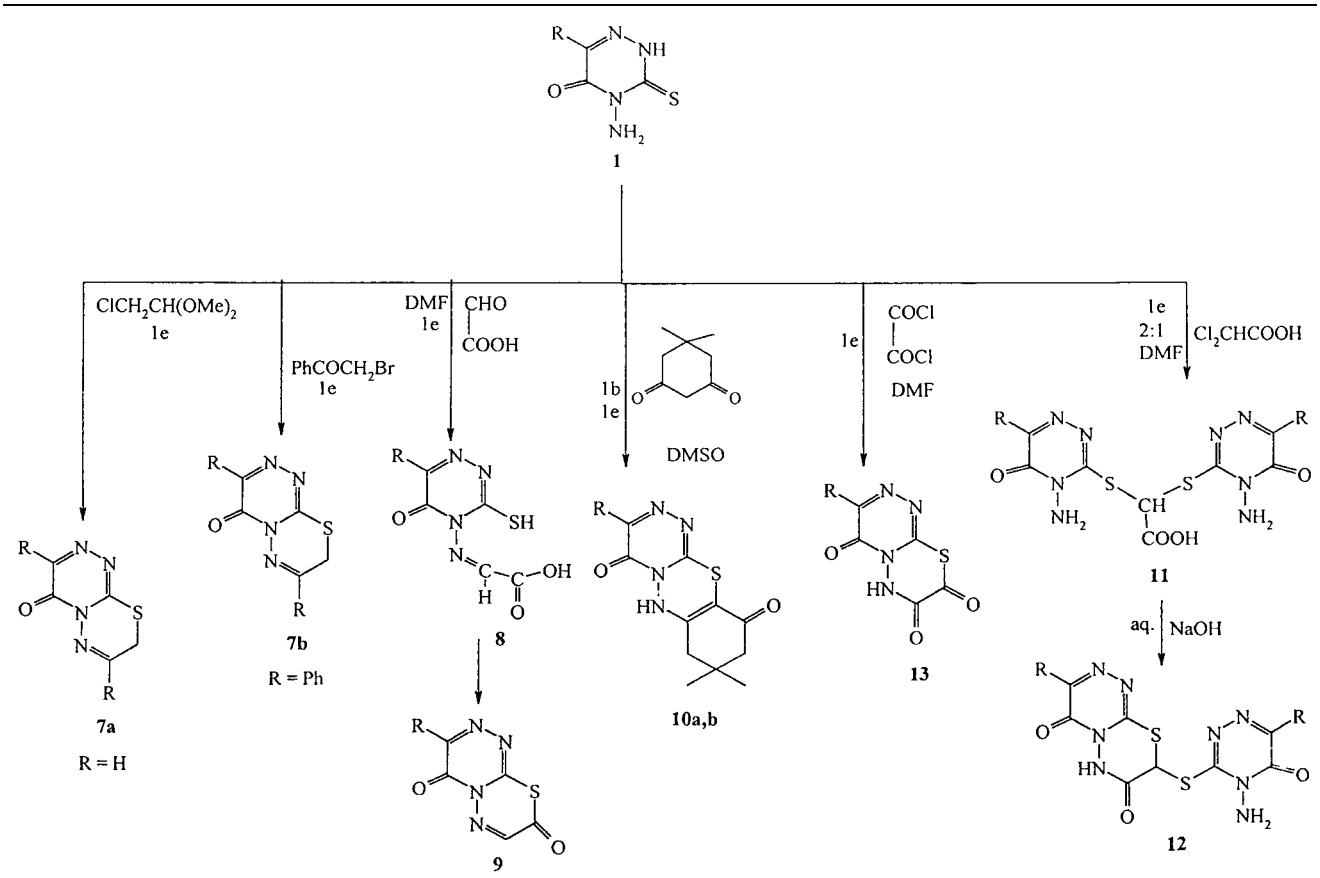
Interestingly, 1,2,4-triazino[3,4-*b*][1,3,4]thiadiazin-4,8-dione (**9**) was obtained from the condensation of compound **1e** with glyoxylic acid followed by dehydration of **8** with conc. H_2SO_4. Similarly cyclo-condensation of compounds **1a/e** with the bioxygen compound dimidone in the presence of DMSO [16] yielded the 8,8-dimethyl-7,8,9,10-tetrahydro-3-substituted-1,2,4-triazino[3,4-*b*][1,3,4]benzo-thiadiazin-4,10-diones **10 a**, **b** (Scheme 2).

The reactions of bi-halogen compounds with 4-amino-3-mercapto-1,2,4-triazin-5-one (**1**) have also been studied.

