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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of some (*E*)-6-[2-(furan-2-yl)ethenyl]-1,2,4-triazin-5-ones and their biological evaluation as antitumor agents

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Abstract The synthesis of some new (*E*)-6-[2-(furan-2-yl)ethenyl]-1,2,4-triazin-5-ones directly linked to either pyrazole, pyrazoline, pyrazolidine counterparts, or to substituted thio and hydrazono functionalities is described. Six of the newly synthesized compounds were selected by the National Cancer Institute (NCI) to be evaluated for their in vitro antitumor activity according to the protocol of the NCI in vitro disease-oriented human cells screening panel assay. The results revealed that the pyrazole derivative **5c** was found to be the most active member in this screen as evidenced by its ability to exert potential growth inhibitory activity against most of the tested subpanel tumor cell lines with selective influence on leukemia subpanel tumor cell lines (GI_{50} values 2.01–3.03 μM). Moreover, a comparative study for log GI_{50} values of both compound **5c** and 5-fluorouracil (5-FU) revealed that compound **5c** showed higher potency than 5-FU against most of the tested subpanel tumor cell lines. Thus compound **5c** could be considered as a suitable lead towards the design of broad spectrum antitumor active agents targeting various human tumor cell lines.

Keywords Synthesis · 1,2,4-Triazin-5-ones · Pyrazole · Antitumor activity

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Introduction

Cancer poses a serious human health problem despite much progress in understanding its biology and pharmacology. Although cancer research has led to a number of new and effective solutions, the medicines used as treatments have clear limitations due to lack of selectivity leading to toxicity, metastatic spreading and the intrinsic or acquired resistance developed after few therapeutic cycles (Braña and Ramos, 2001; Cozzi, 2003). At the same time, random screening remains one of the main routes to discover new leads with antineoplastic activity and the National Cancer Institute (NCI), Bethesda, USA, is still playing an articular role in this field, with special emphasis on novel chemical structures that have not had extensive clinical evaluation (Cocco *et al.*, 2000).

Among the wide variety of heterocycles that have been explored for developing pharmaceutically important molecules, the 1,2,4-triazines have received great attention as chemotherapeutic agents. Azanucleosides (6-azacytosine and 6-azauracil), structurally based on the 1,2,4-triazine scaffold were proved to display antitumor (Creasey *et al.*, 1963), antiviral (Sidwell *et al.*, 1968), and antifungal activities (Sangshetti and Shinde, 2010). In addition, some 1,2,4-triazin-6(1*H*)-ones were reported to display significant broad spectrum antitumor activity against lymphoblastic leukemia CEM, myeloid leukemia K562, and lung adenocarcinoma A549 cancer cell lines (Gucky *et al.*, 2009) while, some 1,2,4-triazine 5-one derivatives were found to exhibit strong antiproliferative effect on human leukemia K-562 cell line (Krauth *et al.*, 2010). Moreover, particular interest has been focused on 6-azaisocytosine (3-amino-1,2,4-triazin-5(2*H*)-one), an isosteric isomer of 6-azacytosine and 6-azauracil as potential transcription inhibitor (Pal chykovska *et al.*, 2004). On the other hand, a

literature survey revealed that some pyrazoles and pyrazole containing compounds have been implemented as antileukemic (Daidone *et al.*, 2004a; Chou *et al.*, 2007; Manetti *et al.*, 2008), antitumor (Li *et al.*, 2006; Xia *et al.*, 2007; Xia *et al.*, 2008; Farag *et al.*, 2008), and antiproliferative agents (Schenone *et al.*, 2004; Daidone *et al.*, 2004b), beside their capability to exert remarkable anticancer effects through inhibiting different types of enzymes that play important roles in cell division (Warshakoon *et al.*, 2006; Huang *et al.*, 2007; Zhu *et al.*, 2007). Furthermore, some thioethers were found to show enhanced antimicrobial and antitumor activities (Gulerman *et al.*, 2001; Khalil *et al.*, 2003) beside being a common structural subunit in SDABOs (dihydroalkylthio-benzyl-oxopyrimidines) which possess antiproliferative as well as antiviral activities (Manetti *et al.*, 2005). Addditionally, hydrazono derivatives with their effective contribution as antineoplastic agents (Remers, 2004) were not far of our attention.

Inspired by the above-mentioned facts and as a continuation of an ongoing research program aimed at the discovery of novel chemotherapeutic agents (Rostom *et al.*, 2009; Ashour and Abdel Wahab, 2009; Rostom *et al.*, 2011), it seemed worthwhile to synthesize new structure hybrids (A–C) incorporating the 1,2,4-triazin-5-one scaffold linked to a pyrazole, pyrazoline, pyrazolidine ring or substituted thioether and hydrazono moieties (Fig. 1) which are believed to be responsible for the biological significance of some relevant natural and synthetic chemotherapeutic agents. This combination was suggested in an attempt to investigate the possible synergistic influence of such structure hybridization on the anticipated biological activity hoping to discover a new lead structure that would have a significant antitumor potential. In addition, variation in the nature and size of substituents was also attempted, as it would offer variable electronic, lipophilic, and steric environment that would influence the targeted biological activity. The present work reports the synthesis and the results of preliminary antitumor screening of compounds selected by the NCI.

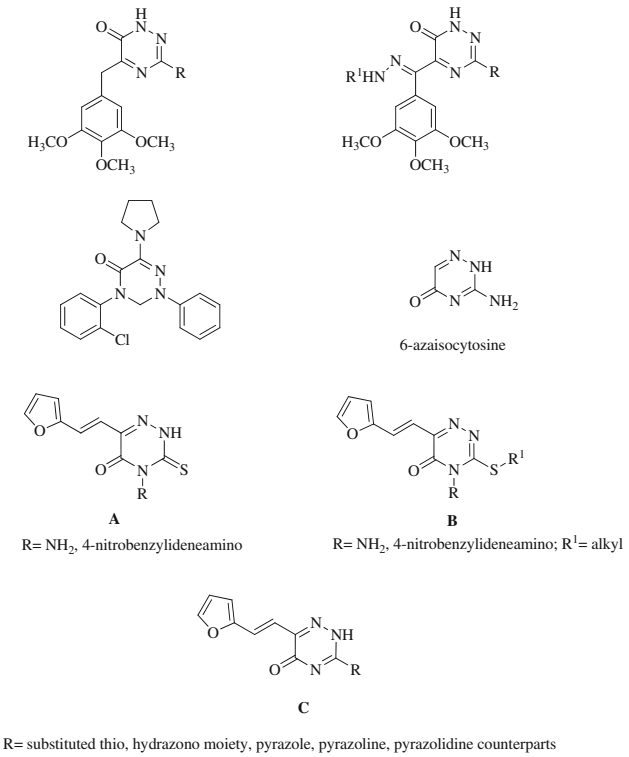


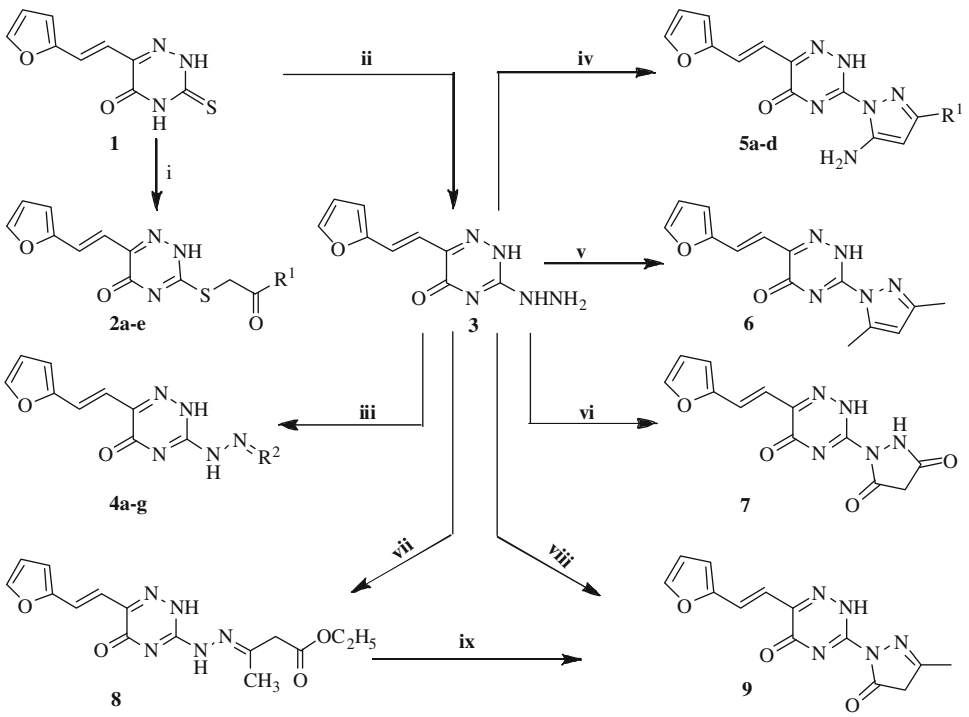
Fig. 1 The structure of some reported antitumor 1,2,4-triazines and the newly synthesized compounds

Chemistry

Synthesis of the intermediate and target compounds was accomplished according to the steps depicted in Schemes 1 and 2. In Scheme 1, the starting thione **1** and the hydrazine intermediate **3** were prepared according to previously reported reaction conditions (Slouka, 1962; Osman *et al.*, 2007). Heating **1** with phenacyl or 4-substituted phenacyl bromide in ethanol gave rise to the substituted 2-oxoethylsulfanyl analogs **2a**–**e**. ^{1}H-NMR spectra of compounds **2a**–**e** revealed singlets at 4.45 and 4.38 ppm for compounds **2a**, **b** and two doublets at 3.67–3.87 ppm for compounds **2c**–**e** attributed to SCH_2 protons. In addition, investigation of ^{1}H-NMR spectra for compounds **2a**–**e** revealed presence of two doublets for ethenyl C_1 and C_2 protons at 6.65–6.81 and 7.56–7.75 ppm with coupling constant 16.05–16.7 Hz indicating their existence as E-isomers (Williams and Fleming, 1980). ^{13}C-NMR spectrum of compound **2e** as an example showed a signal at 50.55 ppm corresponding to SCH_2 carbon and other signals were observed at their expected chemical shifts. Condensation of the hydrazine derivative **3** with 4-substituted benzaldehydes, furfural, 5-nitro-2-furfural, isatin, and *N*-methyl isatin in boiling ethanol resulted in the formation of the corresponding hydrazones **4a**–**g**. ^{1}H-NMR spectra of compounds **4a**–**e** showed singlets for the N=CH protons in the range 7.82–8.08 ppm indicating existence of these compounds in the E configuration around the N=C (Pretsch *et al.*, 2000, pp 211–212). ^{13}C-NMR spectrum of compound **4a** provided further confirmation of the chemical structure. On the other hand, reaction of **3** with phenacyl or 4-substituted phenacyl cyanides in ethanol/acetic acid mixture furnished the target 5-aminopyrazoles **5a**–**d**. Inspection of ^{1}H-NMR spectra of compounds **5a**–**d** indicated presence of a singlet at 5.88–5.92 ppm corresponding to pyrazole C_4-proton in addition to D_2O exchangeable singlets at 7.06–7.10 ppm

