Med Chem Res (2015) 24:4093–4104
DOI 10.1007/s00044-015-1460-3

MEDICINAL CHEMISTRY RESEARCH

CrossMark

ORIGINAL RESEARCH

Potent anticancer and antimicrobial activities of pyrazole, oxazole and pyridine derivatives containing 1,2,4-triazine moiety

Amira S. Abd El-All[1] · Souad A. Osman[2] · Hanaa M. F. Roaiah[1] · Mohamed M. Abdalla[3] · Abeer A. Abd El Aty[1] · Wafaa H. AbdEl-Hady[2]

Received: 18 March 2015 / Accepted: 29 August 2015 / Published online: 5 September 2015
© Springer Science+Business Media New York 2015

Abstract Due to the importance of biological activities of 1,2,4-triazine derivatives, in this manuscript, ten of newly synthesized compounds containing 1,2,4-triazine moiety were prepared. Anticancer and antimicrobial activities of some selected synthesized compounds were screened. Chalcone derivatives **6**, **7** were synthesized via reaction of Mannich base **2** in acetic acid and fused sodium acetate with formyl khellin **5** or vanillin. Treatment of chalcone derivatives **6**, **7** toward hydrazine hydrate, phenyl hydrazine, hydroxylamine hydrochloride or malononitrile, respectively, led to give pyrazoline **8**, **9**, **10**, **11**, oxazole **12**, **13** and pyridine **14**, **15** derivatives, respectively. The structures of the isolated products were established by elementary analysis and spectral data studies. Compounds **1**, **5**, **6**, **8**, **10**, **12** and **14** were tested against different human cancer cell lines, and cytotoxicity in vitro and most of the synthesized compounds were proved to have high activities against cytotoxic test. Then, the compounds **1**, **5**, **6**, **8**, **10**, **12** and **14** were tested against cancer in vivo, and the result was established. Also, antimicrobial activity of compounds **1**, **5**, **6**, **8**, **10**, **12** and **14** was screened in vitro against a panel of gram-positive and gram-negative bacterial pathogens and fungi. The results indicated that compound **5** showed higher antimicrobial activity than compounds **1**, **6**, **8**, **10**, **12** and **14**, and compound **5** exhibited a wide range of antimicrobial activities against gram-positive and gram-negative bacteria and fungi, greater than well-known antibacterial and antifungal agents with minimal inhibitory concentration ranged between 6.25 and 25 µg.

Keywords 1,2,4-Triazine · Chalcone derivatives · Furochromone · Pyrazolines · Oxazoles · Cyanopyridines · Cytotoxicity · Anticancer activity · Antimicrobial activity

Introduction

1,2,4-Triazines and their derivatives have been widely studied in terms of their synthetic methodologies and reactivity, since some of these derivatives were reported to have promising biological activities and used as a drugs in medicinal chemistry (Nyffenegger *et al.*, 2007), including antifungal (Sztanke *et al.*, 2005; Holla *et al.*, 2001), anti-HIV (El-Barbary *et al.*, 2005; Makk *et al.*, 2014), anticancer (Abdel-Monem, 2004; El-Gendy *et al.*, 2003), anti-inflammatory (Kumar *et al.*, 2014; Mullick *et al.*, 2009), analgesic (Makhlouf and Maklad, 2004), antiamebic activity (Singh *et al.*, 2005), antimicrobial activity (El-Barbary *et al.*; 2014; Hegde *et al.*, 2008; Bishnoi *et al.*, 2014), antiasthma agents (Paul *et al.*, 1985) and diuretic and antihypertensive activities (Abdel-Monem, 2004; Paul *et al.*, 1985; Heilman *et al.*, 1979; Ibrahim *et al.*, 2008; Abd-El-All *et al.*, 2013).

In addition, furochromones are very interesting heterocycles, having a wide range of biological activities (Magd-El-Din *et al.* 2012). They have been reported as antimicrobial (Ragab *et al.*, 1993; Kim *et al.*, 2006; Galal *et al.*,

✉ Amira S. Abd El-All
amira19661@hotmail.com

[1] Research Division of Pharmaceutical and Drug Industries, Department of Natural and Microbial Product, National Research Center, El-Behoos Street, Dokki, Giza 12622, Egypt

[2] Division of Inorganic Chemical Industries and Mineral Resources, Department of Organometallic and Organometalloid Chemistry, National Research Center, El-Behoos Street, Dokki, Giza 12622, Egypt

[3] Research Unit, Saco Pharm. Co., 6 October City 11632, Egypt

2009; Hayakawa *et al.*, 2005; Hishmat *et al.*, 1999; Hishmat *et al.*, 1983) and bacteriostatic, bactericidal, fungistatic and fungicidal agents (Hishmat *et al.*, 1999).

On the other hand, chalcone is a biosynthetic product of the shikimate pathway and is a separate class of compounds, having a wide range of biological properties. Chalcones are useful synthons in the synthesis of a large number of bioactive molecules such as pyrazolines that are well-known nitrogen-containing heterocyclic compounds. Considerable interest has been focused on the pyrazoline structure, which possesses a broad spectrum of biological activities such as antiamebic, antimicrobial, monoamine oxidase inhibitors, antimycobacterial, antidepressant, anticonvulsant and anti-inflammatory. Moreover, the pyridine nucleus is prevalent in numerous natural products and is extremely important in the chemistry of biological systems. Pyridine derivatives have been used as bactericides, fungicides and anticancer agents (Bondock *et al.*, 2013).

Based on these findings, our aim is to design and synthesize novel pyrazolines **8**, **9**, *N*-phenyl pyrazolines **10**, **11**, oxazole **12**, **13** and pyridine **14**, **15** compounds from chalcone derivatives **6**, **7** containing the 1,2,4-triazine moiety and to study their anticancer, cytotoxicity, antimicrobial and antifungal activities of the newly synthesized compounds **6**, **8**, **10**, **12** and **14** which have union between 1,2,4-triazine and furochromone moieties.

Results and discussion

Chemistry

Mannich base 2-((4-acetylphenyl amino)methyl)-6-methyl-3-thioxo-1,2,4-triazin-5(2H)-one **2** was prepared as previously reported by condensation of 3,4-dihydro-6-methyl-3-thioxo-1,2,4-triazin-5(*2H*)-one **1** with formaldehyde and 4-amino acetophenone in the presence of absolute ethyl alcohol as solvent (Abd-El-All *et al.*, 2013) (Fig. 1).

Also, the naturally occurring furochromone "khellin" **3** yields khellinone **4** upon hydrolysis with potassium hydroxide. When **4** is subjected to Vilsmeier–Haack reaction, the corresponding 4,9-dimethoxy-5-oxo-5H-furo[3,2-g]chromene-6-carboxaldehyde **5** "formyl khellin" is obtained (Abdel-Aziz *et al.*, 1990; Eidin and Schünemann 1983) (Fig. 2).

It has been found that chalcone derivatives **6**, **7** were prepared via addition of Mannich base **2** and formyl khellin **5** or vanillin in a glacial acetic acid and fused sodium acetate upon refluxing for 4 h (Scheme 1). Structure of compounds **6**, **7** was confirmed on the basis of their analytical and spectral data. IR spectra of compounds **6**, **7** found a new band of (C=O) at 1655 cm^{-1} and 1665 cm^{-1} indicating the formation of chalcone, while IR of compound **7** showed a band at 3567 cm^{-1} of (OH) indicating the attachment of vanillin. Its ^{1}H-NMR spectrum (DMSO-d6, δ ppm) showed doublet at 8.70–8. 76 due to CH=CH aliphatic chalcone, and two singlet peaks appeared at 3.79 and 10.58 are assigned to methoxy and hydroxyl protons of vanillin. ^{13}C NMR spectrum of compound **6** was revealed signal at δ 163.1(C=O), 176.9(C=O) and 178.5(C=S). The other bands of chalcone derivatives **6**, **7** appeared in IR, ^{1}H-NMR and ^{13}C NMR spectra in the expected regions.