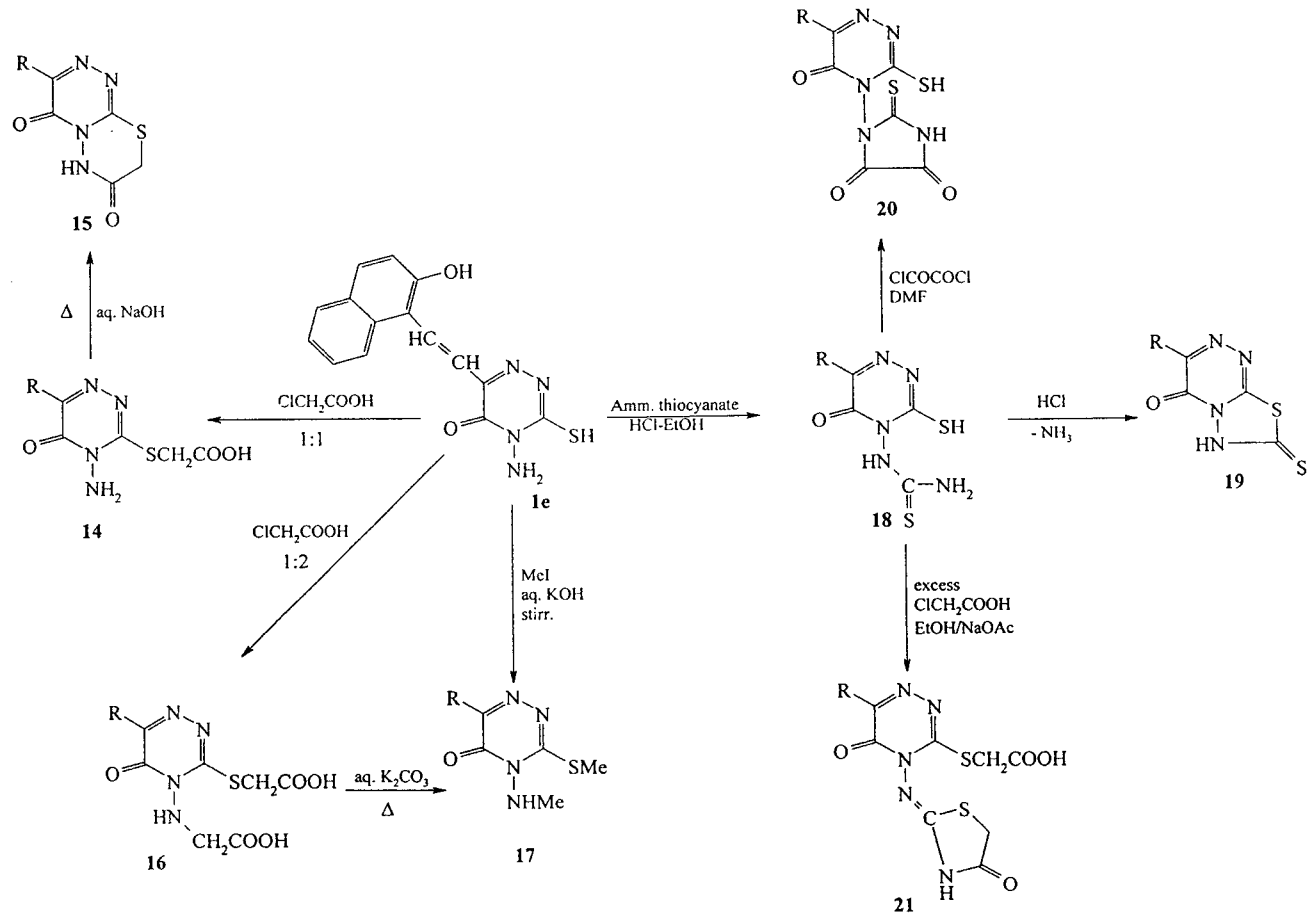
Thus, treatment of compound **1** with 1,1′-dichloroacetic acid in DMF gave 1,1-di[(4′-amino-6′-substituted-5-oxo-1,2,4-triazin-3-yl)thio] acetic acid (**11**) which upon basic cyclization on refluxing with aq. NaOH yielded 1,2,4-triazino[3,4-*b*][1,3,4]thiadiazindione (**12**), while treatment of compound **1e** with oxalyl dichloride in DMF yielded 3-(2′-hydroxynaphthylethenyl)-1,2,4-triazino[3,4-*b*][1,3,4]-thiadiazin-4,7,8-trione (**13**) (Scheme 2).

The greater reactivity of the hydrogen atom of the NH_2 and SH groups of 1,2,4-triazinon **1** is presumably due to its favourable location between the N^2–CS and N^4–C_5 positions. Thus, reaction of compound **1e** with monochloroacetic acid (1:1) in DMF resulted in the formation of 3-carboxymethylthio-1,2,4-triazinone **14** which upon refluxing with aq. NaOH yielded 3-substituted-7,8-dihydro-1,2,4-triazino[3,4-*b*][1,3,4] thiadiazin-4,7-dione (**15**), while when the reaction was carried out in the proportion of 1:2 by moles under the same conditions [18] in yielded 4-carboxymethylamino-3-carboxymethylthio-6-substituted-1,2,4-triazin-5-one **16**. The structure of **16** was deduced from decarboxylation with aq. K_2CO_3 to give 4-methylamino-3-methylthio-1,2,4-triazinone **17** which was also obtained from treatment of compound **1e** with MeI in aq. KOH (Scheme 3).

Scheme 2



Scheme 3



The principal objective of the present work was the formation of fused/isolated heterobicyclic nitrogen systems. Thus, addition of amm. thiocyanate in HCl−EtOH to compound **1e** produced N-substituted thiourea **18** which upon refluxing with conc. HCl, oxalyl chloride and or monochloroacetic acid in different media [18] yielded 6*H*-3-substituted-7-thioxo-1,2,4-triazino[4,3-*b*][1,3,4]-thiadiazol-4-one (**19**), N^1-(3-merca-pto-6-substituted-5-oxo-1,2,4-triazin-4-yl)-2-thioxoimidazol-4,5(3*H*)dione (**20**) and 2-(3-carboxymethylthio-6-substituted-5-oxo-1,2,4-triazin-4-yl-imino)-thiazolidin-4-one (**21**) respectively (Scheme 3).

The structures of compounds **1**–**21** were determined on the basis of elemental analysis and spectral data as well as chemical methods.

Thus, the structures of compounds **1a**–**e** were characterised from UV absorption spectra which showed the para and β-bands in the region from 395–330 and 279–275 nm due to the 1,2,4-triazine moiety in addition to the substitutent's aromatic systems, while in the case of fused heterobicyclic systems **4b** showed λ_{max} at 432, 410, 337 and 268.5 nm due to β- and para-bands correlated with P-π heteroconjugation systems. In the case of an isolated heterobicyclic system **2c** exhibited λ_{max} 376 and 296.5 nm due to inhibition of the p-π conjugated system.

The IR spectrum of the parent compound **1**, showed mainly stretching bands of NH_2 and SH with other characteristic groups while in the case of substituted derivatives, **2, 8**, **11**, **18** showed only the NH_2 and/or SH functional groups. Heterobicyclic systems **4**, **5**, **7a** were marked by the disappearance of both NH_2 and SH groups.