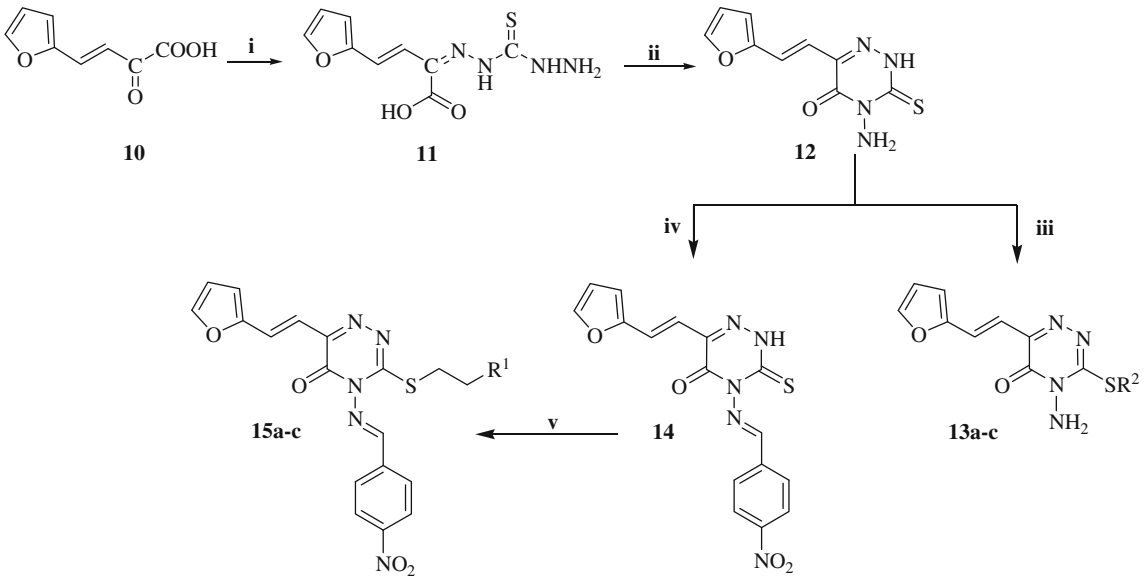
due to NH_2 protons. Additionally, condensation of **3** with an equimolar amount of acetyl acetone in ethanol gave rise to the requisite 3,5-dimethyl pyrazole **6**. ^{1}H-NMR spectrum revealed presence of three singlets at 2.22, 2.46, and 6.24 ppm interpreted for two CH_3 groups and pyrazole C_4-proton. Heating the same intermediate **3** with diethyl malonate in a mixture of ethanol/glacial acetic acid afforded the pyrazolidine-3,5-dione **7**. ^{1}H-NMR spectrum for this compound showed a singlet for the pyrazolidine C_4-protons at 2.45 ppm in addition to a D_2O exchangeable singlet at 8.70 ppm due to pyrazolidine NH proton. Reaction of **3** with an equimolar amount of ethyl acetoacetate in boiling ethanol yielded the ethyl butanoate ester **8**. ^{1}H-NMR spectrum of compound **8** revealed a singlet for the C-3 methyl group at a high chemical shift (1.94 ppm) confirming that the configuration at C-3 is E (Pretsch *et al.*, 2000, pp 211–212). Attempts to cyclize the ethyl butanoate ester to the corresponding pyrazolinone **9** using high boiling point solvents were unsuccessful; however fusion of the ethyl butanoate ester in an oil bath at 160 °C afforded the respective pyrazolinone **9** in a good yield. ^{1}H-NMR spectrum of **9** showed a singlet at 2.47 ppm assigned for CH_3 protons and a singlet at 3.44 ppm attributed to pyrazoline C_4 proton, while its MS spectrum revealed a molecular ion peak at *m*/*z* 285 (21 %) which is in accordance with its molecular formula. It should be noted that the pyrazolinone derivative **9** could be directly prepared from the hydrazine intermediate **3** by fusing the latter compound with ethyl acetoacetate in an oil bath at 160 °C.

Referring to Scheme 2, stirring **10** (Rohmer, 1898) with thiocarbohydrazide in ethanol containing a catalytic amount of glacial acetic acid resulted in the formation of the respective thiocarbohydrazone **11.** IR spectra of the latter compound displayed absorption band characteristic for C=O group at low frequency; 1661 cm^{-1}, while its ^{1}H-NMR spectrum showed a D_2O exchangeable singlet for the NH proton at a high chemical shift; 10.3 ppm confirming presence of a hydrogen bond between the C=O and NH groups (Pretsch *et al.*, 2000, pp. 290–291). This led to the suggestion that the configuration around the C=N is Z. Heating **11** in 1 N sodium hydroxide gave rise to the corresponding 4-amino-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2*H*)-one **12**. IR spectrum for compound **12** lacked absorption bands for OH group and showed absorption



R^1 = C_6H_5, 4-$CH_3C_6H_4$, 4-ClC_6H_4, 4-BrC_6H_4, 4-$NO_2C_6H_4$; R^2 = 4-$CH_3OC_6H_4CH$, 4-ClC_6H_4CH, 4-$NO_2C_6H_4CH$, 2-furylidene, 5-nitro-2-furylidene, isatinylidene, N-methyl isatinylidene.

Scheme 1 Reagents and reaction conditions: *i* R^1COCH_2Br, absolute ethanol, reflux, *ii* hydrazine 98 %, absolute ethanol, reflux, *iii* aldehydes or ketones, absolute ethanol, glacial acetic acid, reflux, *iv* R^1COCH_2CN, ethanol, glacial acetic acid, reflux, *v* acetylacetone, absolute ethanol, reflux, *vi* diethyl malonate, glacial acetic acid, reflux, *vii* ethyl acetoacetate, absolute ethanol, reflux, *viii* ethyl acetoacetate, fusion, oil bath, 160 °C, *ix* fusion, oil bath, 160 °C



R^1 = morpholino, piperidino, pyrrolidino; R^2 = CH_3, C_2H_5, $C_6H_5CH_2$

Scheme 2 Reagents and reaction conditions: *i* thiocarbohydrazide, ethanol, glacial acetic acid, stir, r.t., *ii* 1 N sodium hydroxide, *iii* methyl iodide or ethyl iodide or benzyl chloride, dry dimethyl formamide, anhydrous potassium carbonate, stir, r.t., *iv* 4-nitrobenzaldeyhde, absolute ethanol, glacial acetic acid, reflux, *v* $ClCH_2CH_2R^1$.HCl, potassium hydroxide, stir, r.t.

bands characteristic for NH_2 and N-CS moieties whereas, its ^{1}H-NMR spectrum verified its structure. Stirring the aminothione **12** with an equivalent amount of methyl iodide, ethyl iodide or benzyl chloride in dry DMF containing anhydrous potassium carbonate afforded the S-alkyl derivatives **13a-c** in good yields. IR, ^{1}H-NMR, and ^{13}C-NMR spectral data confirmed the chemical structure of these derivatives. Additionally, synthesis of the azomethine derivative **14** was achieved by condensing the thione **12** with 4-nitrobenzaldehyde in boiling ethanol containing few drops of glacial acetic acid. IR spectrum of **14** lacked absorption bands characteristic for NH_2 group, while its ^{1}H-NMR spectrum revealed singlets attributed to the nitrophenyl group in addition to a singlet for the N=CH proton at 8.89 ppm indicating that the configuration around the C=N is E (Pretsch *et al.*, 2000, pp 211–212). Moreover, the MS spectrum of **14** showed a molecular ion peak at *m/z* 369 (20.5 %) which matches with its molecular formula. Finally, alkylation of the azomethine **14** with hydrochloride salts of 2-substituted ethyl chloride derivatives in aqueous KOH furnished the corresponding 2-substituted ethylsulfanyl derivatives **15a–c**. Inspection of ^{1}H-NMR spectra of the latter compounds indicates presence of signals for SCH_2 and NCH_2 protons at their expected chemical shifts. Furthermore, ^{13}C-NMR spectrum of compound **15b** displayed signals at 28 and 58.20 ppm corresponding to SCH_2 and NCH_2 in addition to signals of piperidine carbons which appeared at their expected values.

In vitro antitumor screening

Primary in vitro one-dose assay

Out of the newly synthesized compounds, six derivatives namely: **2c**, **5c**, **7**, **12**, **14**, and **15a** were selected by the National Cancer Institute (NCI) in vitro disease-oriented human cells screening panel assay to be evaluated for their in vitro antitumor activity.

An effective one-dose assay has been added to the NCI-60 cell screen in order to increase compound throughput and reduce data-turnaround time to suppliers while maintaining efficient identification of active compounds (Weislow *et al.*, 1989; Monks *et al.*, 1991; Boyd and Paull, 1995). All compounds submitted to the NCI-60 cell screen are tested initially at a single high dose (10 μM) in the full NCI-60 cell panel including leukemia, non-small cell lung, colon, CNS melanoma, ovarian, renal, prostate, and breast cancer cell lines. Only compounds which satisfy pre-determined threshold inhibition criteria would proceed to the five-dose screen. The threshold inhibition criteria for proceeding to the five-dose screen was designed to efficiently capture compounds with anti-proliferative activity, and it is based on careful analysis of historical Development Therapeutic Program (DTP) screening data. Data are reported as a mean graph of the percent growth of treated cells, and presented as percentage growth inhibition (GI%) caused by the test compounds (Table 1).