On the other hand, cyclization of chalcone derivatives **6**, **7** with hydrazine hydrate in ethanol and a few drops of hydrochloric acid formed the corresponding new pyrazoline hydrazine derivatives **8**, **9** (Heilman *et al.*, 1979; Shawali and Gomha, 2002; Ismail, 2001). Under the same reaction conditions, chalcone derivatives **6**, **7** react with phenyl hydrazine or hydroxylamine hydrochloride to afford the corresponding new N-phenyl pyrazoline **10**, **11** and oxazole derivatives **12**, **13**, while aminocyanopyridine derivatives **14**, **15** were synthesized by refluxing chalcone derivatives **6**, **7** with malononitrile in the presence of ammonium acetate in ethanol solution (Scheme 2). Structures of **8–15** were established on their analytical and spectral data. IR of compounds **8**, **9** found the disappearance of carbonyl group which was assigned to chalcone and 1,2,4-triazine ring at 1655–1665 and 1672 cm^{-1} and the appearance of a new band at ν 3120–2894 cm^{-1} which was assigned to (3NH, NH_2) which indicated the presence of hydrazinyl group. The ^{1}H-NMR spectrum (DMSO-d6, δ ppm) of compound 9 displayed a doublet peak at 2.51–2.56 which is characteristic to diastereotopic (CH_2), triplet peak at 2.85–3.12 due to pyrazole ring and two singlet peaks present at 7.01 and 8.41 that are corresponding to protons of the NH and NH_2 of hydrazinyl group which were D_2O

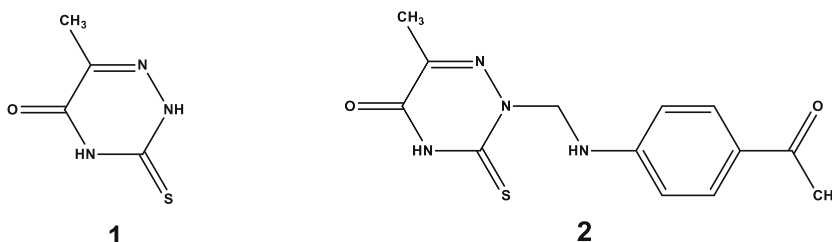


Fig. 1 1,2,4-Triazine derivate and its Mannich with 4-amino acetophenone

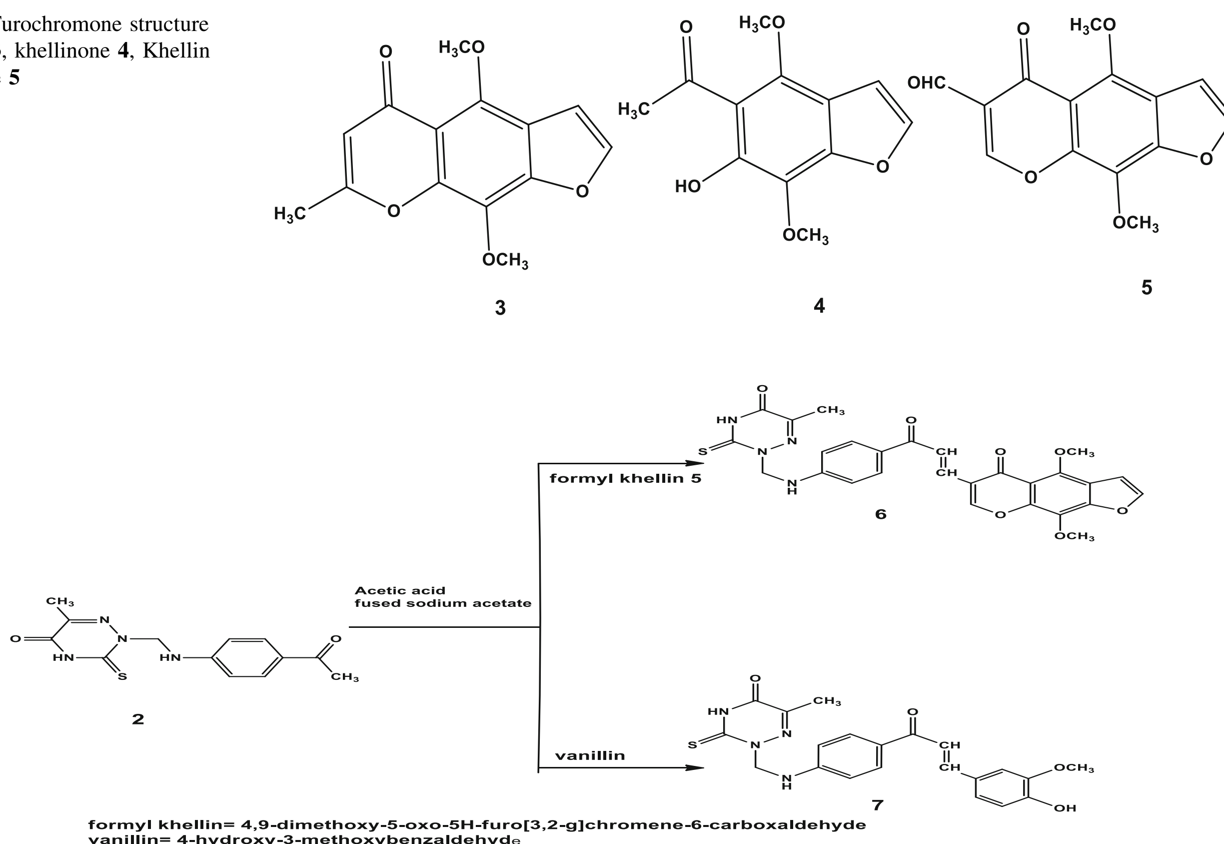


Fig. 2 Furochromone structure khellin **3**, khellinone **4**, Khellin aldehyde **5**

Scheme 1 Preparation of chalcones 6, 7

exchangeable. IR spectra of the compounds **10–15** showed the disappearance carbonyl group which was assigned to chalcone at ν 1655–1665 cm^{-1}, while compounds **14**, **15** showed appearance of cyano group in the expected regions at ν 2218, 2221 cm^{-1}. The ^{1}H-NMR of compounds **10–13** found the disappearance of signals for (CH=CH) aliphatic and appearance of signals of CH and CH_2 refers to pyrazole and oxazole rings in the expected regions. The ^{1}H-NMR(DMSO-d_6, δ ppm) spectrum of compound **15**, taken as example, revealed six singlet peaks at 1.04–1.08, 2.04, 3.88, 4.47, 5.26 and 10.72 due to (NH_2, CH_3, OCH_3, 2NH and OH), doublet peak at 5.48–5.60 related to methane proton and multiplet peak appearing at 7.63–7.69 for seven aromatic protons.