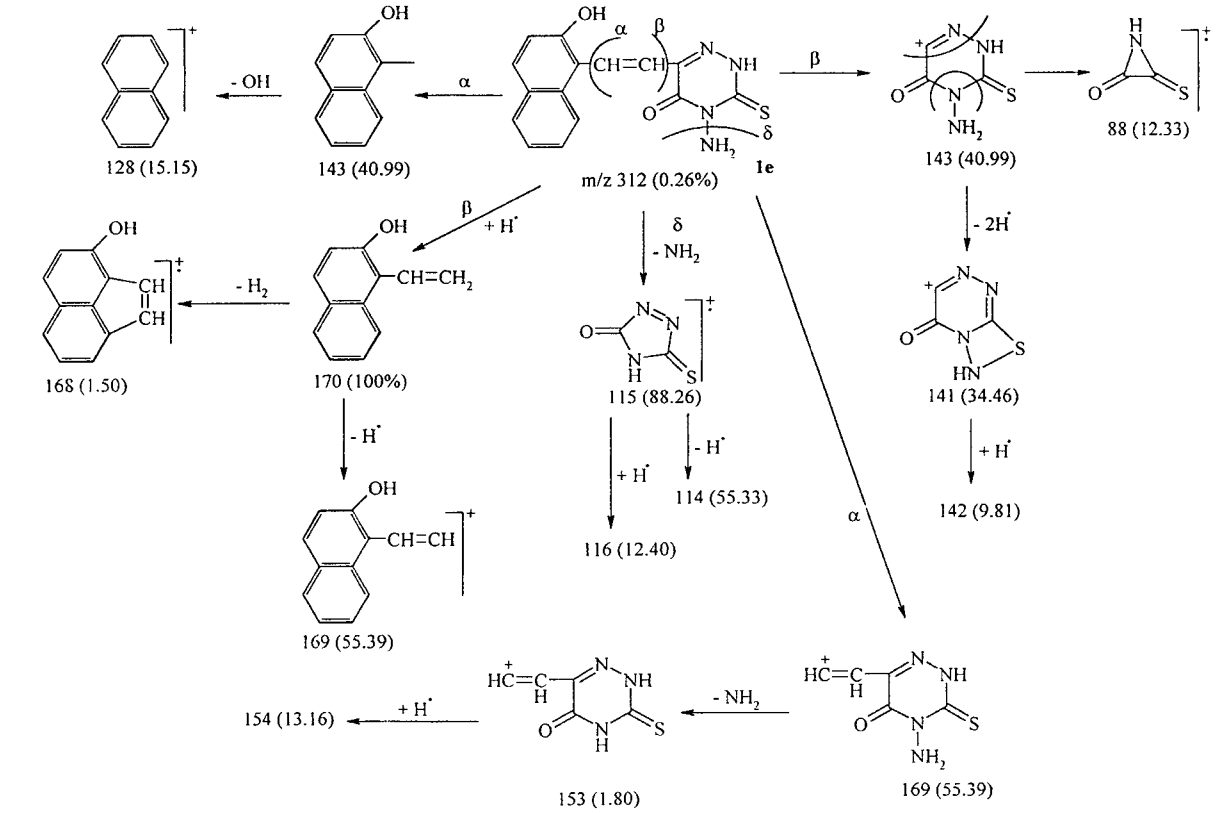
The ^{1}H NMR spectrum of compound **1** showed two different proton signals due to NH_2 and SH with other signals for aromatic and hetero protons; while in the case of heterobicyclic systems, **10b** showed the absence of both NH_2 and SH protons.

M/S of the parent compound **1** and products **2**–**21**, showed the molecular ion has lower percentage intensity, which followed in most cases the splitting off of the 2-hydroxynaphthylethene radical at m/z 170 as a base peak with additional splitting of N−N, C−C, and C−N bonds (Schemes 4, 5). All fragmentation paths leading to various daughter ions were used to deduce the postulated structure.

2.2. Pharmacology

For the past 10 years, the Developmental Therapeutics Program (DTP) of the Division of Cancer Treatment and Diagnosis (DCTD), National Cancer Institute (NCI) has used an *in vitro* model consisting of 60 human tumor cell lines as a primary anticancer screen [J. Natl. Cancer Inst., 83: 757–766, 1991]. An analysis of the data indicated that approximately 95% of the actives from the 60 cell line screen can be identified using only three cell lines. For this reason, the DTP has now begun using, as its primary anticancer assay, a 3-cell line panel consisting of MCF7 (Breast), NCI-H460 (Lung) and SF-268 (CNS). This 3-cell line, one-dose assay has been used by DTP for several years for the evaluation of combinatorial libraries and has proven to be an effective pre-screen. The inclusion of this assay in our decision-making process will allow for more detailed evaluation of agents which have ex-

Scheme 4: Fragmentation pattern of compound **1e**



Scheme 4: Fragmentation pattern of compound **4b**

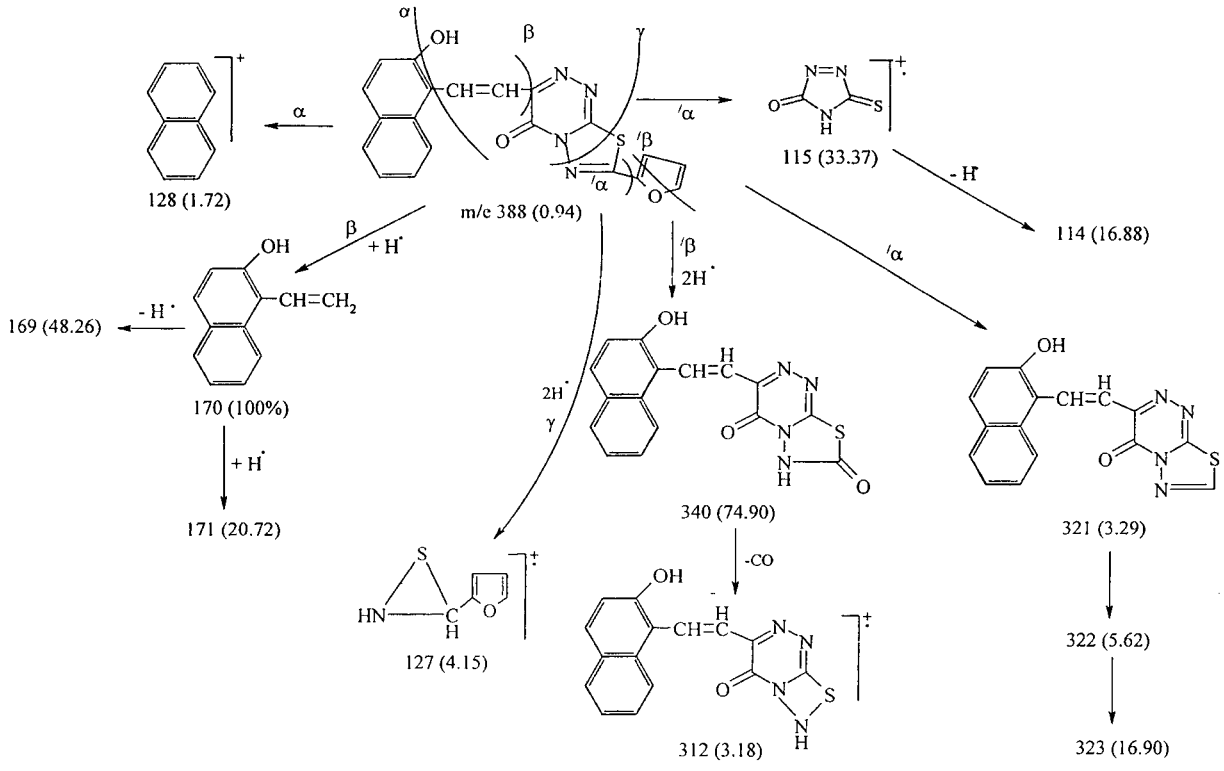


Table 1: *In vitro* model primary anticancer data for some of the new compounds

Compd.	Concentration	Growth percentages			Activity
		(Lung) NCI-H460	(Breast) **MCF_7**	(CNS) **SF-268**	
1b	1 1.00E-04 molar	102	81	97	Inactive
1c	1 1.00E-04 molar	118	33	98	Inactive
1e	1 1.00E-04 molar	−26	−33	−7	Active
4d	1 1.00E-04 molar	95	80	88	Inactive
4e	1 1.00E-04 molar	−3	−33	−1	Active
4f	1 1.00E-04 molar	11	10	21	Active
5	1 1.00E-04 molar	116	41	−22	Active
6	1 1.00E-04 molar	13	−29	40	Active
10a	1 1.00E-04 molar	126	95	109	Inactive
16	1 1.00E-04 molar	21	9	17	Active