Table 1 Mean growth percent, delta values, and in vitro percentage growth inhibition (GI%) caused by the selected compounds against some tumor cell lines at the single-dose assay

Comp. no.	NSC-number	Mean growth percent	Delta	Panel	Subpanel cell lines (growth inhibition percent)
2c	746136/1	97.13	77.96	Breast cancer	MCF7 (80.83), T-47D (38.32), MDA-MB-48 (57.34)
				Ovarian cancer	IGROV 1 (80.44)
				Leukemia	K-562 (38.06)
				Renal cancer	RXF 393 (62.53), UO-31 (33.27)
				Melanoma	SK-MEL-2 (42.31)
5c	748494/1	49.14	48.11	Non-small cell lung cancer	A549/ATCC (62.66), EKVX (58.26), HOP-92 (39.68), NCI-H226 (52.63), NCI-H23 (41.17), NCI-H322 M (75.57), NCI-H460 (79.63)
				Colon cancer	HCC-2998 (51.49), HCT-116 (59.00), HCT-15 (61.94), HT29 (55.77), KM12 (44.01), SW-620 (50.75)
				Breast cancer	MCF7 (54.06), T-47D (37.26)
				Ovarian cancer	IGROV 1 (64.98), OVCAR-3 (62.13), OVCAR-4 (70.9), OVCAR-5 (39.07), OVCAR-8 (75.15), NCI/ADR-RES (94.27), SK-OV-3 (39.34)
				Leukemia	CCRF-CEM (66.06), HL-60(TB) (4.83), K-562 (58.29), MOLT-4 (55.63), RPMI-8226 (56.67), SR (61.94)
				Renal cancer	786-O (53.06), A498(66.15), ACHN (70.57), CAKI-1 (98.97), RXF 393 (32.23), SN 12C (31.83), TK-10 (48.80), UO-31 (96.08)
				Melanoma	LOX IMVI(50.13), M14 (42.64), MDA-MB-435 (37.18), SK-MEL-28 (33.64), SK-MEL-5 (54.04),UACC-257 (30.42), UACC-62 (47.52)
				Prostate cancer	PC-3 (47.44), DU-145 (36.73)
				CNS cancer	SF-268 (47.48), SF-295 (90.1), SF-539 (44.65), SNB-19 (42.23), SNB-75 (32.65), U251 (49.39)
7	752242/1	105.76	60.02	Leukemia	HL-60(TB) (54.26)
12	746140/1	102.26	79.29	Breast cancer	MCF7 (72.68)
				Ovarian cancer	IGROV 1 (77.03), OVCAR-8 (59.10)
				Melanoma	SK-MEL-2 (70.66)
14	746142/1	96.42	71.98	Breast cancer	MCF7 (73.08)
				Ovarian cancer	IGROV 1 (75.56), OVCAR-8 (47.79)
				Melanoma	SK-MEL-2 (70.32)
15a	748493/1	92.86	113.30	Breast cancer	MCF7 (69.03), T-47D (31.84)
				Leukemia	CCRF-CEM (120.44, lethality), RPMI-8226 (32.99), SR(66.41)

Data obtained from NCI in vitro disease-oriented human tumor cell screen at 10 μM concentration

The obtained results revealed that compound **2c** was able to exhibit promising broad spectrum anticancer activity particularly against breast cancer MCF7, MDA-MB-48, Ovarian cancer IGROV 1, and renal cancer RXF 393 cell lines (GI% values 80.83, 57.34, 80.44, and 62.53, respectively). However, its overall antitumor profile did not meet the pre-determined threshold inhibition criteria and therefore was not sufficient to proceed to the five-dose screen. Compounds **12** and **14** displayed remarkable growth inhibitory activity towards breast cancer MCF7, ovarian cancer IGROV 1, and melanoma SK-MEL-2 cell lines (GI% range 70.32–77.03). Moreover, compound **15a** exhibited significant activity against breast cancer MCF7 and SR cell lines (GI% values 69.03, 120.44, and 66.41, respectively). It should be noted here that compound **15a** showed lethal effect towards the CCRF-CEM cell line with 20.44 %. On the other hand, compound **7** was proved to be the weakest anticancer member in this screen owing to its low potency and narrow margin of activity, which was against only leukemia HL-60(TB) and with GI% value of 54.26. Whereas, compound **5c** was found to be the most active member in this preliminary screen as evidenced by its ability to exert potential growth inhibitory activity against most of the tested subpanel tumor cell lines.

Consequently, it passed successfully this assay and was carried over to the five-dose screen against a panel of about 60 different tumor cell lines.

In vitro full panel (five-dose) 60-cell line assay for compound **5c**

About 60 cell lines of nine tumor subpanels, including leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancer cell lines, were incubated with five concentrations (0.01–100 μM) for each compound and were used to create log concentration % growth inhibition curves. Three response parameters (GI_{50}, TGI, and LC_{50}) were calculated for each cell line. The GI_{50} value (growth inhibitory activity) corresponds to the concentration of the compounds causing 50 % decrease in net cell growth, the TGI value (cytostatic activity) is the concentration of the compounds resulting in total growth inhibition and the LC_{50} value (cytotoxic activity) is the concentration of the compounds causing net 50 % loss of initial cells at the end of the incubation period (48 h). Subpanel and full panel mean-graph midpoint values (MG-MID) for certain agents are the average of individual real and default GI_{50}, TGI, or LC_{50} values of all cell lines in the subpanel or the full panel, respectively.

In the present study, compound **5c** exhibited potential antitumor activities against most of the tested subpanel tumor cell lines (GI_{50} and TGI values <100 μM). This compound showed a distinctive pattern of sensitivity against some individual cell lines (Table 1), as well as a broad spectrum (MG-MID) of antitumor activity (Table 2).

A deep insight into the obtained results (Table 2) revealed that compound **5c** exhibited remarkably high activity against renal cancer CAKI-1 cell line with GI_{50} value of 0.37 μM. It also displayed a distinguished sensitivity profile towards all leukemia cell lines with GI_{50} range of 2.01–3.03 μM beside a potential activity against seven cell lines with GI_{50} range of 1.23–1.63 μM. In addition, compound **5c** showed appreciable growth inhibitory potential against 58 cell lines with GI_{50} range of 1.88–18.7 μM. Further interpretation of the obtained data revealed that compound **5c** was able to totally inhibit the growth of 50 cell lines at 4.6–84.8 μM. Moreover, **5c** was cytotoxic against three cell lines; ovarian cancer OVCAR-3, NCI/ADR-RES, and prostate cancer DU-145 (LC_{50} values 31.8, 96.4, and 55.4 μM, respectively).

The results also revealed that compound **5c** displayed high growth inhibitory potential (GI_{50} MG-MID 3.98 μM) (Table 3), together with reasonable cytostatic (TGI MG-MID 35.48 μM) and mild cytotoxic (LC_{50} MG-MID 97.72 μM) activities (Table 4).

The ratio obtained by dividing the compound full panel MG-MID (μM) by its individual subpanel MG-MID (μM)

Table 2 GI_{50}, TGI, and LC_{50} of some selected in vitro tumor cell lines (μM) for compound **5c**

Panel-cell line	Compound **5c**		
	GI_{50}	TGI	LC_{50}
Leukemia			
CCRF-CEM	2.88	16.8	>100
HL-60(TB)	2.83	8.19	>100
K-562	2.79	23	>100
MOLT-4	2.01	18.5	>100
RPMI-8226	3.03	23.2	>100
Non-small cell lung cancer			
A549/ATCC	3.48	34.2	>100
EKVX	3.57	31.1	>100
HOP-62	5.77	76.8	>100
HOP-92	1.61	9.28	>100
NCI-H226	12.2	75.6	>100
NCI-H23	5.13	53.9	>100
NCI-H322 M	1.47	38.2	>100
NCI-H460	1.88	13.9	>100
NCI-H522	5.12	36.8	>100
Colon cancer			
COLO 205	3.44	18.4	>100
HCC-2998	5.59	>100	>100
HCT-116	2.92	15.6	>100
HCT-15	1.89	>100	>100
HT29	7.82	>100	>100
KM12	3.19	32	>100
SW-620	5.02	>100	>100
CNS cancer			
SF-268	1.99	25.1	>100
SF-295	1.58	24.3	>100
SF-539	5.49	26.1	>100
SNB-19	5.81	69.4	>100
SNB-75	10.6	37.2	>100
U251	4.47	84.8	>100
Melanoma			
LOX IMVI	6.69	73.4	>100
MALME-3 M	11	53.1	>100
M14	2.27	31.7	>100
MDA-MB-435	5.22	>100	>100
SK-MEL-2	15.6	77.9	>100
SK-MEL-28	4.06	82.3	>100
SK-MEL-5	3.83	24.8	>100
UACC-257	6.59	71.8	>100
UACC-62	5.07	>100	>100
Ovarian cancer			
IGROV1	4.89	37.8	>100
OVCAR-3	1.48	5.2	31.8
OVCAR-4	3.45	28.8	>100
OVCAR-5	5.49	82	>100

Table 2 continued

Panel-cell line	Compound **5c**		
	GI_{50}	TGI	LC_{50}
OVCAR-8	4.47	65.8	>100
NCI/ADR-RES	1.23	8.11	96.4
SK-OV-3	5.58	7.43	>100
Renal cancer			
786-O	3.98	21.2	>100
A498	1.62	6.62	>100
ACHN	3.8	24.8	>100
CAKI-1	0.37	4.6	>100
RXF 393	13.1	54.9	>100
SN12C	18.7	>100	>100
TK-10	3.64	23.1	>100
UO-31	1.63	13.7	>100
Prostate cancer			
PC-3	5.6	56.2	>100
DU-145	3.01	18.3	55.4
Breast cancer			
MCF7	4.05	50.4	>100
MDA-MB-231/ATCC	13.9	>100	>100
BT-549	4.42	43.8	>100
T-47D	4.37	44.2	>100
MDA-MB-468	3.08	22.2	>100

Data obtained from NCI in vitro disease-oriented human cell screen

is considered as a measure of compound selectivity. Ratios between 3 and 6 refer to moderate selectivity, ratios >6 indicate high selectivity towards the corresponding cell line, while compounds meeting neither of these criteria are rated non-selective (Acton *et al.*, 1994). In this context, compound **5c** was found to be non-selective with broad spectrum antitumor activity against the nine tumor subpanels tested with selectivity ratios ranging between 0.59 and 1.47 at the GI_{50} MG-MID level. Moreover, log GI_{50} values for compound **5c** was illustrated with respect to 5-fluorouracil as a comparative study for anticancer potency (Table 5), where values of −0.4 and less are considered to be of high anticancer activity. The comparative study indicated that compound **5c** displayed antitumor activity against all human tumor cell lines higher than 5-fluorouracil.

The above results revealed that this compound could be an appropriate candidate for further derivatization in order to explore the scope and limitations of its potential hoping to find more selective and active anticancer agents.

Experimental

All reagents and solvents were purchased from commercial suppliers and were dried and purified when necessary by standard techniques. Melting points were determined in

Table 3 Median growth inhibitory concentrations (GI_{50}, μM) of in vitro subpanel tumor cell lines for compound **5c**

MG-MID	Subpanel tumor cell lines GI_{50} MG-MID (μM) (SI)								
	A	B	C	D	E	F	G	H	I
3.98	2.71 (1.47)	4.47 (0.89)	4.27 (0.93)	4.99 (0.80)	6.70 (0.59)	3.80 (1.0)	5.85 (0.68)	4.30 (0.92)	6.39 (0.62)

Median values calculated according to the data obtained from NCI's in vitro disease-oriented human tumor cell screen

A Leukemia, *B* non-small cell lung cancer, *C* colon cancer, *D* CNS cancer, *E* Melanoma, *F* ovarian cancer, *G* renal cancer, *H* prostate cancer, *I* breast cancer; *GI_{50} (μM)* full panel mean-graph mid point (MG-MID) = the average sensitivity of all cell lines towards the test agent; *SI* selectivity index

Table 4 Median total growth inhibitory concentrations (TGI, μM) and lethal concentration (LC_{50}, μM) of in vitro subpanel tumor cell lines for compound **5c**

MG-MID	Subpanel tumor cell lines								
	A	B	C	D	E	F	G	H	I
35.48	17.94	41.08	22.0	44.48	59.29	43.14	37.35	40.15	48.12
(97.72)	–[a]	–	–	–	–	(89.7)[b]	–	(50.0)	–

Median values calculated according to the data obtained from NCI's in vitro disease-oriented human tumor cell screen. For subpanel tumor cell lines, see footnote of Table 3

TGI (μM) full panel mean-graph mid point (MG-MID) = the average sensitivity of all cell lines towards the test agent