The formation of pyrazoline derivatives **8**, **9** is, therefore, assumed to proceed via cyclization, and initial attack of the amine nucleophilic on the carbonyl carbon of the chalcone by nitrogen atom of hydrazine hydrate and the carbonyl oxygen gets hydroxylation. Another end of the nitrogen atom of hydrazine hydrate bonded with β-carbon of chalcone, and the unsaturation was shifted between carbonyl and α-carbon of the chalcones. The hydroxylated group was followed by intramolecular cyclization via elimination of water molecule. Migration of proton of cyclic N-2 to C-4 of azole ring and the π-bond was shifted to N-2 and C-3 of the azole ring (Scheme 3).

Biological assay

In vitro cytotoxicity activities

Mosimann's method was used to evaluate the cytotoxic activity of synthesized compounds **1**, **5**, **6**, **8**, **10**, **12** and **14** against different human cancer cell lines (Table 1) including: cervical carcinoma (KB), ovarian carcinoma (SKOV-3), CNS cancer (SF-268), non-small lung cancer (NCl H460), colon adenocarcinoma (RKOP 27), antileukemia (HL60, U937, K561), melanoma (G361,SK-MEL-28) and neuroblastoma (GOTO, NB-1). The cytotoxic effect of the tested compounds over cell lines of HeLa (cervical), MCF-7(breast), HT1080 (fibrosarcoma) and HEPG2 (liver) was also tested. The results were expressed as the IC50, and the results are given in Table 1, which induces a 50 % inhibition of cell growth of treated cells when compared to the growth of control cells. There was a good reproducibility between replicate wells with standard errors below 10 %.

In vitro inhibition of histone deacetylase

Yoshida's method was used to evaluate the cytotoxic activity of synthesized compounds **1**, **5**, **6**, **8**, **10**, **12** and **14** against antileukemia cell line (K562). The tested compounds showed a high potent activity against antileukemia cell line (K562) (Table 1).

Assay of in vivo acute toxicity

HEpG2 cells

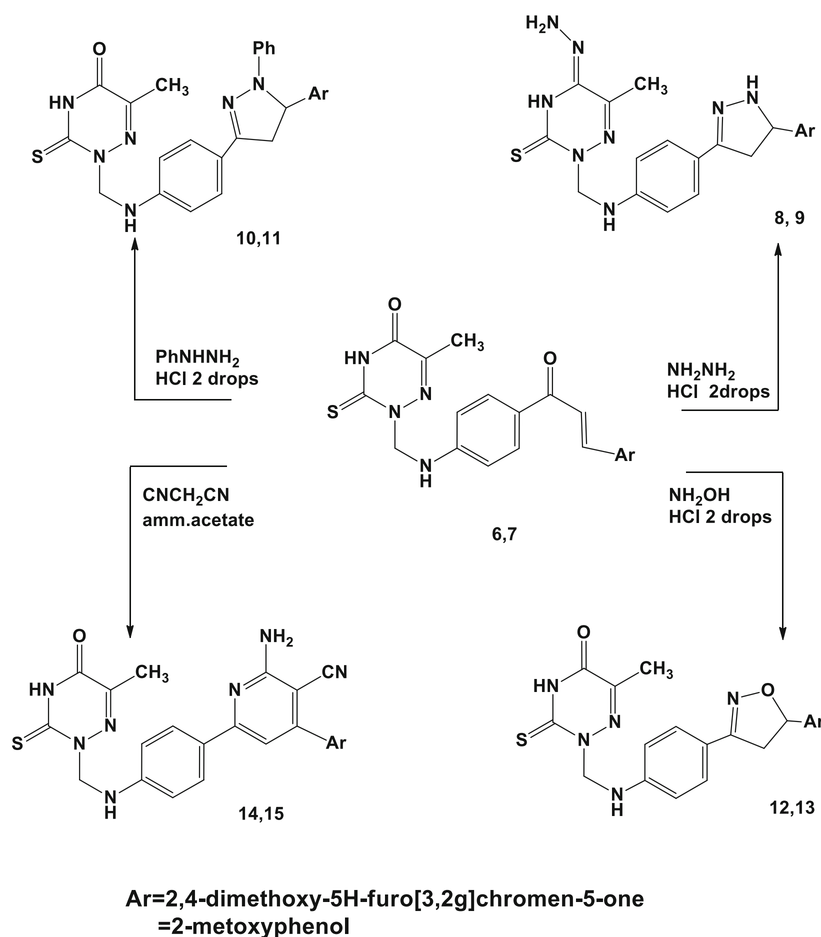
Compounds **1**, **5**, **6**, **8**, **10**, **12** and **14** were tested tumor cell growth inhibition against HEpG2 cells (1×10^5 cells in 0.1 ml PBS) (Table 3). Tested compounds were injected subcutaneously (10 µg/50 µl) daily for four doses. Monoclonal antiplatelet antibody (PY-13) and sterile PBS were used as isotype and negative controls, respectively. Tumor growth was observed every 3 days by measuring its diameter with vernier calipers. Tumor weight (TW) was calculated by TW (g) = tumor volume (cm^3) = d2 × D/2, where d is the shortest and D is the longest diameter, respectively. Mice were killed when the tumor size reached 2.0 cm in diameter, and samples were collected.

The results are presented in Table 2.

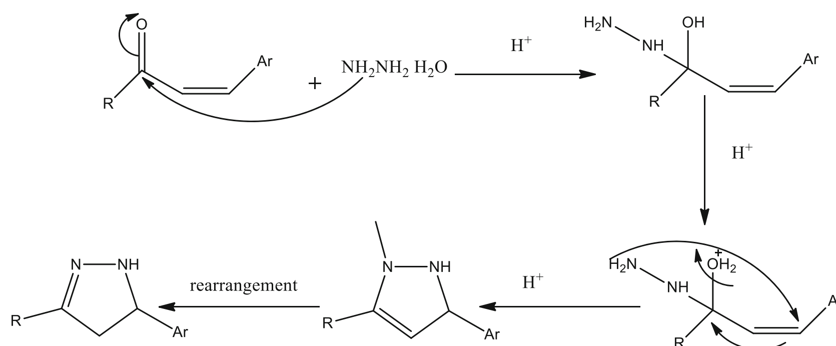
All tested compounds **1**, **5**, **6**, **8**, **10**, **12** and **14** were indicating a significance activity tumor cell growth inhibition against HEpG2 compared with control drug doxorubicin. Concerning the new pyridine derivative combining with 1,2,4-triazine and furochromone moieties **14** was the most active compared with the other synthesized chalcone **6**, pyrazoles **8**, **10** and oxazole **12** combining with 1,2,4-triazine and furochromone moieties, and it is obvious that the least active compound in this tested compounds is start material 1,2,4-triazine **1**.

MCF-7 xenograft model

Tested compounds **1**, **5**, **6**, **8**, **10**, **12** and **14** were evaluated against MCF-7 mouse xenograft model of breast cancer (Table 3).



Scheme 2 Derivatives preparation of pyrazole **8–11**, oxazole **12**, **13** and pyridine **14**, **15**



Scheme 3 The suggested mechanism of preparation pyrazoline derivatives **8**, **9**

The results are presented in Table 3.

Seven compounds **1**, **5**, **6**, **8**, **10**, **12** and **14** showed marked acute activity against MCF-7 mouse xenograft model of breast cancer. It is obvious that new pyridine derivative union with 1,2,4-triazine and furochromone moieties **14** was the most active, while the least active compound was the start material 1,2,4-triazine **1**.