* Results for each test agent are reported as the percent age growth of the treated cell compared to the untreated control cells.
** Compounds which reduce the growth of any one of the cell lines to 32% or less (negative numbers indicate cell kill) are passed on for evaluation in the full panel of 60 cell lines over a 5-log dose range.

hibited some level of ability to inhibit the growth of human tumor cell in culture.
In the current protocol, each cell line is inoculated and preincubated on a microtiter plate. Test agents are then added at a single concentration and the culture incubated for 48 h. End-point determinations are made with sulforhodamine B, a protein-binding dye.
The compounds selected have been evaluated in the 3-cell line, one dose primary anticancer assay (Table 1) and for in vitro antitumor activity (Table 2).
Anti HIV screening is currently under study at the NCI, U.S.A.

3. Discussion

The aim of this investigation was to study the drug profiles and structure-activity relations of the compounds and to compare the percentage growth of treated cells compared to untreated control cells.
Generally, compounds **4e**, **1e**, **6**, **5** and **16** are more active than **1b**, **1c** and **10a**. Among the former, 7-(4′-bromophenyl)-3-(2′-hydroxynaphthyl-ethenyl)-1,2,4-triazino-[3,4-*b*][1,3,4]thiadiazol-4-one (**4e**) showed significant activity against all cell lines of the Lung, Breast and Central Nervous System evaluation relates to the toxophoric >N−C−S moiety of 1,3,4-thiadiazoles [23, 24]. Also, the higher activity of compound **4e** is due to combining two biolabile 1,2,4-triazine and thiadiazole rings to give a compact and planner a system.
On the other hand, the weak activity of the compound 8,8-dimethyl-7,8,9,10-tetrahydro-3-substituted-1,2,4-triazino[3,4-2*b*][1,3,4]benzothia-diazine-4,10-dione (**10a**) was due to inhibition of electron mobility from 1,2,4-triazine to 1,3,4-thiadiazinone.

Table 2: *In vitro* activity data for some of the new compounds

Compd.	Response parameters* (mean log 10)			Selectivity analysis Subpanel selectivity***
	(Δ)**/Range			
	GI_{50}	TGI	LC_{50}	
1e	1.44 2.15	0.98 1.38	0.45 0.48	Leukemia, Ovarian Cancer
4e	1.29 1.92	0.64 0.80	0.12 0.13	Leukemia, Colon Cancer, Melanoma
4f	0.65 1.58	1.02 1.14	0.09 0.09	Leukemia, Colon Cancer, Ovarian Cancer
5	0.80 1.06	0.38 0.40	0.00 0.00	CNS Cancer, Melanoma, Renal Cancer, Breast Cancer
6	1.08 1.68	0.87 1.04	0.43 0.46	Leukemia, Non-small Cell Lung Cancer, CNS Cancer, Melanoma, Breast Cancer
16	2.13 2.82	1.89 2.02	0.26 0.27	Leukemia, Non-small cell lung cancer, CNS cancer, Ovarian Cancer, Prostate Cancer

* GI_{50}: Concentration giving 50% inhibition; TGI: concentration giving total growth inhibition; LC50: concentration having 50% lethal effect; Mean values in 60 different tumor cells are reported.
** The reported data represent the logarithmic difference between the parameter values referring to the most sensitive cell line and the mean parameter, Δ is considered low if <1, moderate if >1 and <3, high if >3.

The results of *in vitro* antitumor activity tests for the compounds synthesized indicated that compounds **1e**, **4e**, **6** and **16** were moderately active especially towards Leukemia, Ovarian, CNS and Breast cancers (Table 2).
Regarding the results obtained from compounds **4e**, **1e**, **6**, **5** to **16**, these showed that in spite of the closed structure the difference of activity is due to different substituents on the 1,2,4-triazine molecule.
A fall in activity between isomeric structures **1c** and **1e** can likewise be observed and on comparing the styryl (**1e**) and ethanyl (**1c**) moieties, those which appear as diprotonic (**1c**) seemed to display only very weak activity. Dithioxo derivative **6** apparently has a higher activity than compound **5** and the driving force for **6** deprotonation may be the resonance stabilization of the originating anion reminiscent of the conjugate anion of thiadiazolium salts [25].
Also, introduction of a $-CH_2COOH$ group on the SH and NH_2 of the 1,2,4-triazine ring (**16**) causes fundamental changes in antitumor activity.
An increasing percentage of sulfur and nitrogen elements led to increasing antitumor activity **4e > 16 > 1e** while a decreasing percentage of these elements led to a decreasing antitumor activity **10a > 1b** (Table 3).
It can be concluded from these results that significant antitumor activity was only associated with both the molecular distribution and the electronic mobility of a 1,2,4-triazine moiety which has thiol↔thione (**6**).

Table 3: Percentages of sulfur and nitrogen elements with respect to growth percentages of tumor cells

Compd.	Active Antitumor					Compd.	Inactive Antitumor				
	S [%]	N [%]	Growth percentages				S [%]	N [%]	Growth %		
			Lung	Breast	CNS				Lung	Breast	CNS
4e	6	12	−3	−33	−1	**1c**	10.65	18.65	118	33	98
4f	6.8	11.8	11	10	21	**1b**	15	26	102	81	97
16	7.4	14	21	9	17	**10a**	12	29	126	95	109
1e	9	14	−26	−33	−7						

4. Experimental

M.p.s were determined on a Boetius apparatus and were uncorrected. TLC was carried out by paper chromatography using $CHCl_3/CH_3OH$ (9 : 1). IR spectra in KBr were recorded on a Perkin-Elmer 293 FT spectrometer (ν_{max} in cm^{-1}). UV absorption spectra in DMF were recorded on a Perkin-Elmer 550 S UV-Vis spectrometer (λ_{max} in nm), ^{1}H NMR spectra were recorded on an EMNMR spectrometer 200 MHz PMR using DMSO as a solvent and TMS as internal reference (Chemical shifts in ppm) and MS were recorded on a Gas Chromatographic $GCMS_{qp}$ 1000_{ex} Schimadzu instrument at 70 ev. Compounds **1a** [18], **1c** and **1d** [14] were prepared following a reported procedure. The physical data of the compounds synthesized are given in Table 4.