[a] LC_{50} (MG-MID) value > 100 μM

[b] LC_{50} (μM) full panel mean-graph mid point (MG-MID) = the average sensitivity of all cell lines towards the test agent

Table 5 Log GI_{50} values (μM) of compound **5c** with respect to 5-FU

Panel/cell line	Log GI_{50}	
	5c	5-FU
Leukemia		
CCRF-CEM	−5.54	−4.5
HL-60(TB)	−5.55	−4.7
K-562	−5.56	−4.7
MOLT-4	−5.7	−4.9
RPMI-8226	−5.52	−5.3
Non-small cell lung cancer		
A549/ATCC	−5.46	−5.7
EKVX	−5.45	−3.5
HOP-62	−5.24	−4.7
HOP-92	−5.79	−3.8
NCI-H226	−4.91	−3.6
NCI-H23	−5.29	−4.9
NCI-H322 M	−5.83	−4.7
NCI-H460	−5.73	−6
NCI-H522	−5.29	−4.4
Colon cancer		
COLO 205	−5.46	−5.2
HCC-2998	−5.25	−5.8
HCT-116	−5.54	−5.4
HCT-15	−5.72	−5.2
HT29	−5.11	−5.2
KM12	−5.5	−5
SW-620	−5.3	−4.6
CNS cancer		
SF-268	−5.7	−4.3
SF-295	−5.8	−4.3
SF-539	−5.26	−5.9
SNB-19	−5.24	−3.9
SNB-75	−4.97	−3.7
U251	−5.35	−4.4
Melanoma		
LOX IMVI	−5.17	−5.2
MALME-3M	−4.96	−4.7
M14	−5.64	−4.3
SK-MEL-2	−4.81	−3.4
SK-MEL-28	−5.39	−4.3
SK-MEL-5	−5.42	−4.9
UACC-257	−5.18	−4
UACC-62	−5.3	−4.9
Ovarian cancer		
IGROV1	−5.31	−4.9
OVCAR-3	−5.83	−4.6
OVCAR-4	−5.46	−4.2
OVCAR-5	−5.26	−3.8
OVCAR-8	−5.35	−4.7
SK-OV-3	−5.25	−3.8
Renal cancer		
786-O	−5.4	−4.9
A498	−5.79	−5
ACHN	−5.42	−5
CAKI-1	−6.43	−5.4
RXF 393	−4.88	−4.3
SN12C	−4.73	−4.6
TK-10	−5.44	−3.9
UO-31	−5.79	−5.3
Prostate cancer		
PC-3	−5.25	−4.3
DU-145	−5.52	−5
Breast cancer		
MCF7	−5.39	−5.8
MDA-MB-231/ATCC	−4.86	−3.3
HS 578T	−5.07	−3.6
BT-549	−5.35	−4
T-47D	−5.36	−4.1

open glass capillaries using Stuart capillary melting point apparatus (Stuart Scientific Stone, Staffordshire, UK) and are uncorrected. Infrared (IR) spectra were recorded on Perkin-Elmer 1430 infrared spectrophotometer (Perkin Elmer, Beaconsfield, UK) and measured by υ cm^{-1} scale using KBr cell. ^{1}H-NMR spectra were scanned on Jeol-500 MHz spectrometer (Jeol, Tokyo, Japan) and Varian Mercury VX-300 using tetramethylsilane (TMS) as internal standard and DMSO-d_6 as the solvent (chemical shifts are given in δ ppm). Splitting patterns were designated as follows: s: singlet; brs: broad singlet; d: doublet; dd: doublet of doublet; t: triplet; m: multiplet. ^{13}C-NMR proton decoupled spectra were recorded on a Varian Mercury VX-300 spectrometer in DMSO-d_6 and measured in δ scale. Mass spectra were run on a Finnigan mass spectrometer model SSQ/7000 (70 eV) or on a gas chromatograph/mass spectrometer Schimadzu GCMS-QP 2010 Plus (70 eV). Elemental analyses were performed on Elementar Vario E1 and were found within ±0.4 % of the theoretical values. Follow up of the reactions and checking the purity of the compounds was made by thin layer chromatography (TLC) on silica gel-precoated aluminum sheets (Type 60 GF254; Merck; Germany) and the spots were detected by exposure to UV lamp at λ 254 nm for few seconds. Compounds **1** (Slouka, 1962), **3** (Osman *et al.*, 2007), and **10** (Rohmer, 1898) were prepared according to previously reported reaction conditions.

3-[(2-Aryl-2-oxoethyl)sulfanyl]-6-[(*E*)-2-(furan-2-yl)ethenyl]-1,2,4-triazin-5(2*H*)-ones (**2a**–**e**)

A mixture of the thione **1** (2.2 g, 10 mmol) and the appropriate phenacyl bromide (10 mmol) in absolute ethanol (10 ml) was heated under reflux for 2 h. The reaction mixture was cooled to room temperature and the separated product was filtered, washed with ethanol, dried and crystallized from dioxane/water.

*(E)-6-[2-(Furan-2-yl)ethenyl]-3-[(2-phenyl-2-oxoethyl)sulfanyl]-1,2,4-triazin-5(2H)-one (**2a**)*

Brown solid (76 %); m.p.: 192–194 °C; IR (KBr, cm^{-1}): 3146 (NH), 3095 (CH furan), 2964, 2916 (CH_2), 1692, 1659(C=O), 1625 (C=N), 1552, 1526, 1482 (C=C, δ NH), 1290, 1076 (C–S–C), 1232, 1014 (C–O–C), 744 (*oop* furan); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 4.45 (s, 2H, SCH_2), 6.54–6.59 (m, 1H, furan C_4-H), 6.77 (d, $J = 3.9$ Hz, 1H, furan C_3-H), 6.81 (d, $J = 16.7$ Hz, 1H, ethenyl C_1-H), 7.23–7.34 (m, 5H, phenyl-H), 7.56 (d, $J = 16.7$ Hz, 1H, ethenyl C_2-H), 7.78 (s, 1H, furan C_5-H), 12.40 (s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{17}H_{13}N_3O_3S$ (339.37): C, 60.17; H, 3.86; N, 12.38; found: C, 59.78; H, 3.47; N, 12.73.

*(E)-6-[2-(Furan-2-yl)ethenyl]-3-[{2-(4-methylphenyl)-2-oxoethyl}sulfanyl]-1,2,4-triazin-5(2H)-one (**2b**)*

Yellow solid (63 %); m.p.: 218–220 °C; IR (KBr, cm^{-1}): 3275 (NH), 3075 (CH furan), 2921, 2850 (CH_2, CH_3), 1705, 1653 (C=O), 1628 (C=N), 1529, 1473 (C=C, δ NH), 1281, 1087 (C–S–C), 1194, 1017 (C–O–C), 750 (*oop* furan); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.34 (s, 3H, CH_3), 4.38 (s, 2H, SCH_2), 6.57–6.62 (m, 1H, furan C_4-H); 6.75 (d, $J = 3.9$ Hz, 1H, furan C_3-H), 6.79 (d, $J = 16.7$ Hz, 1H, ethenyl C_1-H), 7.33 (d, $J = 8.4$ Hz, 2H, methylphenyl $C_{3,5}$-H), 7.56 (d, $J = 16.7$ Hz, 1H, ethenyl C_2-H), 7.75 (s, 1H, furan C_5-H), 7.85 (d, $J = 8.4$ Hz, 2H, methylphenyl $C_{2,6}$-H), 12.39 (s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{18}H_{15}N_3O_3S$ (353.39): C, 61.18; H, 4.28; N, 11.89; found: C, 61.53; H, 3.88; N, 11.53.

*(E)-3-[{2-(4-Chlorophenyl)-2-oxoethyl}sulfanyl]-6-[2-(furan-2-yl)ethenyl]-1,2,4-triazin-5(2H)-one (**2c**)*

Yellow solid (78 %); m.p.: 228–230 °C; IR (KBr, cm^{-1}): 3171 (NH), 3097 (CH furan), 2962, 2915 (CH_2), 1672 (C=O), 1615 (C=N), 1588, 1533, 1483 (C=C, δ NH), 1286, 1091 (C–S–C), 1257, 1013 (C–O–C), 748 (*oop* furan); ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 3.67, 3.78 (2 × d, $J = 12.2$ Hz, each 1H, S-CH_2), 6.51–6.54 (m, 1H, furan C_4-H), 6.65–6.80 (m, 2H, ethenyl C_1-H and furan C_3-H), 7.48, 7.57 (2 × d, $J = 8.4$ Hz, each 2H, chlorophenyl $C_{3,5}$-H and chlorophenyl $C_{2,6}$-H), 7.69–7.74 (m, 2H, ethenyl C_2-H and furan C_5-H), 8.32 (s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{17}H_{12}ClN_3O_3S$ (373.81): C, 54.62; H, 3.24; N, 11.24; S, 8.58; found: C, 54.62; H, 3.13; N, 11.06; S, 8.59.

*(E)-3-[{2-(4-Bromophenyl)-2-oxoethyl}sulfanyl]-6-[2-(furan-2-yl)ethenyl]-1,2,4-triazin-5(2H)-one (**2d**)*

Yellow solid (80 %); m.p.: 218–220 °C; IR (KBr, cm^{-1}): 3164 (NH), 3018 (CH furan), 2964, 2901 (CH_2), 1673 (C=O), 1608 (C=N), 1582, 1506, 1478 (C=C, δ NH), 1287, 1069 (C–S–C), 1255, 1011 (C–O–C), 732 (*oop* furan); ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 3.67, 3.77 (2 × d, $J = 12.2$ Hz, each 1H, S-CH_2), 6.51–6.56 (m, 1H, furan C_4-H), 6.67 (d, $J = 16.05$ Hz, 1H, ethenyl C_1-H), 6.79 (d, $J = 3.1$ Hz, 1H, furan C_3-H), 7.50, 7.61 (2 × d, $J = 8.0$ Hz, each 2H, bromophenyl $C_{3,5}$-H and bromophenyl $C_{2,6}$-H), 7.69–7.75 (m, 2H, ethenyl C_2-H and furan C_5-H), 8.32 (s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{17}H_{12}BrN_3O_3S$ (418.26): C, 48.82; H, 2.89; N, 10.05; S, 7.67; found: C, 49.09; H, 2.52; N, 9.87; S, 7.67.