Antimicrobial activity

In vitro antimicrobial screening

The results given in Table 4 revealed that compound **5** exhibited a wide range of antimicrobial activities against both gram-positive and gram-negative bacteria and fungi, and its inhibitory activity against fungi (especially *A. alternata* and *F. oxysporium*) was higher than of the bacteria, while compound **5** exhibited a moderate antimicrobial activity against all the tested pathological strains except A. *niger* and *A. tenuissima*, in comparison with the standard drugs. Compounds **6**, **8**, **10**, **12** and **14** exhibited low inhibitory effect against gram-negative bacteria *E. coli* and *P. aeruginosa* (zones of inhibition range from 7 to 9 mm). Results indicated that compound **5** was the most effective against all tested microorganisms with zones of inhibition ranging from 11 to 30 mm and minimum inhibitory concentrations (MIC) ranging between 6.25 and 25 µg. Compared with the standard antifungal drug (triflucan), compound **5** exhibited excellent antifungal activity against all tested fungi, with minimum inhibitory concentration better than that of the standard drug (Table 5).

Experimental

Chemistry

All melting points were uncorrected. Elemental analysis made at the Micro-analytic Center, Cairo University. The IR spectra (4000–400 cm^{-1}) were recorded using KBr pellets in a Jasco FT/IR 300 E Fourier. The ^{1}H-NMR spectra were measured on ^{1}H-NMR spectrophotometers and recorded using Joel EX-200, 500 MHz. The mass spectra were performed using Finnigan MAT SSQ 7000 (Thermo. Inst. Sys. Inc., USA) spectrophotometer at 70 and 20 eV.

*Preparation of chalcone (**6**, **7**)*

General procedure Mannich base **2** (10 mmol) and (10 mmol) of furochromone-6-carboxaldehyde **5** or vanillin in 30 ml of glacial acetic acid and 10 mmol of fused sodium acetate was heated under reflux for 4 h and then poured onto ice water, and the resulting solid was collected by filtration and crystallized from ethanol.

(E)-2-(((4-(3-(4,9-dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6-yl)acryloyl)phenyl)amino) methyl)-6-methyl-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2H)-one ***6*** A beige powder was crystallized from ethanol, yield 70 %, m.p. 220–222 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 2.14 (s,3H, CH_3); 3.91 (s, 3H, OCH_3); 4.25 (s, 3H, OCH_3); 4.90 (s, 1H, NH exchangeable with D_2O); 5.32–5.52 (d, 2H, CH_2); 6.42 (d, 1H, $J = 2$ Hz, furan C-2); 6.85 (d, 1H, $J = 2$ Hz, furan C-3); 7.03–7.96 (m, 5H, aromatic and 4-pyranone moiety); 8.70–8.76 (2d, 2H, CH=CH aliphatic, $J = 12.6$ Hz); 8.99 (s, 1H, NH exchangeable with D_2O). ^{13}C NMR (DMSO-d_6) δ 15.8 (CH_3), 61.5 ($O\underline{C}H_3$), 62.4($O\underline{C}H_3$), 65.0 (CH_2), 128.2, 149.0 (C=C aliphatic), 101.8, 110.5, 112.5, 119.5, 120.3, 125.1, 129.0, 130.3, 132.6, 140.9, 145.7, 147.6, 150.3, 150.8, 153.2. 154.7, 163.1 (C=O), 176.9 (C=O), 178.5 (C=S). IR (KBr, ν/cm^{-1}): (NH) 3320 br, (NH) 3216 s, (C=O triazine ring) 1672 s, (C=O chalcone) 1665, (C=O pyrane ring) 1641 s, (C=N triazine ring) 1564 s, (C=S) 1245 s. Anal. Calc. for $C_{27}H_{22}N_4O_7S$: C, 59.33; H, 4.06; N, 10.25; S, 5.87. Found: C, 59.57; H, 3.85; N, 10.07.; S, 6.02.

(E)-2-(((4-(3-(4-hydroxy-3-methoxyphenyl)acryloyl)phenyl) amino)methyl)-6-methyl-3-thioxo-3,4-dihydro-1,2,4-triazin-

Table 1 Cytotoxicity activities of compounds

Compound	IC50 Pico M tumor cell growth inhibition				
	KB	SKOV-3	SF-268	NCI H460	RKOP27
1	0.27	0.34	0.22	0.16	0.29
5	0.38	0.25	0.71	0.25	0.40
6	0.48	0.36	0.80	0.64	0.39
8	0.27	0.17	0.90	0.53	0.28
10	0.36	0.25	0.85	0.95	0.15
12	0.45	0.24	0.87	0.87	0.56
14	0.34	0.23	0.88	0.77	0.67

	Leukemia			Melanoma	
	HL60	U937	K561	G361	SK-MEL-28
1	0.23	0.26	0.23	0.32	0.023
5	0.43	0.35	0.46	0.43	0.021
6	0.57	0.44	0.78	0.56	0.053
8	0.65	0.53	0.80	0.76	0.054
10	0.87	0.65	0.90	0.89	0.076
12	0.90	0.76	0.98	0.09	0.028
14	0.70	0.79	0.96	0.06	0.034

	Neuroblastoma		Cervical	Breast	Fibrosarcoma	Liver
	GOTO	NB-1	HeLa	MCF-7	HT1080	HepG2
1	0.29	0.33	0.25	0.0097	0.25	0.00085
5	0.38	0.54	0.46	0.0086	0.43	0.00074
6	0.57	0.75	0.53	0.0062	0.12	0.00063
8	0.67	0.66	0.24	0.0043	0.34	0.00062
10	0.76	0.57	0.23	0.0034	0.56	0.00052
12	0.55	0.98	0.57	0.0023	0.78	0.00051
14	0.44	0.09	0.64	0.0012	0.76	0.00049

	IC50 (nM)
Against K562 cell line	
1	0.0096
5	0.0076
6	0.0055
8	0.0044
10	0.0043
12	0.0032
14	0.0021

5(2H)-one *7* A yellow powder was crystallized from ethanol, yield 75 %, m.p. 137–140 °C. ^{1}H-NMR (500 MHz, DMSO-d$_6$) δ 2.01 (s,3H, CH_3); 3.79 (s, 3H, OCH_3); 4.72 (s, 1H, NH exchangeable with D_2O); 5.82–5.86 (d, 2H, CH_2, $J = 8.4$ Hz); 6.05 (s, 1H, NH exchangeable with D_2O); 7.67–7.90 (m, 7H, aromatic); 8.70–8.76(2d, 2H, CH=CH aliphatic, $J = 12.6$ Hz); 10.58 (s, 1H, OH exchangeable with D_2O). IR (KBr, ν/cm^{-1}): ν (OH) 3567, ν (NH) 3257 br, ν (NH) 3120 s, ν (C=O triazine ring) 1672 s, ν (C=O chalcone) 1655 s, ν (C=N triazine ring) 1563 s, ν (C=S) 1239 s. Anal. Calc. for $C_{21}H_{20}N_4O_4S$: C, 59.42; H, 4.75; N, 13.20; S, 7.55. Found: C, 59.76; H, 4.49; N, 13.01.; S, 7.84.