4.1. Preparation of **1b**

A mixture of α-oxoglutaric acid (0.01 mol) and thiocarbohydrazide (0.01 mol) in abs. EtOH (100 ml) with drops of conc. H_2SO_4 (0.5 ml) was refluxed for 4 h, cooled then filtered and crystallized to give **1b** UV λ_{max} (log E): 330.5 sh (1.66), 279.5 (4.12); IR: 3568 (OH), 3448, 3335 (NH_2, NH), 3061 (aromatic CH), 2922 (aliphatic CH), 1710 (C=O), 1662 (def. NH_2), 1526 (C=N), 1443 (def. CH_2), 1343 (NCS), 1149 (C−S).

4.2. Preparation of **1e**

A mixture of α,β-unsaturated-oxo-acid (0.01 mol) (prepared from condensation of pyruvic acid and 2-hydroxy-naphthaldehyde in alkaline medium) and thiocarbohydrazide (0.01 mol) in abs. EtOH (20 ml) and K_2CO_3 was refluxed for 4 h, cooled and poured onto ice and dil AcOH. The solid obtained was filtered off and recrystallized to give **1e**. UV λ_{max} (log E): 395 (4.20), 278(3.85); IR: 3744 (OH), 3207 (NH_2), 3055 (aromatic CH), 2923 (aliphatic CH), 1660 (C=O), 1622 (def. NH_2), 1579 (C=N), 1318 (NCS), 1183 (C−S). ^{1}H NMR (**1e**): 3.2, 3.4 (s, NH_2, NH), 7.0–7.5 (m, CH=CH), 8.6 (s, NH), 10 (s, OH), 10.8, 11.3 (coupling CH=CH), 12.9 (s, SH). M/e (Int.%): 88 (12.33), 114 (55.33), 115 (88.26), 116 (12.40), 128 (15.15), 141 (34.46), 142 (9.81), 143 (40.99), 153 (1.80), 154 (13.16), 168 (1.50), 169 (55.39), 170 (100), 181 (0.4), 312 (0.26).

4.3. N^4-Acyl/aroylamino-3-mercapto-6-substituted-1,2,4-triazin-5-ones **(2a–c)**

A mixture of **1a** or **1e** (0.01 mol) and acyl/aroylchloride (0.01 mol) in DMF (15 ml) was refluxed for 1 h, cooled and poured onto crushed ice. The solid obtained was filtered off and recrystallized to give **2a–c**. UV (**2c**) λ_{max} (log E): 436.5 sh (1.08), 378.5 (3.96), 279 (3.56); IR (**2c**): 3432 (OH), 3214 (NH), 3054 (aromatic CH), 2922 (aliphatic CH), 1710, 1683 (2C=O), 1589 (C=N), 1322 (NCS), 1188 (C−S), 817, 744 (aromatic ring).

4.4. 6-[3-Thioxo-1,2,4-triazin-5(2H)one-4-yl]amino-2-thioxo-4-phenylhexahydropyrimidine **(3)**

A mixture of **2b** (0.01 mol) and thiourea (0.01 mol) in EtOH (20 ml) with NaOH (10%) was refluxed for 4 h, cooled and poured onto ice. The solid obtained was filtered off and recrystallized to give **3** (Table 3). IR: 3203(NH), 3058 (aromatic CH), 2925 (aliphatic CH), 1716 (C=O), 1583 (C=N), 1465 (def. CH_2), 1318 (NCS), 1181, 1141 (2C−S), 818, 744 (phenyl ring).

4.5. 3-(2-Hydroxynaphthylethenyl)-7-methyl-1,2,4-triazino[3,4-b][1,3,4] thiadiazol-4-one **(4a)** *and 3-(2-hydroxynaphthylethenyl)-7-(furyl-2-yl)-1,2,4-triazino [3,4-b][1,3,4] thiadiazol-4-one* **(4b)**

A mixture of **2a** and **2c** (0.01 mol) was conc. H_2SO_4 (5 ml) was stirred for 1 h, cooled and poured onto ice. The solid obtained was filtered off and recrystallized to give **4a** and **4b**. UV λ_{max} (log E): 432 sh. (1.76), 410.5 2.20), 337.5 (2.04), 268.5 (2.80); IR (**4b**): 3477 (OH), 1717 (C=O), 1622 (C=C), 1576 (C=N), 1320 (NCS), 1178 (C-S), 788, 748 (phenyl ring). M/e (Int.%): 114 (16.85), 115 (33.87), 127 (4.15), 128 (1.72), 169 (48.26), 171 (20.72), 312 (3.18), 321 (3.29), 322 (5.62), 323 (16.90), 340 (74.90), 388 (0.94).

Table 4: Phsyical data for the new heterobicyclic nitrogen systems 1–21

Compd.	Crystallized from	M.p. (°C)	Yield (%)	Mol. Formula*	M.wt.	Sulfur analysis	
						Found %	Calcd. %
1a	MeOH	210	80	$C_3H_4N_4OS$	144	21.91	22.22
1b	MeOH	190	70	$C_6H_8N_4O_3S$	216	14.9	14.81
1c	EtOH	260	75	$C_{14}H_{12}N_4O_2S$	300	10.0	10.66
1d	MeOH	185	50	$C_7H_8N_4O_2S$	212	14.50	15.09
1e	DMF	265	90	$C_{15}H_{12}N_4O_2S$	312	10.88	11.25
2a	MeOH	240	60	$C_{17}H_{14}N_4O_3S$	354	8.5	9.03
2b	MeOH	195	55	$C_{12}H_{10}N_4O_2S$	274	11.60	11.67
2c	MeOH	150	60	$C_{20}H_{14}N_4O_4S$	406	7.50	7.88
3	MeOH	125	70	$C_{13}H_{12}N_6S_2O$	332	18.4	19.27
4a	MeOH	220	90	$C_{17}H_{12}N_4O_2S$	336	9.2	9.52
4b	MeOH	285	88	$C_{20}H_{12}N_4O_3S$	388	8.0	8.24
4c	EtOH	260	95	$C_{10}H_5N_4OSCl$*	264	11.90	12.12
4d	MeOH	215	80	$C_{22}H_{13}N_4O_2SCl$**	432.5	6.62	7.39
4e	MeOH	110	85	$C_{22}H_{13}N_4O_2SBr$***	477	6.2	6.70
4f	MeOH	200	95	$C_{22}H_{13}N_5O_4S$	443	7.1	7.22
5	MeOH	250	70	$C_{30}H_{18}N_8O_4S_2$	309	9.5	10.35
6	MeOH	180	65	$C_4H_2N_4OS_2$	186	34.0	34.38
7a	MeOH	235	70	$C_{17}H_{11}N_4O_2S$	335	9.3	9.55
7b	MeOH	140	75	$C_{23}H_{16}N_4O_2S$	412	7.7	7.76
8	MeOH	250	60	$C_{17}H_{12}N_4O_4S$	368	8.6	8.69
9	DMF	280	60	$C_{17}H_{11}N_4O_3S$	351	8.8	9.11
10a	MeOH	170	40	$C_{11}H_{12}N_4O_2S$	264	11.2	12.12
10b	MeOH	210	55	$C_{23}H_{20}N_4O_3S$	432	6.8	7.40
11	MeOH	120	70	$C_{32}H_{24}N_8O_6S_2$	680	9.1	9.41
12	MeOH	180	65	$C_{32}H_{22}N_8O_5S_2$	562	8.9	9.66
13	Dil. DMF	300	55	$C_{17}H_{10}N_4O_4S$	366	8.5	8.74
14	MeOH	250	60	$C_{17}H_{14}N_4O_4S$	370	7.4	8.64
15	MeOH	160	70	$C_{17}H_{12}N_4O_3S$	352	8.5	9.09
16	MeOH	260	50	$C_{19}H_{16}N_4O_6S$	428	6.6	7.47
17	MeOH	250	55	$C_{17}H_{16}N_4O_2S$	340	9.0	9.41
18	MeOH	270	60	$C_{16}H_{13}N_5O_2S_2$	371	16.2	17.25
19	EtOH	220	70	$C_{16}H_{10}N_4O_2S_2$	344	17.8	18.07
20	MeOH	250	60	$C_{18}H_{11}N_5O_4S_2$	425	14.5	15.05
21	MeOH	210	50	$C_{20}H_{15}N_5O_5S_2$	469	13.3	13.64