*(E)-6-[2-(Furan-2-yl)ethenyl]-3-[{2-(4-nitrophenyl-2-oxoethyl}sulfanyl]-1,2,4-triazin-5(2H)-one (**2e**)*

Orange solid (60 %); m.p.: >300 °C; IR (KBr, cm^{-1}): 3113 (NH), 3025 (CH furan), 2900, 2856 (CH_2), 1675 (C=O), 1621 (C=N), 1575, 1472 (C=C, δ NH), 1519, 1347 (NO_2), 1281, 1077 (C–S–C), 1219, 1019 (C–O–C), 754 (*oop* furan); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.74, 3.87 (2 × d, $J = 12.6$ Hz, each 1H, S-CH_2), 6.51–6.57 (m, 1H, furan C_4-H), 6.68 (d, $J = 16.2$ Hz, 1H, ethenyl C_1-H), 6.81 (d, $J = 3.3$ Hz, 1H, furan C_3-H), 7.72 (d, $J = 16.2$ Hz, 1H, ethenyl C_2-H), 7.75 (s, 1H, furan C_5-H), 7.89, 8.29 (2 × d, $J = 8.7$ Hz, each 2H, nitrophenyl $C_{2,6}$-H and $C_{3,5}$-H), 8.59 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (300 MHz, DMSO-d_6) δ 50.55 (CH_2), 122.11 (furan C_4), 122.99 (furan C_3), 127.26 (ethenyl C_1), 132.90 (nitrophenyl $C_{3,5}$), 134.49 (ethenyl C_2), 137.64 (nitrophenyl $C_{2,6}$), 152.0 (nitrophenyl C_1), 154.54 (furan C_5), 156.33 (furan C_2), 157.21 (nitrophenyl C_4), 158.5 (triazine C_6), 161.01 (triazine C_3), 175.18 (triazine C_5), 175.98 (C=O); Anal. Calcd for $C_{17}H_{12}N_4O_5S$ (384.37): C, 53.12; H, 3.15; N, 14.58; S, 8.34; found: C, 52.79; H, 2.89; N, 14.84; S, 8.22.

6-[(*E*)-2-(Furan-2-yl)ethenyl]-3-(2-substituted hydrazono)-1,2,4-triazin-5(2*H*)-ones (**4a**–**g**)

A mixture of the hydrazine **3** (0.48 g, 2.2 mmol) and the proper aldehyde or ketone (2.2 mmol), in absolute ethanol (10 ml) containing a catalytic amount of glacial acetic acid,

was heated under reflux for 1 h. The reaction mixture was cooled and the precipitated product was filtered, washed with ethanol, dried and crystallized from the proper solvent.

*6-[(E)-2-(Furan-2-yl)ethenyl]-3-[(E)-2-(4-methoxybenzylidene)hydrazono]-1,2,4-triazin-5(2H)-one (**4a**)*

Yellow solid (98 %); m.p.: 282–284 °C (ethanol); IR (KBr, cm^{-1}): 3350, 3250 (NH), 3050 (CH furan), 2912, 2838 (CH_3), 1663 (C=O), 1620 (C=N), 1558, 1505, 1464 (C=C, δ NH), 1243, 1025 (C–O–C), 738 (*oop* furan); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.82 (s, 3H, OCH_3), 6.54–6.58 (m, 1H, furan C_4-H), 6.73 (d, $J = 3.3$ Hz, 1H, furan C_3-H), 6.89 (d, $J = 16.2$ Hz, 1H, ethenyl C_1-H), 6.99 (d, $J = 8.7$ Hz, 2H, methoxyphenyl $C_{3,5}$-H), 7.74 (s, 1H, furan C_5-H), 7.75–7.85 (m, 3H, ethenyl C_2-H and methoxyphenyl $C_{2,6}$-H), 8.05 (s, 1H, N = CH), 11.6, 12.9 (2 × s, each 1H, 2 NH, D_2O exchangeable). ^{13}C-NMR (300 MHz, DMSO-d_6) δ 64.76 (OCH_3), 121.38 (furan C_4), 121.85 (furan C_3), 123.55 (methoxyphenyl $C_{3,5}$), 128.95 (ethenyl C_1), 132.12 (ethenyl C_2), 136.01 (methoxyphenyl C_1), 138.58 (methoxyphenyl $C_{2,6}$), 152.80 (N = CH), 153.63 (furan C_5), 154.51 (furan C_2), 160.87 (triazine C_6), 161.56 (methoxyphenyl C_4), 170.21 (triazine C_3), 174.0 (C=O); Anal. Calcd for $C_{17}H_{15}N_5O_3$ (337.33): C, 60.53; H, 4.48; N, 20.76. Found: C, 60.19; H, 4.17; N, 20.52.

*3-[(E)-2-(4-Chlorobenzylidene)hydrazono]-6-[(E)-2-(furan-2-yl)ethenyl]1,2,4-triazin-5(2H)-one (**4b**)*

Yellow solid (97 %); m.p.: 276–278 °C (ethanol); IR (KBr, cm^{-1}): 3393, 3300 (NH), 3050 (CH furan), 1663 (C=O), 1636 (C=N), 1553, 1505 (C=C, δ NH), 1273, 1017 (C–O–C), 741 (*oop* furan); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 6.57–6.62 (m, 1H, furan C_4-H), 6.76 (d, $J = 3.6$ Hz, 1H furan C_3-H), 6.88 (d, $J = 15.9$ Hz, 1H, ethenyl C_1-H), 7.51 (d, $J = 8.4$ *Hz*, 2H, chlorophenyl $C_{3,5}$-H), 7.76 (s, 1H, furan C_5-H), 7.84 (d, $J = 15.9$ Hz, 1H ethenyl C_2-H), 7.98 (d, $J = 8.4$ Hz, 2H, chlorophenyl $C_{2,6}$-H), 8.08 (s, 1H, N=CH), 11.9, 13.1 (2 × s, each 1H, 2 NH, D_2O exchangeable); Anal. Calcd for $C_{16}H_{12}ClN_5O_2$ (341.75): C, 56.23; H, 3.54; N, 20.49; found: C, 56.08; H, 3.54; N, 20.35.

*6-[(E)-2-(Furan-2-yl)ethenyl]-3-[(E)-2-(4-nitrobenzylidene)hydrazono]-1,2,4-triazin-5(2H)-one (**4c**)*

Yellow solid (95 %); m.p.: >300 °C (dimethyl formamide); IR (KBr, cm^{-1}): 3361 (NH), 3045 (CH furan), 1675 (C=O), 1621 (C=N), 1587, 1503 (C=C, δ NH), 1558, 1338 (NO_2), 1250, 1016 (C–O–C), 738 (*oop* furan); ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 6.52–6.56 (m, 1H, furan C_4-H), 6.69 (d, $J = 3.05$ *Hz*, 1H, furan C_3-H), 6.84 (d, $J = 20$ *Hz*, 1H, ethenyl C_1-H), 7.82 (s, 1H, CH = N), 7.84 (d, $J = 20$ *Hz*, 1H, ethenyl C_2-H), 8.11 (d, $J = 8.4$ *Hz*, 2H, nitrophenyl $C_{2,6}$-H), 8.14 (s, 1H, furan C_5-H), 8.22 (d, $J = 8.4$ *Hz*, 2H, nitrophenyl $C_{3,5}$-H), 12.24, 13.40 (2 × s, each 1H, 2 NH, D_2O exchangeable); Anal. Calcd for $C_{16}H_{12}N_6O_4.1/2\ H_2O$ (361.31): C, 53.19; H, 3.63; N, 23.26; found: C, 53.44; H, 3.50; N, 23.16.

*6-[(E)-2-(Furan-2-yl)ethenyl]-3-[(E)-2-{(furan-2-yl)methylene}hydrazono]-1,2,4-triazin-5(2H)-one (**4d**)*

Yellow orange solid (90 %); m.p.: 264–266 °C (dimethyl formamide); IR (KBr, cm^{-1}): 3325, 3182 (NH), 3025 (CH furan), 1663 (C=O), 1615 (C=N), 1563, 1509 (C=C, δ NH), 1275, 1016 (C–O–C), 745 (*oop* furan); ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 6.55-6.56 (m, 1H, furan C_4-H), 6.62–6.63 (m, 1H, furan C_4-H), 6.72 (d, $J = 3.1$ Hz, 1H, furan C_3-H), 6.83 (d, $J = 16.1$ Hz, 1H, ethenyl C_1-H), 7.03 (d, $J = 3.05$ Hz, 1H, furan C_3-H), 7.73 (s, 1H, furan C_5-H), 7.78 (d, $J = 16.05$ Hz, 1H, ethenyl C_2-H), 7.82 (d, $J = 1.6$ *Hz*, 1H, furan C_5-H), 7.97 (s, 1H, CH = N), 11.71, 12.88 (2 × s, each 1H, 2 NH, D_2O exchangeable); Anal. Calcd for $C_{14}H_{11}N_5O_3.\ 2H_2O$ (333.30): C, 50.45; H, 4.54; N, 21.01; found: C, 50.06; H, 4.32; N, 21.13.

*6-[(E)-2-(Furan-2-yl)ethenyl]-3-[(E)-2-{(5-nitrofuran-2-yl)methylene}hydrazono]-1,2,4-triazin-5(2H)-one (**4e**)*

Orange solid (96 %); m.p.: >300 °C (dioxane); IR (KBr, cm^{-1}): 3400, 3150 (NH), 3025 (CH furan), 1663 (C=O), 1628 (C=N), 1595, 1497 (C=C, δ NH), 1567, 1349 (NO_2), 1249, 1018 (C–O-C), 738 (*oop* furan); ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 6.56–6.57 (m, 1H, furan C_4-H), 6.74 (d, $J = 3.8$ Hz, 1H, furan C_3-H), 6.84 (d, $J = 16.1$ Hz, 1H, ethenyl C_1-H), 7.42 (d, $J = 3.8$ Hz, 1H, nitrofuran C_3-H), 7.73 (s, 1H, furan C_5-H), 7.78 (d, $J = 16.1$ Hz,1H, ethenyl C_2-H), 7.80 (d, $J = 3.8$ Hz, 1H, nitrofuran C_4-H), 8.01 (s, 1H, CH = N), 12.28, 13.14 (2 × s, each 1H, 2 NH, D_2O exchangeable); Anal. Calcd for $C_{14}H_{10}N_6O_5.1H_2O$: C, 46.67; H, 3.36; N, 23.33; found: C, 46.29; H, 3.07; N, 23.43.

*3-[2-{6-((E)-2-(Furan-2-yl)ethenyl)-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl}hydrazono]indolin-2-one (**4f**)*

Orange solid (80 %); m.p.: >300 °C (dimethyl formamide/water); IR (KBr, cm^{-1}): 3357, 3181, 3105 (NH), 3042 (CH furan), 1688 (C=O), 1624 (C=N), 1556, 1491 (C=C, δ NH), 1231, 1046 (C–O–C), 741 (*oop* furan); ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 6.56–6.59 (m, 1H, furan C_4-H), 6.76–6.82 (m, 3H, furan C_3-H, isatin C_4-H and ethenyl C_1-H), 6.94–7.23 (2 × t, $J = 7.5$ Hz, each 1H, isatin $C_{5,6}$-H), 7.62 (d,

$J = 16.1$ Hz, 1H, ethenyl C_2-H), 7.75 (d, $J = 1.6$ *Hz*, 1H, furan C_5-H), 8.40–8.45 (m, 1H, isatin C_7-H), 10.50, 12.90, 13.9 (3 × s, each 1H, 3 NH, D_2O exchangeable); Anal. Calcd for $C_{17}H_{12}N_6O_3 \cdot 2H_2O$ (384.35): C, 53.12; H, 4.20; N, 21.87; found: C, 52.98; H, 3.87; N, 21.94.