Table 2 In vivo acute toxicity of prepared compounds against HEpG2 cells of liver cancer

Compound	Tumor growth Vt/Vo			
	16	20	24	28
Doxorubicin	3.66	4.38	5.07	7.12
1	1.56	1.88	2.34	2.88
5	1.45	1.76	2.21	2.74
6	1.37	1.65	2.10	2.65
8	1.31	1.54	2.00	2.48
10	1.27	1.43	1.89	2.33
12	1.25	1.39	1.77	2.21
14	1.18	1.34	1.63	1.88

Table 3 Evaluated compounds **1**, **5**, **6**, **8**, **10**, **12** and **14** in an MCF-7 mouse xenograft model of breast cancer

Compound	Tumor growth Vt/Vo for compounds at time in days										
	0	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**
1	1.00	1.38	1.81	4.75	9.88	12.64	24.75	27.66	29.00	38.90	40.21
5	1.00	1.22	1.45	1.67	1.89	1.98	2.34	2.56	2.89	3.11	4.84
6	1.00	1.19	1.41	1.59	1.81	1.90	2.23	2.48	2.76	3.08	4.45
8	1.00	1.15	1.38	1.55	1.75	1.80	2.18	2.37	2.61	3.00	4.31
10	1.00	1.14	1.24	1.49	1.69	1.70	2.08	2.26	2.49	2.89	4.12
12	1.00	1.12	1.18	1.35	1.56	1.60	2.01	2.14	2.34	2.78	4.09
14	1.00	1.09	1.15	1.29	1.48	1.50	1.88	2.05	2.22	2.66	4.00

Table 4 Antimicrobial activity of compounds (**1**, **5**, **6**, **8**, **10**, **12**, **14**)

Compound	Gram-positive bacteria		Gram-negative bacteria		Fungi					
	B. subtilis ATCC6633	*S. aureus* ATCC29213	*E. coli*ATCC 25922	*P. aeruginosa* ATCC27953	*C. albicans* ATCC 10321	*A. niger* NRRL-363	*F. solani* NRC15	*F. oxysporium* NRC23	*A. alternata* NRC43	*A. tenuissima* KM651985
1	7	8	9	8	8	N.A.	8	10	7	N.A.
5	13	15	14	11	13	15	17	20	30	15
6	N.A.	N.A.	8	8	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
8	N.A.	N.A.	7	9	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
10	N.A.	N.A.	8	9	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
12	N.A.	N.A.	9	7	N.A.	N.A.	7	N.A.	N.A.	N.A.
14	N.A.	N.A.	9	7	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Thiophenicol	13	12	10	11	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Triflucan	N.A.	N.A.	N.A.	N.A.	12	9	11	13	10	11

Antimicrobial activity expressed as inhibition diameter zones in millimeters (mm) of the tested compounds against the pathological strains based on the agar diffusion technique

N.A. No activity

Table 5 Minimal inhibitory concentration (μg/disk) of compound **5** against the pathological strains

Compound	Gram-positive bacteria		Gram-negative bacteria		Fungi					
	B. subtilis ATCC6633	*S. aureus* ATCC29213	*E. coli*ATCC 25922	*P. aeruginosa* ATCC27953	*C. albicans* ATCC 10321	*A. niger* NRRL-363	*F. solani* NRC15	*F. oxysporium* NRC23	*A. alternata* NRC43	*A. tenuissima* KM651985
5	25	6.25	12.5	25	6.25	6.25	6.25	6.25	12.5	25
Thiophenicol	3.13	3.13	25	25	–	–	–	–	–	–
Triflucan	–	–	–	–	25	100	50	50	100	100

*Preparation of compounds **8–15***

General procedure A mixture contains compound **6** or **7** (10 mmol) with (10 mmol) each of (hydrazine hydrate, phenyl hydrazine, hydroxylamine hydrochloride or malononitrile in case of malononitrile, ammonium acetate is used) dissolve in 50 ml ethanol as solvent and a few drops of HCl. Then, the reaction mixture was heated under reflux and poured onto ice water, the resulting solid was collected by filtration and recrystallized from proper solvent.

*(E)-6-(-(4-(((5-Hydrazono-6-methyl-3-thioxo-4,5-dihydro-1,2,4-triazin-2(3H)-yl)methyl)amino)phenyl)-4,5-dihydro-1H-pyrazol-5-yl)-4,9-dimethoxy-5H-furo[3,2-g]chromen-5-one **8*** Brown powder was crystallized from ethanol, yield 77 %, m.p. >300 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 2.08 (s, 3H, CH_3); 2.56, 2.63 (dd, 2H, diastereotopic CH_2, $J = 2.4$ Hz); 3.02–3.30 (t, 1H, CH, $J = 12.5$ Hz); 3.93 (s, 3H, OCH_3); 4.04 (s, 1H, NH exchangeable with D_2O); 4.42 (s, 3H, OCH3); 5.38 (s, H, NH triazine ring exchangeable with D_2O); 5.97–6.01 (d, 2H, CH_2 methane proton, $J = 8.2$ Hz); 6.8(d, 1H, $J = 2.2$ Hz, C-2 furan); 7.21 (s, 2H, NH_2 pyrazoline ring exchangeable with D_2O); 7.41–7.69 (m, 5H, aromatic and 4-pyranone moiety); 8.3 (d, 1H, $J = 2.6$ Hz, C-2 furan); 8.48 (s, 1H, NH pyrazoline ring exchangeable with D_2O);. IR (KBr, ν/cm^{-1}): ν (NH_2) 3262, 3294 s, ν (NH) 3222 s, ν (C=O pyrane ring) 1640 s, ν (C=N ring) 1590 s, ν (C=S) 1296 s. Anal. Calc. for $C_{27}H_{26}N_8O_5S$: C, 56.44; H, 4.56; N, 19.50; S, 5. 58. Found: C, 56.66; H, 4.25; N, 19.27; S, 5.80.

*(E)-6-(5-Hydrazono-2-(((4-(5-(4-hydroxy-3-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)amino)methyl)-6-methyl-3,4-dihydro-1,2,4-triazine-3(2H)-thione **9*** Deep red powder was washed with DMF, yield 73 %, m.p. >300 °C. ^{1}H-NMR (270 MHz, DMSO-d6) δ 2.08 (s, 3H, CH_3); 2.51, 2.56 (dd, 2H, diastereotopic CH_2, $J = 2.4$ Hz); 2.85–3.12 (t, 1H, CH, $J = 12.5$ Hz); 3.84 (s, 1H, NH exchangeable with D_2O); 3.90–4.02 (m, 3H, OCH_3); 5.34 (s, H, NH triazine ring exchangeable with D_2O); 5.97–6.01 (d, 2H, CH_2 methane proton, $J = 8.4$ Hz); 7.01 (s, 2H, NH2 pyrazoline ring exchangeable with D_2O); 7.41–7.69 (m, 7H, aromatic); 8.41 (s, 1H, NH pyrazoline ring exchangeable with D_2O); 11.4 (s, 1H, OH exchangeable with D_2O). MS: m/z (M^- 451.55, 0.47 %). IR (KBr, ν/cm^{-1}): ν (OH) 3626 s, ν (NH2) 3257, 3289 s, ν (NH) 3120 s, ν (C=N rings) 1590 s, ν (C=S) 1296 s. Anal. Calc. for $C_{21}H_{28}N_8O_2S$: C, 55.24; H, 6.18; N, 24.54; S, 7. 02. Found: C, 55.56; H, 5.97; N, 24.15; S, 7.36.