* Elemental analysis, (C, H, N) are within ± 0.5 of the theoretical values
* Cl Found: 13.1%; Calcd: 13.44%
** Cl Found: 7.3%; Calcd: 8.19%
*** Br Found: 16.5%; Calcd: 16.77%

4.6. Triazinothiadiazolone (**4c**)

A mixture of **1a** (0.01 mol) and *p*-chlorobenzaldehyde (0.01 mol) in abs. EtOH (10 ml) with conc. HCl (2 ml) was heated under reflux for 4 h, cooled and poured onto crushed ice. The solid obtained was filtered off and recrystallized to give **4c**.

4.7. 7-Aryl-3-(2′-hydroxynaphthylethenyl)-1,2,4-triazino[3,4-b][1,3,4]-thiadiazol-4-ones (**4d–f**)

A mixture of **1e** (0.01 mol) and aromatic acids (0.01 mol) in $POCl_3$ (7 ml) was heated under reflux for 4 h, cooled and poured onto ice with stirring for 1 h, and the solid obtained was filtered off and recrystallized to give **4d–f**. UV (**4f**) λ_{max} (log E): 355.5 (3.62), 279 (3.38); IR (**4d**): 3384 (OH), 3059 (aromatic CH), 1734 (C=O), 1626 (C=C), 1594 (C=N), 1340 (NCS), 1131 (C−S), 813, 750, 711 (aromatic rings), 668 (C−Cl); (**4f**): 3442 (OH), 3088 (aromatic CH), 1717 (C=O), 1623 (C=C), 1527 (C=N), 1349 (NCS), 1527, 1395 (asy. and sy. NO_2), 1160 (C−S), 819, 750 (phenyl rings). M/e (Int.%) (**4e**): 81(18), 114(61.62), 115(90.50), 128 (53.69), 143 (5.04), 144 (40.99), 157 (24.55), 158 (4.88), 169 (100), 170 (38.81), 183 (46.43), 185 (33.47), 196 (2.34), 199 (13.17), 213 (1.85), 242 (1.63), 478 (0.13). ^{1}H NMR (**4e**): 6.0–6.5 (m, CH=CH), 7.2–8.4 (m, aromatic and/OH protons).

4.8. 7,7′-Bis[3-(2′-hydroxynaphthylmethyl)-1,2,4-triazino[3,4-b][1,3,4] thiadiazol-4-one] (**5**)

A mixture of **1c** (0.01 mol) and glyoxalic acid (0.01 mol) in $POCl_3$ (5 ml) was refluxed for 4 h, and poured onto ice with stirring for 1 h. The solid obtained was filtered off and recrystallized to give **5**. IR: 3459 (OH), 3334 (NH), 1711 (C=O), 1620 (C=C), 1572 (C=N), 1344 (NCS), 1149 (C−S), 1092 (C−S−C), 994, 869, 814 (aromatic ring). M/e (Int.%): 105 (59.27), 115 (1.38), 140 (98.92), 141 (11.42), 153 (8.42), 182 (22.80), 242 (1.57), 270 (9.90), 298 (100), 321 (0.79), 618 (0.0).

4.9. 4-Oxo-1,2,4-triazino [3,4-b][1,3,4] thiadiazol-7(6H) thione (**6**)

A mixture of **1a** (0.01 mol) and carbon disulphide (0.01 mol) in EtOH (15 ml) with aq. KOH (5%, 20 ml) was refluxed for 6 h, cooled and poured onto ice-HCl. The solid obtained was filtered off and recrystallized to give **6** (Table 3). IR: 3773 (OH), 3500–2920 (b, OH, NH, aromatic and aliphatic CH), 1721 (C=O), 1630 (C=C), 1583 (C=N), 1311 (NCS), 1162 (C−S), 1083 (C−S−C), 823, 751 (aromatic ring).

4.10. 8H-3-(2′-Hydroxynaphthylethenyl)-1,2,4-triazino[3,4-b][1,3,4]-thiadiazin-4-one (**7a**)

A mixture of **1e** (0.01 mol) and chloroacetaldehyde dimethyl acetal (0.01 mol) in DMF (10 ml) was refluxed for 6 h, cooled and poured onto ice. The solid obtained was filtered off and recrystallized to give **7a**.

4.11. 1,2,4-Triazino [3,4-b][1,3,4] thiadiazin-4-one (**7b**)

A mixture of **1e** (0.01 mol) and phenacyl bromide (0.01 mol) in DMF (20 ml) was refluxed for 6 h, cooled and poured onto ice. The solid obtained was filtered off and recrystallized to give **7b**.

4.12. 4-(Carboxymethylidene)amino-6-(2′-hydroxynaphthylethenyl)-3-thioxo-1,2,4-triazin-5(2H)one (**8**)

A mixture of **1e** (0.01 mol) and glyoxylic acid (0.01 mol) in DMF (20 ml) was refluxed for 8 h, cooled and poured onto ice. The solid obtained was filtered off and recrystallized to give **8**. UV λ_{max} (log E): 430.5 sh (2.8), 410 (3.75), 329.5 (3.20), 276.5 (3.35); IR: 3745 (OH), 3800–2862 (b, OH, NH, aromatic and aliphatic CH), 1700, 1665 (2C=O) 1618 (C=C), 1577 (C=N), 1463 (def. CH), 1315 (NCS), 1177 (C−S), 822, 789, 744 (aromatic ring).