*3-[2-{6-((E)-2-(Furan-2-yl)ethenyl)-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl}hydrazono]-1-methylindolin-2-one (**4g**)*

Orange solid (87 %); m.p.: 260–262 °C (dimethyl formamide/water); IR (KBr, cm^{-1}): 3344, 3310(NH), 3024 (CH furan), 2932, 2875 (CH_3), 1676 (C=O), 1620 (C=N), 1550, 1520 (C=C, δ NH), 1245, 1042 (C–O–C), 741 (*oop* furan); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.7 (s, 3H, CH_3), 6.54–6.60 (m, 1H, furan C_4-H), 6.78–6.84 (m, 3H, furan C_3-H, isatin C_4-H and ethenyl C_1-H), 6.93–7.21 (2 × t, $J = 7.5$ Hz, each 1H, isatin $C_{5,6}$-H), 7.65 (d, $J = 16.2$ Hz, 1H, ethenyl C_2-H), 7.76 (d, $J = 1.6$ *Hz*, 1H, furan C_5-H), 8.37–8.42 (m, 1H, isatin C_7-H), 10.40, 14.0 (2 × s, each 1H, 2NH, D_2O exchangeable); Anal. Calcd for $C_{18}H_{14}N_6O_3$ (362.34): C, 59.67; H, 3.89; N, 23.19; found: C, 59.45; H, 3.64; N, 23.04.

3-(5-Amino-3-aryl-1*H*-pyrazol-1-yl)-6-[(*E*)-2-(furan-2-yl)ethenyl]-1,2,4-triazin-5-(2*H*)-ones (**5a**–**d**)

A solution of the hydrazine **3** (0.48 gm, 2.2 mmol) and the selected phenacyl cyanide (2.2 mmol) in ethanol/glacial acetic acid mixture (10 ml) (4:1) was refluxed for 3 h. The reaction mixture was cooled and the precipitated product was filtered, washed with ethanol, dried and crystallized from dioxane.

*(E)-3-(5-Amino-3-phenyl-1H-pyrazol-1-yl)-6-[2-(furan-2-yl)ethenyl]-1,2,4-triazin-5-(2H)-one (**5a**)*

Pale yellow solid (80 %); m.p.: 262–264 °C; IR (KBr, cm^{-1}): 3418, 3344, 3306, 3178 (NH), 3058 (CH furan), 1660 (C=O), 1617(C=N), 1576, 1546 (C=C, δ NH), 1245, 1016 (C–O–C), 752 (*oop* furan); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 5.92 (s, 1H, pyrazole C_4-H), 6.60–6.65 (m, 1H, furan C_4-H), 6.86 (d, $J = 3.6$ Hz, 1H furan C_3-H), 6.93 (d, $J = 16.2$ Hz, 1H, ethenyl C_1-H), 7.1 (br.s, 2H, NH_2, D_2O exchangeable), 7.40–7.50 (m, 3H, phenyl $C_{3,4,5}$-H), 7.81 (d, J = 1.5 Hz, 1H, furan C_5-H), 7.90–7.97 (m, 3H, ethenyl C_2-H and phenyl $C_{2,6}$-H), 13.9 (br.s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{18}H_{14}N_6O_2$ (346.34): C, 62.42; H, 4.07; N, 24.27; found: C, 62.27; H, 3.77; N, 24.0.

*(E)-3-[5-Amino-3-(4-methylphenyl)-1H-pyrazol-1-yl]-6-[2-(furan-2-yl)ethenyl]-1,2,4-triazin-5-(2H)-one (**5b**)*

Yellow solid (60 %); m.p.: 272–274 °C; IR (KBr, cm^{-1}): 3398, 3347, 3301 (NH), 3023 (CH furan), 2918 (CH_3), 1680 (C=O), 1624 (C=N), 1548, 1486 (C=C, δ NH), 1248, 1012 (C–O–C), 743 (*oop* furan); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.35 (s, 3H, CH_3), 5.88 (s, 1H, pyrazole C_4-H), 6.56-6.62 (m, 1H, furan C_4-H), 6.88 (d, $J = 3.6$ Hz, 1H furan C_3-H), 6.93 (d, $J = 16.2$ Hz, 1H, ethenyl C_1-H), 7.1 (br.s, 2H, NH_2, D_2O exchangeable), 7.27 (d, $J = 8.2$ Hz, 2H, methylphenyl $C_{3,5}$-H), 7.82–7.89 (m, 4H, furan C_5-H, ethenyl C_2-H and methylphenyl $C_{2,6}$-H), 13.9 (br.s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{19}H_{16}N_6O_2$ (360.37): C, 63.32; H, 4.48; N, 23.32; found: C, 63.65; H, 4.27; N, 23.13.

*(E)-3-[5-Amino-3-(4-chlorophenyl)-1H-pyrazol-1-yl]-6-[2-(furan-2-yl)ethenyl]-1,2,4-triazin-5-(2H)-one (**5c**)*

Pale yellow solid (85 %); m.p.: 280–282 °C; IR (KBr, cm^{-1}): 3415, 3300, 3177 (NH), 3025 (CH furan), 1658 (C=O), 1620 (C=N), 1546, 1488 (C=C, δ NH), 1250, 1016 (C–O–C), 762 (*oop* furan); ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 5.89 (s, 1H, pyrazole C_4-H), 6.58–6.61 (m, 1H, furan C_4-H), 6.81 (d, $J = 3.8$ Hz, 1H, furan C_3-H), 6.89 (d, $J = 16.8$ Hz, 1H, ethenyl C_1-H), 7.07 (br.s, 2H, NH_2, D_2O exchangeable), 7.48 (d, $J = 8.4$ *Hz*, 2H, chlorophenyl $C_{3,5}$-H), 7.76 (s, 1H, furan C_5-H), 7.87 (d, $J = 16.8$ Hz, 1H, ethenyl C_2-H), 7.94 (d, $J = 7.3$ Hz, 2H, chlorophenyl $C_{2,6}$-H), 13.98 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (500 MHz, DMSO-d_6) δ: 85 (pyrazole C_4), 112.51 (furan C_4), 112.92 (ethenyl C_1), 118.41 (furan C_3), 124.11(ethenyl C_2), 127.83 (chlorophenyl $C_{2,6}$), 128.56 (chlorophenyl $C_{3,5}$), 130.70 (chlorophenyl C_1), 133.60 (chlorophenyl C_4), 140.01 (triazine C_6), 144.65 (furan C_5), 145.58 (pyrazole C_3), 149.93 (furan C_2), 151.84 (pyrazole C_5), 152.02 (triazine C_3), 153.02 (C=O); Anal. Calcd for $C_{18}H_{13}ClN_6O_2$ (380.79): C, 56.78; H, 3.44; N, 22.07; found: C, 56.75; H, 3.74; N, 22.02.

*(E)-3-[5-Amino-3-(4-bromophenyl)-1H-pyrazol-1-yl]-6-[2-(furan-2-yl)ethenyl]-1,2,4-triazin-5-(2H)-one (**5d**)*

Yellowish white solid (78 %); m.p.: 288–290 °C; IR (KBr, cm^{-1}): 3396, 3361, 3294 (NH), 3023 (CH furan), 1662 (C=O), 1624(C=N), 1548, 1488 (C=C, δ NH), 1249, 1012 (C–O–C), 753 (*oop* furan). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 5.89 (s, 1H, pyrazole C_4-H), 6.58–6.62 (m, 1H, furan C_4-H), 6.83 (d, $J = 3.8$ Hz, 1H, furan C_3-H), 6.90 (d, $J = 16.4$ Hz, 1H, ethenyl C_1-H), 7.06 (br.s, 2H, NH_2, D_2O exchangeable), 7.43 (d, $J = 7.3$ Hz, 2H, bromophenyl $C_{3,5}$-H), 7.75 (s, 1H, furan C_5-H), 7.89 (d, $J = 16.4$ Hz, 1H, ethenyl C_2-H), 7.90 (d, $J = 7.3$ Hz, 2H, bromophenyl $C_{2,6}$-H), 13.95 (s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{18}H_{13}BrN_6O_2$ (425.24): C, 50.84; H, 3.08; N, 19.76; found: C, 50.93; H, 2.82; N, 19.62.

(*E*)-3-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-6-[2-(furan-2-yl) ethenyl]-1,2,4-triazin-5(2*H*)-one (**6**)

A mixture of the hydrazine **3** (0.2 g, 0.9 mmol) and acetylacetone (0.1 g, 0.11 ml, 1 mmol) in absolute ethanol was heated under reflux for 3 h. The reaction mixture was cooled, and the formed precipitate was filtered, washed with ethanol, dried and crystallized from ethanol. Shiny yellow crystals (68 %); M.p. 180–182 °C; IR (KBr, cm^{-1}): 3288, 3116 (NH), 3069 (CH furan), 2988, 2923 (CH_3), 1676 (C=O), 1619 (C=N), 1588, 1558, 1540, 1493 (C=C, δ NH), 1239, 1018 (C–O–C), 746 (*oop* furan); ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 2.22, 2.46 (2 × s, each 3H, pyrazole CH_3), 6.24 (s, 1H, pyrazole C_4-H), 6.54–6.57 (m, 1H, furan C_4-H), 6.80 (d, 1H, $J = 2.3$ Hz, furan C_3-H), 6.86 (d, $J = 16.1$ Hz, 1H, ethenyl C_1-H), 7.76 (s, 1H, furan C_5-H), 7.86 (d, $J = 16.1$ Hz, 1H, ethenyl C_2-H), 14.16 (s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{14}H_{13}N_5O_2$ (283.29): C, 59.36; H, 4.63; N, 24.72. Found: C, 59.27; H, 4.92; N, 24.42.

(*E*)-3-(3,5-Dioxopyrazolidin-1-yl)-6-[2-(furan-2-yl)ethenyl]-1,2,4-triazin-5(2*H*)-one (**7**)

A mixture of the hydrazine **3** (0.2 g, 0.9 mmol) and diethyl malonate (0.16 g, 0.15 ml, 1 mmol) in a mixture of ethanol/glacial acetic acid (10 ml) (1:1) was heated under reflux for 8 h and then cooled. The precipitated solid was filtered, washed with ethanol, dried and crystallized from glacial acetic acid. Red crystals (57 %); m.p.: 165–167 °C; IR (KBr, cm^{-1}): 3341, 3101 (NH), 3059 (CH furan), 2903 (CH_2), 1707, 1662 (C=O), 1628 (C=N), 1583, 1552, 1500 (C=C, δ NH), 1205, 1029 (C–O–C), 761 (*oop* furan); ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 2.45 (s, 2H, pyrazolidine C_4-H), 3.36 (br.s., 1H, pyrazolidine NH), 6.56–6.60 (m, 1H, furan C_4-H), 6.87 (d, $J = 3.8$ Hz, 1H,furan C_3-H), 7.0 (d, $J = 16.3$ Hz, 1H, ethenyl C_1-H), 7.79 (s, 1H, furan C_5-H), 7.86 (d, $J = 16.3$ Hz, 1H, ethenyl C_2-H), 8.70 (s, 1H, NH, D_2O exchangeable), 13.59 (s, 1H, triazine NH, D_2O exchangeable); Anal. Calcd for $C_{12}H_9N_5O_4$ (287.23): C, 50.18; H, 3.16; N, 24.38; found: C, 49.77; H, 3.49; N, 24.56.