*(E)-(((4-(5-(4,9-Dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)amino)methyl)-6-methyl-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2H)-one **10*** Brown powder was crystallized from ethanol, yield 75 %, m.p. 110–112 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 2. 03 (s, 3H, CH_3); 2.55, 2.63 (dd, 2H, diastereotopic CH_2, $J = 2.4$ Hz); 2.89–3.11 (t, 1H, CH, $J = 12.5$ Hz); 3.65 (s, 3H, OCH_3); 3.84 (s, 1H, NH exchangeable with D_2O); 3.92 (s, 3H, OCH_3); 5.24 (s, 1H, NH triazine ring exchangeable with D_2O); 5.55–5.63 (d, 2H, CH_2 methane proton, $J = 6.4$ Hz); 6.42 (d, 1H, $J = 2$ Hz, furan C-2); 7.07–7.78 (m, 10H aromatic); 8.94 (d, 1H, $J = 2$ Hz, furan C-2). IR (KBr, ν/cm^{-1}): ν (NH) 3235, ν (NH) 3056 s, ν (C=O triazine ring) 1656 s, ν (C=O pyrane ring) 1641 s ν (C=N rings) 1597 and 1494 s, ν (C=S) 1285 s. MS: m/z (M^- 637, 17.96 %). Anal. Calc. for $C_{33}H_{28}N_6O_6S$: C, 62.25; H, 4. 43; N, 13.20; S, 5.04. Found: C, 62.45; H, 4.19; N, 13.00; S, 529.

*2-(((4-(5-(4-Hydroxy-3-methoxyphenyl)-1phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)amino) methyl) 6-methyl–3-thioxo-3,4dihydro-1,2,4-triazin-5(2H)-one **11*** Yellowish powder was crystallized from ethanol, yield 70 %, m.p. 155–158 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 2. 05 (s, 3H, CH_3); 2.56–2.60 (dd, 2H, diastereotopic CH_2, $J = 2.6$ Hz); 2.71–3.03(t, 1H, CH, $J = 12.5$ Hz), 3.35 (s, 3H, OCH_3); 3.88 (s, 1H, NH exchangeable with D_2O); 5.25 (s, 1H, NH triazine ring exchangeable with D_2O); 5.95–6.13 (d,2H, CH_2 methane proton, $J = 6.00$ Hz); 7.17–7.71 (m, 12H aromatic); D_2O); 11.4 (s, 1H, OH exchangeable with D_2O). IR (KBr, ν/cm^{-1}): ν (OH) 3403 br, ν (NH) 3235, ν (NH) 3056 s, ν (C=O triazine ring) 1656 s, ν (C=N rings) 1597 and 1494 s, ν (C=S)

1285 s.MS: m/z (M $^{+}$ 513, 0, 17 %). Anal. Calc. for $C_{27}H_{28}N_6O_3S$: C, 62.77; H, 5. 46; N, 16.27; S, 6.21. Found: C, 62.89; H, 5.19; N, 16.10; S, 6.56.

2-(((4-(5-(4,9-Dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6-yl)-4,5-dihydroisoxazol-3-yl)phenyl)amino)methyl)-6-methyl-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2H)-one ***12*** Brown powder was crystallized from ethanol, yield 78 %, m.p. 160–162 °C. ^{1}H-NMR (270 MHz, DMSO-d_6) δ 2.05 (s, 3H, CH_3); 2.62,2.72 (dd, 2H, diastereotopic CH_2, $J = 4.2$ Hz); 3.07–3.29 (t, 1H, CH, $J = 12.5$ Hz); 3.47 (s, 3H, OCH_3); 3.80 (s, 1H, NH exchangeable with D_2O); 3.90 (s, 3H, OCH_3); 5.28 (s, 1H, NH triazine ring exchangeable with D_2O); 5.59–5.75 (d, 2H, CH_2 methane proton $J = 6.6$ Hz); 6.42 (d, 1H, $J = 2$ Hz, furan C-2); 7.07–7.78 (m, 5H aromatic); 8.94 (d, 1H, $J = 2$ Hz, furan C-2). IR (KBr, ν/cm^{-1}): ν (NH) 3220 s, ν (NH) 3106 s, ν (C=O triazine ring) 1656 s, (C=O pyrane ring) 1645 s, ν (C=N rings)· 1597 s and 1495 s, ν (C=S) 1226 s. Anal. Calc. for $C_{27}H_{23}N_5O_7S$: C, 57.75; H, 4.13; N, 12.47; S, 5.71. Found: C, 57.99; H, 4.01; N, 12.22; S, 5.82.

2-(((4-(5-(4-Hydroxy-3-methoxyphenyl)-4,5-(4-dihydroisoxazol-3-yl)phenyl)amin) methyl) 6-methyl-3-thioxo-3, 4dihydro-1,2,4-triazin-5(2H)-one ***13*** Brown powder was crystallized from ethanol, yield 69 %, m.p. 210–212 °C. ^{1}H-NMR (270 MHz, DMSO-d_6) δ 2.03 (s, 3H, CH_3); 2.54, 2.61 (dd, 2H, CH_2 diastereotopic CH_2, $J = 2.1$ Hz); 3.09–3.30 (t, 1H, CH, $J = 12.5$ Hz); 3.47 (s, 3H, OCH_3); 3.81 (s, 1H, NH exchangeable with D_2O); 5.23 (s, 1H, NH triazine ring exchangeable with D_2O); 5.38–5.52 (d, 2H, CH_2 methane proton, $J = 4.6$ Hz); 7.63–7.82 (m, 7H aromatic); 11.4 (s, 1H, OH exchangeable with D_2O). IR (KBr, ν/cm^{-1}): ν (OH) 3366 br., ν (NH) 3220 s, ν (NH) 3106 s, ν (C=O triazine ring) 1656 s, ν (C=N rings)· 1597 s and 1495 s, ν (C=S) 1226 s. MS: m/z (M$^+$ 513, 0.17 %). Anal. Calc. for $C_{21}H_{23}N_5O_4S$: C, 57.13; H, 5. 25; N, 15.86; S, 7.26. Found: C, 57.49; H, 5.05; N, 15.39; S, 7.46.

2-Amino-4-(4,9-dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6-yl)-6-(4-(((6-methyl-5-oxo-3-thioxo-4,5-dihydro-1,2,4-triazin-2(3H)-yl)methyl)amino)phenyl)nicotinonitrile ***14*** A brown powder was crystallized from ethanol, yield 72 %, m.p. 255–257 °C. ^{1}H-NMR (270 MHz, DMSO-d_6) δ 1.12–1.26 (s, 2H, NH_2 exchangeable with D_2O); 2.43 (s, 3H, CH_3); 3.43 (s, 3H, OCH_3); 3.92 (s, 3H, OCH_3); 4.92 (s, 1H, NH exchangeable with D_2O); 5.26 (s, 1H, NH triazine ring exchangeable with D_2O); 5.37–5.50 (d, 2H, CH_2 methane proton, $J = 6.4$ Hz); 5.99–6.20 (d, 1H, $J = 1$ Hz, C-5 pyridine); 6.80 (d, 1H, $J = 2$ Hz, C-2 furan); 7.23(d, 1H, $J = 2$ Hz, C-3 furan); 7.63–7.69 (m, 5H aromatic); IR (KBr, ν/cm^{-1}): ν (NH_2) 3325, 3290, ν (NH triazine ring) 3265, ν (NH olefinic) 3222, ν (C ≡ N) 2218 s, ν (C=O) 1672 s, ν (C=O pyrane ring) 1640 s, ν (C=N rings) 1563 and 1496 s, ν (C=S) 1245 s. Anal. Calc. for $C_{30}H_{23}N_7O_6S$: C, 59.11; H, 3.80; N, 16.08; S, 5.26. Found: C, 59.33; H, 3.62; N, 16.32; S, 5.48.