4.13. 1,2,4-Triazino [3,4-b][1,3,4]thiadiazin-4,8-dione (**9**)

A mixture of **8** (0.01 mol) and conc. H_2SO_4 (5 ml) was stirred for 1 h, and poured onto ice and Na_2CO_3. The solid obtained was filtered off and recrystallized to give **9**. IR: 3631–3419 (b, OH), 1803, 1622 (2C=O), 1573 (C=N), 1316 (NCS), 1180 (C−S), 1088 (C−S−C), 820, 787, 741 (aromatic rings).

4.14. 8,8-Dimethyl-7,8,9,10-tetrahydro-3-substituted-1,2,4-triazino[3,4-b] [1,3,4] benzothiadiazin-4,10-diones (**10a, b**)

A mixture of **1a** or **1e** (0.01 mol) and dimidone (0.01 mol) in DMSO (10 ml) was refluxed for 12 h. The reaction mixture was concentrated and cooled. The solid obtained was filtered off and recrystallized to give **10a, b**. UV (**10b**): 440, 415 and 270 nm ^{1}H NMR (**10a**): δ 1.23–1.29 (m, $2CH_3$), 3.5 (CH_2−C−), 4.14–4.18 (CH_2−C=O), 7.23 (2H CH=CH), 7.60–7.73 (m, aromatic proton), 9.3 (OH), 10.03 (NH). M/e (Int.%): 436 (12.18; M + 4), 410 (45.51), 341 (87.82), 250 (53.21), 185 (56.41), 116 (75.00), 93 (67.95) and 69 (100%).

4.15. 1,1-Di[(4′-amino-6′-substituted-5-oxo-1,2,4-triazin-3-yl)thio]acetic acid (**11**)

A mixture of **1e** (0.02 mol) and 1,1-dichloroacetic acid (0.01 mol) in DMF (50 ml) was refluxed for 1 h, cooled then poured onto ice.
The solid thus obtained was filtered and crystallized to give **11**. M/e (Int.%): 239(3), 250 (39), 277 (100), 302 (2), 317 (15), 331 (15) 334 (6), 370 (8), 402 (22), 456 (10), 470 (20), 472 (10), 481 (5), 499 (10), 548 (10), 562 (2).

4.16. 3-(2′-Hydroxynaphthylethenyl)-8[(6-(2′-hydroxynaphthylethen-yl)-4-amino-5-oxo-1,2,4-triazino-3-thioxo-3-yl)]1,2,4-triazin [3,2-b][1,3,4]thiadiazin-4,7-dione (**12**)

A mixture of **11** (0.01 mol) and aq. NaOH (10%) was refluxed for 4 h, cooled and poured onto ice −HCl. The solid obtained was filtered off and recrystallized to give **12**. M/e (Int%): 684 (2, M + 4 + H_2O), 513 (20), 499 (18), 456 (10), 291 (10), 277 (35), 268 (98), 250 (100) and 171 (20).

4.17. 3-(2′-Hydroxynaphthylethenyl)-1,2,4-triazino[3,4-b][1,3,4]thiadiazin-4,7,8-trione (**13**)

A mixture of **1e** (0.01 mol) and oxalyl chloride (0.01 mol) in DMF (15 ml) was refluxed for 6 h, cooled and poured onto ice. The solid obtained was filtered off and recrystallized to give **13**.

4.18. 4-Amino-6-(2′-hydroxynaphthylethenyl)-3-(carboxymethylthio)-1,2,4-triazin-5-one (**14**)

A mixture of **1e** (0.01 mol) and monochloroacetic acid (0.01 mol) in DMF (20 ml) was refluxed for 8 h, cooled and poured onto ice. The solid obtained was filtered off and recrystallized to give **14**. IR: 3774 (OH), 3202 (NH_2), 3058 (aromatic CH), 2925 (aliphatic CH), 1754–1716, 1682 (2C=O), 1621 (def. NH_2), 1583 (C=N), 1465 (def. CH_2), 1319 (NCS), 1181 (C−S), 818, 744 (aromatic rings).

4.19. 8H-3-(2′-Hydroxynaphthylethenyl)-1,2,4-triazino[3,4-b]thiadiazin-4,7(6H)diones (**15**)

A mixture of **14** (0.01 mol) in aq. NaOH (5%, 20 ml) was refluxed for 2 h, cooled and poured onto ice and HCl. The solid obtained was filtered off and recrystallized to give **15**. IR: 3774, 3703 (OH), 3500–3250 (b, OH↔NH), 3058 (aromatic CH), 2850 (aliphatic CH), 1815, 1721 (2C=O), 1625 (C=C), 1577 (C=N), 1463 (def. CH_2), 1312 (NCS), 1176 (C−S), 817, 790, 743 (aromatic rings).

4.20. 4-Carboxymethylamino-3-carboxymethylthio-6-(2′-hydroxynaphthylethenyl)-1,2,4-triazin-5(2H) one (**15**)

A mixture of **1e** (0.01 mol) and monochloroacetic acid (0.02 mol) in DMF (20 ml) was refluxed for 8 h, cooled and poured onto ice. The solid obtained was filtered off and recrystallized to give **16**. M/e (Int.%): 135 (10), 254 (15), 268 (50), 271 (25), 285 (100), 299 (5), 313 (60), 335 (10), 397 (5), 428 (2).

4.21. 4-Methylamino-3-methylmercapto-6-(2′-hydroxynaphthylethenyl)-1,2,4-triazin-5-one (**17**)

4.21.1. Method A

A mixture of **16** (0.01 mol) in aq. K_2CO_3 (10%, 20 ml) was refluxed for 2 h, cooled and poured onto ice and HCl. The solid obtained was filtered off and recrystallized to give **17**. IR: 3500–3150 (b, OH, NH), 3056 (aromatic CH), 2920 (aliphatic CH), 1721 (C=O), 1626 (C=C), 1574 (C=N), 1463 (def. CH_2), 1313 (NCS), 1176 (C−S), 818, 790, 741 (aromatic rings). ^{1}H NMR: 2.1–2.3 (s, CH_3−S), 3.2 (s, CH_3−N), 4.1–4.3 (s, NH), 6.5–6.6 (m, CH=CH), 7.5–7.99 (m, aromatic and OH protons).

4.21.2. Method B

A mixture of **1e** (0.01 mol) and MeI (0.02 mol) was stirred with an aq. solution of KOH (1%, 20 ml) for 1 h and left for 24 h, then acidified with CH_3COOH. The solid thus obtained was filtered off and crystallized to give **17**. M.p. for **17** from methods A and B combined gave no depression.