(*E*)-Ethyl 3-[2-{6-(2-(furan-2-yl)ethenyl)-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl} hyrazono]butanoate (**8**)

A mixture of the hydrazine **3** (0.2 g, 0.9 mmol) and ethyl acetoacetate (0.13 g, 0.12 ml, 1 mmol) in absolute ethanol was heated under reflux for 3 h. The reaction mixture was left to cool to room temperature and the obtained product was filtered, washed with ethanol, dried and crystallized from ethanol. Pale yellow crystals (76 %); m.p.: 156–158 °C; IR (KBr, cm^{-1}): 3296, 3100 (NH), 3038 (CH furan), 2986, 2901 (CH_2, CH_3), 1732, 1685 (C=O), 1628 (C=N), 1572, 1550, 1500 (C=C, δ NH), 1185, 1016 (C–O–C), 742 (*oop* furan); ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 1.16 (t, $J = 6.9$ Hz, 3H, CH_2-CH_3), 1.94 (s, 3H, CH_3), 4.07 (q, $J = 6.9$ Hz, 2H, CH_2-CH_3), 6.50–6.54 (m, 1H, furan C_4-H), 6.70 (d, $J = 3.1$ Hz, 1H, furan C_3-H), 6.81 (d, $J = 16.1$ Hz, 1H, ethenyl C_1-H), 7.71 (s, 1H, furan C_5-H), 7.77 (d, $J = 16.1$ Hz, 1H, ethenyl C_2-H), 10.72, 12.74 (2 × s, each 1H, 2 NH, D_2O exchangeable); Anal. Calcd for $C_{15}H_{17}N_5O_4$ (331.33): C, 54.38; H, 5.17; N, 21.14 Found: C, 54.04; H, 5.51; N, 20.78.

(*E*)-6-[(2-(Furan-2-yl)ethenyl]-3-(3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazol-1-yl)-1,2,4-triazin-5(2*H*)-one (**9**)

Method A Compound **8** (0.5 g, 1.5 mmol) was heated in an oil bath at 160 °C for 20 min. The reaction mixture was left to cool and the obtained product was triturated with EtOH, filtered, dried, and crystallized from dimethyl formamide/water. Reddish brown solid (72 %).

Method B The hydrazine derivative **3** (0.2 g, 0.9 mmol) was heated with ethyl acetoacetate (0.13 g, 0.12 ml, 1 mmol) in an oil bath at 160 °C for 1 h. The reaction mixture was left to cool and the solidified residue was triturated with ethanol, filtered, dried, and crystallized from dimethyl formamide/water. (65 %); m.p.: 190–192 °C; IR (KBr, cm^{-1}): 3433 (NH), 3066 (CH furan), 2923 (CH_3), 1679 (C=O), 1637 (C=N), 1542, 1487 (C=C, δ NH), 1210, 1012 (C–O–C), 743 (*oop* furan); ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 2.47 (s, 3H, pyrazoline C_3-CH_3), 3.44 (s, 2H, pyrazoline C_4-H), 6.58–6.61 (m, 1H, furan C_4-H), 6.81 (d, $J = 3.1$ Hz, 1H, furan C_3-H), 6.86 (d, $J = 16.1$ Hz, 1H, ethenyl C_1-H), 7.77 (s, 1H, furan C_5-H), 7.89 (d, $J = 16.1$ Hz, 1H, ethenyl C_2-H), 13.38 (s, 1H, NH, D_2O exchangeable); MS (*m*/*z*, %): 285 (M^+, 21.0), 98 (100.0); Anal. Calcd for $C_{13}H_{11}N_5O_3 \cdot ½H_2O$ (294.27): C, 53.06; H, 4.11; N, 23.80; found: C, 52.91; H, 4.29; N, 23.96.

(2*Z*, 3*E*)-4-(Furan-2-yl)-2-[2-(thiocarbohydrazono)]butenoic acid (**11**)

A mixture of the 2-furylidene pyruvic acid **10** (5 g, 30 mmol) and thiocarbohydrazide (3.2 g, 30 mmol) in a mixture of ethanol (20 ml) and glacial acetic acid (0.5 ml) was stirred at room temperature for 15 min. It was filtered, washed with water, dried and crystallized from dimethyl formamide/water. Orange red solid (79 %); m.p.: 238–240 °C; IR (KBr, cm^{-1}): 3437, 3290, 3200 (OH, NH), 3078 (CH furan), 1661 (C=O), 1626 (C=N), 1568, 1551, 1501 (C=C, δ NH), 1534, 1277, 1119, 965 (N–C=S), 1232, 1016 (C–O–C), 753 (*oop* furan); ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 6.50 (s, 2H, NH_2, D_2O exchangeable), 6.55–6.59 (m, 1H, furan C_4-H), 6.78–6.83 (m, 2H, furan

C_3-H and ethenyl C_1-H), 7.64 (d, $J = 16.1$ Hz, 1H, ethenyl C_2-H), 7.77 (s, 1H, furan C_5-H), 9.68, 10.30 (2s, each 1H, 2NH, D_2O exchangeable); Anal. Calcd for $C_9H_{10}N_4O_3S$ (254.27): C, 42.51; H, 3.96; N, 22.03; found: C, 42.74; H, 3.95; N, 22.35.

(*E*)-4-Amino-6-[2-(furan-2-yl)ethenyl]-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2*H*)-one (**12**)

A solution of the thiocarbohydrazone **11** (2.5 g, 10 mmol) in 1 N NaOH (20 ml) was heated for 20 min. After cooling, the solution was acidified with dil. H_2SO_4 to pH 6. The formed precipitate was filtered, washed with water, dried, and crystallized from dioxane.

Pale brown crystals (93 %); m.p.: 230–232 °C; IR (KBr, cm^{-1}): 3437, 3254 (NH), 3078 (CH furan), 1660 (C=O), 1625 (C=N), 1593, 1517, 1499 (C=C, δ NH), 1542, 1278, 1104, 961 (N–C=S), 1235, 1013 (C–O–C), 754 (*oop* furan); ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 6.51 (s, 2H, NH_2, D_2O exchangeable), 6.55–6.59 (m, 1H, furan C_4-H), 6.78–6.83 (m, 2H, furan C_3-H and ethenyl C_1-H), 7.64 (d, $J = 16.1$ Hz, 1H, ethenyl C_2-H), 7.76 (s, 1H, furan C_5-H), 13.90 (s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_9H_8N_4O_2S$ (236.25): C, 45.75; H, 3.41; N, 23.72; found: C, 46.02; H, 3.64; N, 23.47.

4-Amino-6-[(*E*)-2-(furan-2-yl)ethenyl]-3-(substituted sulfanyl)-1,2,4-triazin-5(4*H*)-ones (**13a**–**c**)

A mixture of the thione **12** (0.38 g, 1.6 mmol), anhydrous K_2CO_3 (0.33 g, 2.4 mmol) and dimethylsulfate, ethyl iodide or benzyl chloride (2.4 mmol) in dry dimethyl formamide (4 ml) was stirred at room temperature for 12 h. The reaction mixture was then diluted with water and the obtained product was filtered, washed with water, dried, and crystallized from ethanol.

*(E)-4-Amino-6-[2-(furan-2-yl)ethenyl]-3-(methylsulfanyl)-1,2,4-triazin-5(4H)-one (**13a**)*

Yellow solid (72 %); m.p.: 230–232 °C; IR (KBr, cm^{-1}): 3242, 3180 (NH_2), 3075 (CH furan), 2947 (CH_3), 1661(C=O), 1621 (C=N), 1544, 1494 (C=C, δ NH), 1286, 1078 (C–S–C), 1231, 1010 (C–O–C), 755 (*oop* furan); ^{1}H-NMR (500 MHz, DMSO-d_6) δ 3.32 (s, 3H, CH_3, under DMSO), 6.50 (s, 2H, NH_2, D_2O exchangeable), 6.57 (dd, $J = 3.8$, 1.55 *Hz*, 1H, furan C_4-H), 6.80 (d, $J = 3.8$ Hz, 1H, furan C_3-H), 6.81, 7.64 (2 × d, $J = 16.05$ *Hz*, each 1H, ethenyl C_1-H and ethenyl C_2-H), 7.77 (s, 1H, furan C_5-H); Anal. Calcd for $C_{10}H_{10}N_4O_2S$ (250.28): C, 47.99; H, 4.03; N, 22.39; S, 12.81. Found: C, 47.65; H, 4.39; N, 22.07; S, 13.12.

*(E)-4-Amino-3-(ethylsulfanyl)-6-[2-(furan-2-yl) ethenyl]-1,2,4-triazin-5(4H)-one (**13b**)*

Brown solid (55 %); m.p.: 118–120 °C; IR (KBr, cm^{-1}): 3293, 3201 (NH_2), 3075 (CH furan), 2973, 2926 (CH_2, CH_3), 1676 (C=O), 1624 (C=N), 1536, 1486 (C=C, δ NH), 1287, 1072 (C–S-C), 1231, 1018 (C–O–C), 737(*oop* furan); ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 1.33 (t, $J = 7.0$ *Hz*, 3H, CH_2–*CH_3*), 4.45 (q, $J = 7.0$ Hz, 2H, *CH_2*–CH_3), 6.59 (dd, $J = 3.1$, 1.5 Hz, 1H, furan C_4-H), 6.67 (s, 2H, NH_2, D_2O exchangeable), 6.82–6.85 (m, 2H, ethenyl C_1-H and furan C_3-H), 7.71 (d, $J = 16.8$ Hz, 1H, ethenyl C_2-H), 7.78 (d, $J = 1.6$ Hz, 1H, furan C_5-H); Anal. Calcd for $C_{11}H_{12}N_4O_2S.1/2\ H_2O$ (273.31): C, 48.34; H, 4.79; N, 20.50; S, 11.73; found: C, 48.66; H, 4.39; N, 20.17; S, 11.64.

*(E)-4-Amino-3-(benzylsulfanyl)-6-[2-(furan-2-yl) ethenyl]-1,2,4-triazin-5(4H)-one (**13c**)*

Pale yellow solid (60 %); m.p.: 142–144 °C; IR (KBr, cm^{-1}): 3290, 3190 (NH_2), 3025 (CH furan), 2947(CH_2), 1676 (C=O), 1629 (C=N), 1563, 1530, 1484 (C=C, δ NH), 1291, 1081 (C–S–C), 1253, 1015 (C–O–C), 730 (*oop* furan); ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 5.65 (s, 2H, S-CH_2), 6.59 (dd, $J = 3.4$, 1.93 Hz, 1H, furan C_4-H), 6.66 (s, 2H, NH_2, D_2O exchangeable), 6.82–6.86 (m, 2H, ethenyl C_1-H and furan C_3-H), 7.24–7.37 (m, 5H, phenyl-H), 7.71 (d, $J = 16.1$ Hz, 1H, ethenyl C_2-H), 7.78 (s, 1H, furan C_5-H); ^{13}C-NMR (300 MHz, DMSO-d_6) δ: 44.21 (CH_2), 123.01 (furan C_4), 124.97 (furan C_3), 127.0 (ethenyl C_1), 128.50 (ethenyl C_2), 134.29 (phenyl C_4), 137.14 (phenyl $C_{3,5}$), 139.13 (phenyl $C_{2,6}$), 143.8 (phenyl C_1), 145.71 (furan C_5), 152.85 (furan C_2), 154.48 (triazine C_3), 158.0 (triazine C_6), 163.0 (C=O); Anal. Calcd for $C_{16}H_{14}N_4O_2S$ (326.37): C, 58.88; H, 4.32; N, 17.17; found: C, 58.64; H, 4.07; N, 16.88.