2-Amino-4(4-hydroxy-3-methoxyphenyl)-6-(4-(((6-methyl-5oxo-3-thioxo-3,4-dihydro-1,2,4-triazin-2(3H)-yl) methyl) amino)phenyl)nicotinonitrile ***15*** A brown powder was crystallized from toluene, yield 70 %, m.p. 165–168 °C. ^{1}H-NMR (270 MHz, DMSO-d_6) δ 1.04–1.08 (s, 2H, NH_2 exchangeable with D_2O); 2.04 (s, 3H, CH_3); 3.88 (s, 3H, OCH_3); 4.47 (s, 1H, NH exchangeable with D_2O); 5.26 (s, 1H, NH exchangeable with D_2O); 5.48–5.60 (d, 2H, CH_2 methane proton, $J = 8.00$ Hz); 7.63–7.69 (m, 7H aromatic); 10.72 (s, 1H, OH exchangeable with D_2O);. IR (KBr, ν/cm^{-1}): ν (OH) 3377 br, ν (NH_2) 3320, 3290, ν (NH triazine ring) 3262, ν (NH olefinic) 3210, ν (C ≡ N) 2221 s, ν (C=O) 1669 s, ν (C=N rings) 1596 and 1496 s, ν (C=S) 1288 s. MS: m/z (M$^+$468, 1.18 %). Anal. Calc. for $C_{24}H_{23}N_7O_3S$: C, 58.88; H, 4.74; N, 20.03; S, 6.55. Found: C, 58.99; H, 4.55; N, 19.83; S, 6.88.

Biological assays

Anticancer

The cytotoxicity of the newly synthesized compounds **1**, **5**, **6**, **8**, **10**, **12 and 14** against cancer cell lines in vitro was performed with the MTT assay according to the Mosimann's method. The MTT assay is based on the reduction of the soluble 3-(4,5-methyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) into a blue-purple formazan product, mainly by mitochondrial reductase activity inside living cells. The cells used in cytotoxicity assay were cultured in RPMI 1640 medium supplemented with 10 % fetal calf serum. Cells suspended in the medium (2 × 104/ml) were plated in 96-well culture plates and incubated at 37 · C in a 5 % CO_2 incubator. After 12 h, the test sample (2 µL) was added to the cells (2Ý 104) in 96-well plates and cultured at 37 · C for 3 days. The cultured cells were mixed with 20 µL of MTT solution and incubated for 4 h at 37 · C. The supernatant was carefully removed from each well, and 100 µL of DMSO was added to each well to dissolve the formazan crystals which were formed by the cellular reduction of MTT. After mixing with a mechanical plate mixer, the absorbance of each well was measured by a micro-plate reader using a test wavelength of 570 nm. The results were expressed as the IC50, which induces a 50 % inhibition of cell growth of treated cells when compared to the growth of control cells. Each experiment was performed at least three times. There was a good reproducibility between replicate wells with standard errors below 10 %.

In vivo and in Vitro Screening

Inhibition of Human leukemia K562 *(Histone Deacetylase)* (Finney 1962; Yoshida *et al.*, 1990).

Histone deacetylase fraction was prepared as described by Yoshida *et al.*, and human leukemia K562 (2.5×10^8) cells were disrupted in buffer A (15 mM potassium phosphate buffer, pH 7.5, containing 5 % glycerol and 0.2 mM EDTA, 15 ml). The nuclei were collected by centrifugation (35000 *g*, 10 min) and resuspended with buffer A (15 ml) containing 1 M $(NH_4)_2SO_4$. After sonication, the supernatant was collected by centrifugation, and ammonium sulfate was added to make the final concentration 3.5 M. After stirring for 1 h at 0 °C, the precipitate was collected by centrifugation, dissolved with buffer A (4 ml) and dialyzed against buffer A (2000 ml). The dialyzate was loaded onto a Mono Q HR5/5 column (Pharmacia) equilibrated with buffer A and eluted with a linear gradient of 0–1 M NaCl in buffer A (30 ml). A single peak of histone deacetylase activity was eluted around 0.4 M NaCl, and the fraction was stored at −80 °C until use. Inhibition histone deacetylase was estimated as described by Yoshida *et al.*, 1990 with slight modifications. 3H-labeled histone was prepared by the method of Yoshida *et al.*: 3 K562 cells (108 cells) were incubated in growth medium (25 ml) containing 0.5 mg/ml [3H] sodium acetate (152.8 GBq/mmol; NEN) and 5 mM sodium butyrate at 37 °C. Histone deacetylase inhibitory activity of test compound was measured as follows: the mixture (total volume 50 µl) containing the above histone deacetylase fraction (2 µl), 3H-labeled histone (100 µg/ml) and test compound (5 µl) was incubated for 10 min at 37 °C. [3H] Acetic acid, which was liberated from 3H-labeled histone, was extracted with ethyl acetate, and radioactivity was measured by a liquid scintillation counter.

In vivo cytotoxicity bioassay

Assay of in vivo acute toxicity

The animal study was approved by the Experimental Animal Committee of Faculty of Medicine, Chiang Mai University. All animal experiments met the animal welfare guidelines. Male BALB/c nude mice (6 weeks old) were purchased from the Institute of Experimental Animal, Mahidol University, Bangkok, Thailand. Mice were housed in laminar flow cabinets under specific pathogen-free conditions at room temperature with a 24-h night–day cycle and fed with pellets and water ad libitum. Log growth-phase HepG2 cells (1 × 105 cells in 0.1 ml PBS) were injected subcutaneously into the right flank of athymic nude mice ($n = 4$) to establish a model of tumor-bearing mice. On day 4 after implanting, tested compounds were injected subcutaneously (10 µg/50 µl) daily for four doses. Monoclonal antiplatelet antibody (PY-13) and sterile PBS were used as isotype and negative controls, respectively. Tumor growth was observed every 3 days by measuring its diameter with vernier calipers. Tumor weight (TW) was calculated by TW (g) = tumor volume (cm^3) = d2 × D/2, where d is the shortest and D is the longest diameter, respectively. Mice were killed when the tumor size reached 2.0 cm in diameter, and samples were collected.

Human breast cancer xenograft models and animal treatment

The animal protocol was approved by the Institutional Animal Use and Care Committee of the University of Alabama at Birmingham. Female athymic pathogen-free nude mice (nu/nu, 4–6 weeks) were purchased from Frederick Cancer Research and Development Center (Frederick, MD). To establish MCF-7 human breast cancer xenografts, each of the female nude mice was first implanted with a 60-day s.c. slow-release estrogen pellet (SE-121, 1.7 mg 17β-estradiol/pellet; Innovative Research of America, Sarasota, FL). The next day, cultured MCF-7 cells were harvested from confluent monolayer cultures, washed twice with serum-free medium, resuspended and injected subcutaneously (s.c.) (5×10^6 cells, total volume 0.2 ml) into the left inguinal area of the mice. All animals were monitored for activity, physical condition, body weight and tumor growth. Tumor size was determined by caliper measurement in two perpendicular diameters of the implant every other day. Tumor weight (in g) was calculated by the formula, $1/2a \times b^2$ where "*a*" is the long diameter and "*b*" is the short diameter (in cm). The animals bearing human cancer xenografts were randomly divided into various treatment groups and a control group (7–10 mice/group). The untreated control group received the vehicle only. For the MCF-7 xenograft model, tested compounds were dissolved in PEG400/ethanol/saline (57.1: 14.3: 28.6, v/v/v) and were administered by intraperitoneal (i.p.) injection at dose of 1 mg/kg/day, 3 day/week for 3 weeks.