4.22. 4-(Thiourido-1-yl)-3-mercapto-6-(2′-hydroxynaphthylethenyl)-1,2,4-triazin-5-one (**18**)

A mixture of **1e** (0.01 mol) and amm. thiocyanate (0.01 mol) in EtOH (20 ml) and HCl (2 ml) was refluxed for 6 h, and the reaction mixture was concentrated and cooled. The solid obtained was filtered off and recrystallized to give **18**.

4.23. 6-(2′-Hydroxynaphthylethenyl)-4-oxo-1,2,4-triazino-[3,4-b][1,2,4]-thiadiazol-7(6H)-thione (**19**)

A mixture of **18** (0.01 mol) and HCl (10%, 10 ml) was heated under for 1 h, cooled and poured onto ice. The solid obtained was filtered off and recrystallized to give **19**.

4.24. 2-Thioxo-imidazol-4,5(3H)dione (**20**)

A mixture of **18** (0.01 mol) and oxalylchloride (0.01 mol) in DMF (20 ml) was refluxed for 8 h, cooled and poured onto ice. The solid obtained was filtered off and recrystallized to give **20**

4.25. 6-(2′-Hydroxynaphthylethenyl-3-carboxymethlthio-4-(5-thiazolidinone-2-yl)imino-1,2,4-triazin-5-one (**21**)

A mixture of **18** (0.01 mol) and monochloroacetic acid (0.02 mol, in NaOAc (3 g) and EtOH (20 ml) was heated under reflux for 2 h, cooled and poured onto ice. The solid obtained was filtered off and recrystallized to give **21**. IR: 3637 (OH), 3340 (OH), 3242 (OH), 3200 (NH), 3060 (aromatic CH), 2987 (aliphatic CH), 1816, 1748, 1738 (3C=O), 1622 (C=C), 1586 (C=N), 1536 (C=N), 1462 (def. CH_2), 1321 (NCS), 1178 (C−S), 820, 742 (aromatic rings). ^{1}H NMR (δ): 2.48–2.51 (s, acyclic CH_2−S), 2.7–2.9 (s, cyclic CH_2−S), 3.5 (s, NH), 7.2–7.6 (m, CH=CH), 7.9–8.2 (m, aromatic protons), 8.4 (s, OH), 10.5 (OH).

Acknowledgement: The authors are very grateful to Prof. Dr. V. L. Narayanan and Edward Sausville; Drug synthesis, Chemistry Branch, National Cancer Institute, Bethesda, Maryland 20892 U.S.A., for evalution of the in vitro antitumor tests as Development Program. Thanks are also due to the members of the NCI staff.

References

1 Blackledge, J. A.; Fu Min Xin; Thorps, S. R.; Baynes, J. W.: J. Org. Chem. **58**, 2001 (1993); C.A. **119**, 28107 g (1993)
2 Findeisen, K.; Luerssen, K.; Santel, H. J.; Schmidt, R. R. (Bayer A. G.): C.A. **116**, 59408 f (1992)
3 Abdel-Rahman, R. M.; Morsy, J. M.; Allimony, H. A.; Abdel-Monem, W. R.: Boll. Chim. Farm. **138**, 19 (1999)
4 Abdel-Rahman, R. M.; Seada, M.; Fawzy, M.; El-Baz, I.: Pharmazie **49**, 729 (1994)
5 Abdel-Rahman, R. M.; Seada, M.; Fawzy, M.; El-Baz, I.; Pharmazie **49**, 811 (1994)
6 Abdel-Rahman, R. M.; Morsy, J. M.; Hanafy, F.; Amene, H. A.: Pharmazie **54**, 347 (1999)
7 Abdel-Rahman, R. M.; Morsy, J. M.; El-Edfawy, S.; Amene, H. A.; Pharmazie **54**, 667 (1999)
8 Remmel, R. P.; Sinz, M. W.: Drug Metab. Dispos. **19**, 630 (1991)
9 Wootton, R.; Soullawton, J. R.; Posno, P. E.: Br. J. Clin. Pharmacol. **43**, 23 (1997)
10 Boehner, B. (Ciba-Geigy A.G.): Eur. Pat. 1986; C.A. **105**, 6522e (1986)
11 Chemosphere **17**(10), 2043 (1988); C.A. **110**, 192777 (1989)
12 Boehner, B.; Tobler, H. (Ciba-Geigy A.G.): Eur. Pat Appl. **150**, 677 (1985); C.A. **104**, 19607 (1986)
13 Saha, G. C.; Khayer, K.; Islam, Md. R.; Chowdhury, Md. S. K.: Indian J. Chem. **31B**, 547 (1992)
14 Holla, B. S.; Gonsalves, R.; Shivanada, M. K.: Boll. Chim. Farm. **137** (3), 93 (1998)
15 Jayamma, Y.; Sarangapani, M.; Reddy, V. M.: Indian J. Heterocycl. Chem. **4**, 289 (1995)
16 Talawar, M. B.; Laddi, U. V.; Somannavar, Y. S.; Bennur, R. S.; Bennur, S. C.; Indian J. Heterocycl. Chem. **4**, 297 (1995)
17 Gakhar, H. K.; Prabha, U.; Gill, J. K.: J. Indian Chem. Soc. **61**, 5523 (1984)
18 Abdel-Rahman, R. M.: Pak J. Sci. Ind. Res. **30**, 490 (1987)
19 Fawzi, M. M.; Fr. pat. 1.547, 854 (1968); C.A. **171**, 91538 m (1969)
20 Weislow, O. S.; Kiser, R.; Fine, D. L.; Bader, J.; Shoemaker, R. M.; Boyed, M. R.: J. Nat. Cancer Inst. **81**, April 19 (1989)
21 Boyed, M. R.: Proc. Am. Assoc. Cancer Res. **30**, 652 (1989)
22 Grever, M. R.; Schepartz, S. A.; Chabner, B. A.: The National Cancer Institute, Cancer Drug Discovery and Development Program. Seminars Oncology, 19(6), (December) 622 (1992)
23 John, S. W.; Ger Offen, 2704288 (1977); C.A. **87**, 201546 (1977)
24 Suzuki, F.; Kawakami, J.; Yamamoto, S.; Kosai, Y.: Japan Kokai, 7776432 (1977); C.A. **88**, 100351 (1977)
25 Schachtner, J. E.; Nienaber, J.; Stachel, H. D.; Waisser, K.: Pharmazie **54**, 335 (1999)

Received November 30, 1999
Accpeted September 25, 2000

Prof. Dr. R. M. Abdel-Rahman
Chemistry Department
Faculty of Education
Ain Shams University
Roxy, Cairo
Egypt