*6-[(E)-2-(Furan-2-yl)ethenyl]-4-[(E)(4-nitrobenzylidene)amino]-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2H)-one (**14**)*

A mixture of the amino thione **12** (0.5 g, 2.1 mmol) and 4-nitrobenzaldehyde (0.32 g, 2.1 mmol) in absolute ethanol (20 ml) containing few drops of glacial acetic acid was heated under reflux for 1 h. The reaction mixture was left to cool to room temperature and the formed precipitate was filtered, washed with ethanol, dried and crystallized from glacial acetic acid. Yellow solid (61 %); m.p.: 256-258 °C. IR (KBr, cm^{-1}): 3207 (NH), 3094 (CH furan), 1701 (C=O), 1620 (C=N), 1595, 1525, 1482 (C=C, δNH), 1520, 1260, 1137, 973 (N–C=S), 1525, 1342 (NO_2), 1260, 1015 (C–O–C), 747 (*oop* furan); ^{1}H-NMR (500 MHz, DMSO-d_6) δ:

6.56–6.59 (m, 1H, furan C_4-H), 6.81 (d, $J = 3.8$ Hz, 1H, furan C_3-H), 6.84, 7.63 (2 × d, $J = 16.1$ Hz, each 1H, ethenyl C_1-H and ethenyl C_2-H), 7.78 (s, 1H, furan C_5-H), 8.18, 8.39 (2 × d, $J = 8.4$ Hz, each 2H, nitrophenyl $C_{2,6}$-H and nitrophenyl $C_{3,5}$-H), 8.89 (s, 1H, N = CH), 14.06 (s, 1H, NH, D_2O exchangeable); MS (*m/z*, %): 369 (M^+, 20.5), 119 (100.0). Anal. Calcd for $C_{16}H_{11}N_5O_4S$ (369.35): C, 52.03; H, 3.00; N, 18.96; found: C, 52.35; H, 3.06; N, 18.62.

6-[(*E*)-2-(Furan-2-yl)ethenyl]-4-[(*E*)(4-nitrobenzylidene)amino]-3-[(2-substituted ethyl)sulfanyl]-1,2,4-triazin-5(4*H*)-ones (**15a-c**)

To a suspension of compound **14** (0.48 g, 1.3 mmol) in aqueous KOH solution (0.22 g, 2 ml, 4 mmol), the appropriate alkylating agent (1.3 mmol) was added. The reaction mixture was stirred at room temperature for 24 h then acidified with glacial acetic acid till pH 6. The separated solid product was filtered, dried, and crystallized from the proper solvent.

*6-[(E)-2-(Furan-2-yl)ethenyl]-3-[(2-(morpholin-1-yl)ethyl)sulfanyl]-4-[(E)-(4-nitrobenzylidene)amino]-1,2,4-triazin-5(4H)-one (**15a**)*

Orange solid (52 %); m.p.: 132–134 °C (dimethylformamide); IR (KBr, cm^{-1}): 3068 (CH furan), 2924, 2853, (CH_2), 1686 (C=O), 1620 (C=N), 1591, 1557, 1491 (C=C), 1523, 1343 (NO_2), 1262, 1069 (C–S–C), 1220, 1012 (morpholine and furan C–O–C), 745 (oop furan); ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 2.63 (t, $J = 6.9$ Hz, 2H, S-CH_2), 3.16 (t, $J = 6.9$ Hz, 2H, N-CH_2), 3.54–3.58 (m, 8H, morpholine $C_{2,3,5,6}$-H_2), 6.56–6.59 (m, 1H, furan C_4-H), 6.74 (d, $J = 3.1$ Hz, 1H, furan C_3-H), 6.85, 7.54 (2 × d, $J = 16.1$ Hz, each 1H, ethenyl C_1-H and ethenyl C_2-H), 7.74 (s, 1H, furan C_5-H), 8.12, 8.34 (2 × d, $J = 8.4$ Hz, each 2H, nitrophenyl $C_{2,6}$-H and nitrophenyl $C_{3,5}$-H), 8.99 (s, 1H, N = CH); Anal. Calcd for $C_{22}H_{22}N_6O_5S$ (482.51): C, 54.76; H, 4.60; N, 17.42; S, 6.65; found: C, 54.39; H, 4.79; N, 17.69; S, 6.75.

*6-[(E)-2-(Furan-2-yl)ethenyl]-4-[(E)-(4-nitrobenzylidene)amino]-3-[(2-(piperidin-1-yl)ethyl)sulfanyl]-1,2,4-triazin-5(4H)-one (**15b**)*

Brown solid (60 %); m.p.: 141–143 °C (ethanol/water); IR (KBr, cm^{-1}): 3035 (CH furan), 2933, 2850 (CH_2), 1690 (C=O), 1619 (C=N), 1591, 1523, 1497, (C=C), 1523, 1344 (NO_2), 1280, 1073 (C–S–C), 1226, 1016 (C–O–C), 757 (oop furan); ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 1.39–1.50 (m, 6H, piperidine $C_{3,4,5}$-H_2), 1.73–1.79 (m, 4H, piperidine $C_{2,6}$-H_2), 2.67 (m, 2H, SCH_2), 2.89 (m, 2H, NCH_2), 6.60–6.66 (m, 1H, furan C_4-H), 6.87 (d, $J = 3.0$ Hz, 1H, furan C_3-H), 7.18 (d, $J = 16.5$ Hz, 1H, ethenyl C_1-H), 7.82 (s, 1H, furan C_5-H), 7.87 (d, $J = 16.5$ Hz, 1H, ethenyl C_2-H), 8.20, 8.42 (2 × d, $J = 8.7$ Hz, each 2H, nitrophenyl $C_{2,6}$-H and $C_{3,5}$-H), 9.58 (s, 1H, N=CH); ^{13}C-NMR (300 MHz, DMSO-d_6) δ: 28.0 (SCH_2), 30.71 (piperidine C_4), 31.92 (piperidine $C_{3,5}$), 58.20 (NCH_2), 61.65 (piperidine $C_{2,6}$), 122.01 (furan C_4), 122.80 (furan C_3), 133.92 (nitrophenyl $C_{3,5}$), 135.6 (ethenyl C_1), 138.3 (ethenyl C_2), 139.57 (nitrophenyl $C_{2,6}$), 144.30 (nitrophenyl C_1), 148.5 (N=CH), 152.0 (furan C_5), 154.0 (nitrophenyl C_4), 155.0 (furan C_2), 158.32 (triazine C_3), 161.46 (triazine C_6), 165.29 (C=O); Anal. Calcd for $C_{23}H_{24}N_6O_4S$ (480.54): C, 57.49; H, 5.03; N, 17.49; S, 6.67; found: C, 57.19; H, 4.79; N, 17.46; S, 6.58.

*6-[(E)-2-(Furan-2-yl)ethenyl]-4-[(E)-(4-nitrobenzylidene)amino]-3-[(2-(pyrrolidin-1-yl)ethyl)sulfanyl]-1,2,4-triazin-5(4H)-one (**15c**)*

Orange brown solid (40 %); m.p.: 120–122 °C (methanol/water); IR (KBr, cm^{-1}): 3050 (CH furan), 2926, 2854 (CH_2), 1687 (C=O), 1620 (C=N), 1591, 1558, 1493 (C=C), 1523, 1343 (NO_2), 1260, 1075 (C–S–C), 1224, 1014 (C–O–C), 746 (*oop* furan); ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 1.87–1.89 (m, 4H, pyrrolidine $C_{3,4}$-H_2), 2.97–3.20 (m, 4H, pyrrolidine $C_{2,5}$-H_2), 3.37 (t, $J = 6.9$ Hz, 2H, S-CH_2), 3.38 (t, $J = 6.9$ Hz, 2H, N-CH_2), 6.60–6.63 (m, 1H, furan C_4-H), 6.84 (d, 1H, $J = 3.1$ Hz, furan C_3-H), 7.08 (d, $J = 16.1$ Hz, 1H, ethenyl C_1-H), 7.80 (s, 1H, furan C_5-H), 7.83 (d, $J = 16.1$ Hz, 1H, ethenyl C_2-H), 8.18, 8.40 (2 × d, $J = 8.4$ Hz, each 2H, nitrophenyl $C_{2,6}$-H and nitrophenyl $C_{3,5}$-H), 9.50 (s, 1H, N=CH); Anal. Calcd for $C_{22}H_{22}N_6O_4S$ (466.51): C, 56.64; H, 4.75; N, 18.01; S, 6.87; found: C, 56.29; H, 4.79; N, 17.70; S, 7.12.

In vitro antitumor screening

Preliminary in vitro one-dose antitumor screening

Anti-tumor activity screening for compounds **2c**, **5c**, **7**, **12**, **14**, and **15a** at a dose of 10 μM utilizing 55 different human tumor cell lines, representing leukemia, melanoma and cancers of the lung, colon, brain, ovary, breast, prostate, and kidney was carried out according to standard procedure (Skehan *et al.*, 1990; Rubinstein *et al.*, 1990). The human tumor cell lines of the cancer screening panel are grown in RPMI 1640 medium containing 5 % fetal bovine serum and 2 mmol L-glutamine. For a typical screening experiment, the tumor cells were inoculated into 96-well microtiter plates in 100 μl at plating densities ranging from 5,000 to 40,000 cells/well. Density of the

inoculum depends on the type of tumor cell and its growth characteristics. After cell inoculation, the microtiter plates were incubated at 37 °C, 5 % CO_2, 95 % air, and 100 % relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell lines were fixed in situ with trichloroacetic acid (TCA), to represent a measurement of the cell population for each cell line at the time of test compound addition (time zero, Tz). Tested compounds were solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of test compound addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μg/ml gentamicin. The percentage growth of the tumor cells were calculated relative to time zero.

Full in vitro five-dose antitumor assay

For compounds passed on for the five-dose assay, the compounds were tested at five different concentrations (10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} M). Following drug addition, the plates were incubated for an additional 48 h at 37 °C, 5 % CO_2, 95 % air, and 100 % relative humidity. The cells were assayed by using the sulforhodamine B assay. Sulforhodamine B (SRB) solution (100 μl) at 0.4 % (w/v) in 1 % acetic acid was added to each well, and plates were incubated for 10 min at room temperature. After staining, unbound dye was removed by washing five times with 1 % acetic acid and the plates were air dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay was terminated by fixing settled cells at the bottom of the wells by gently adding 50 μl of 80 % TCA.

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