Antimicrobial assay

In vitro Antimicrobial Screening

Antimicrobial activity of compounds **1**, **5**, **6**, **8**, **10**, **12** and **14** was screened in vitro against a panel of gram-positive and gram-negative bacterial pathogens and fungi, in comparison with control drugs thiophenicol (Thiamphenicol, Sanofi-Aventis, France) as an antibacterial agent and triflucan (Fluconazole, Egyptian International Pharmaceutical

Industries Company (EIPICO)) as an antifungal agent, by the agar diffusion technique (Domig *et al.*, 2007; Ajaiyeoba *et al.*, 2003). The compounds **1**, **5**, **6**, **8**, **10**, **12** and **14** were individually tested against gram-positive bacteria (*Bacillus subtilis* ATCC6633 and *Staphylococcus aureus* ATCC29213), gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC27953) and fungi (*Candida albicans* ATCC 10321, *Aspergillus niger* NRRL-363, *Fusarium solani* NRC15, *Fusarium oxysporium* NRC23, *Alternaria alternata* NRC43 and *Alternaria tenuissima* KM651985). All microorganisms used were obtained from the culture collection of the Department of Chemistry of Natural and Microbial Products, National Research Center, Cairo, Egypt. The microorganisms were passaged at least twice to ensure purity and viability. The compounds were mounted on a paper disk prepared from blotting paper (5 mm diameter) on a concentration of 100 μg/5μL DMSO/disk. Thiophenicol and triflucan were used as positive controls for antibacterial and antifungal activities in a concentration of 50 μg/disk, and DMSO showed no inhibition zone used as a negative control.

Agar plates were prepared by using 100 ml of suspension containing 1×10^8 CFU/ml of pathological tested bacteria and 1×10^6 CFU/ml of fungi spread on nutrient agar (NA) and potato dextrose agar (PDA), respectively. After the media had cooled and solidified, the disks were applied on the inoculated agar plates and incubated for 24 h at 30 °C for bacteria and 72 h at 28 °C for fungi. After incubation, antimicrobial activity was evaluated by measuring the zone of inhibition around the disk in millimeters (mm) and compared with that of the controls. The observed zones of inhibition against the test microorganisms are presented in Table 4.

Minimal inhibitory concentration (MIC) measurement

The active compounds [having inhibition zones (IZ) >10 mm] were then evaluated for its minimal inhibitory concentration (MIC). The final concentrations tested were: 50, 25, 12.5, 6.25 and 3.13 μg. The lowest concentration showing inhibition zone around the disk was taken as the minimum inhibitory concentration (MIC).

Acknowledgments The authors wish to express our thanks to the National Research Center for providing the facilities.

References

Abd-El-All AS, Labib AA, Mousa HA, Bassyouni FA, Hegab KH, El-Hashash M, Atta-Allah SR, AbdEl-Hady WH, Osman SAM (2013) Synthesis of Ag(I), Cu(II), La(III) complexes of some new Mannich bases incorporating 1, 2, 4-triazine moiety and studying their antihypertensive and diuretic activities. J Appl Sci Res 9(1):469–481

Abdel-Aziz MA, Hishmat OH, El-Naem ShI, Fawzy NM (1990) Reaction of 3-formylvisnagin and 3-formylkhellin with 2-thiohydantoin derivatives. Sulfur Lett 10(6):255–267

Abdel-Monem WR (2004) Synthesis and biological evaluation of some new fused heterobicyclic derivatives containing. Chem Pap 58:276–285

Ajaiyeoba EO, Onocha PA, Nwozo SO, Sama W (2003) Antimicrobial and cytotoxicity evaluation of Buchholzia coriacea stem bark. Fitoterapia 74(7–8):706–709. doi:10.1016/S0367-326X(03)00142-4

Bishnoi A, Singh S, Tiwari AK, Rani A, Jain S, Tripathi CKM (2014) Synthesis and antimicrobial activity of some new 1, 2, 4-triazine and benzimidazole derivatives. Ind J Chem 53B:325–331

Bondock S, Naser T, Ammar YA (2013) Synthesis of some new 2-(3-pyridyl)-4,5-disubstituted thiazoles as potent antimicrobial agents. Eur J Med Chem 62:270–279

Domig KJ, Mayrhofer S, ZitzU Mair C, Petersson A, Amtmann E, Mayer HK, Kneifel W (2007) Antibiotic susceptibility testing of Bifidobacterium thermophilum and Bifidobacterium pseudolongum strains. Int J Food Microbiol 120(1–2):191–195

Eidin F, Schünemann J (1983) Darstellung und Reaktionen von 6-Acylkhellin-Derivaten. Arch Pharm (Weinheim) 316:201–209

El-Barbary AA, Sakran MA, El-Madani AM, Nielsen C (2005) Synthesis, characterization and biological activity of some 1,2,4-triazine derivatives. J Heterocycl Chem 42(5):935–941

El-Barbary AA, El-Shehawy AA, Abdo NI (2014) Synthesis and antimicrobial activities of some 6-methyl-3-thioxo-2,3-dihydro-1,2,4-triazine derivatives. Phosphorus Sulfur Silicon Relat Elem 189(3):400–409

El-Gendy Z, Morsy JM, Allimony HA, Abdel-Monem WR, Abdel-Rahman RM (2003) Synthesis of heterobicyclic nitrogen systems bearing a 1,2,4-triazine moiety as anticancer drugs: part IV. Phosphorus Sulfur Silicon Relat Elem 178:2055–2071

Finney DJ (1962) Graded responses, chap 10. In: Probit analyses, 2nd edn., Cambridge University Press, Cambridge

Galal SA, Abd EL-All AS, Abdallah MM, EL-Diwani HI (2009) Synthesis of potent antitumor and antiviral benzofuran derivatives. Bioorg Med Chem Lett 19:2420–2428

Hayakawa I, Shioya R, Agatsuma T, Sugano Y (2005) Synthesis and evaluation of 3-methyl-4-oxo-6-phenyl-4, 5, 6,7-tetrahydrobenzofuran-2-carboxylic acid ethyl ester derivatives as potent antitumor agents. Chem Pharm Bull 53(6):638–640

Hegde JC, Girisha KS, Adhikari A, Kalluraya B (2008) Synthesis and antimicrobial activities of a new series of 4-S-[4(1)-amino-5(1)-oxo-6(1)-substituted benzyl-4(1),5(1)-dihydro-1(1),2(1),4(1)-triazin-3-yl]mercaptoacetyl-3-arylsydnones. Eur J Med Chem 43(12):2831–2834

Heilman WP, Heilman RD, Scozzie JA, Wayner RJ, Gullo JM, Riyan ZS (1979) Synthesis and antihypertensive activity of novel 3-hydrazino-5-phenyl-1,2,4-triazinesJ. Med Chem 22: 671–677

Hishmat OH, Abdel Rahman AH, El-Ebrashi NMA, EL-Diwani HI (1983) Synthesis and microbial activates of some new benzofuran derivatives. Indian J Chem Sec 22B:313–315

Hishmat OH, Fawzy NM, Farrg DS, Abd El-All AS, Abdel Rahman AH (1999) Reaction of formylvisnagin and formylkhellin with secondary amines, active nitriles and hydrazines. Boll Chim Farmaceutico Anno 138(8):427–431

Holla BS, Gonsalves R, Rao BS, Shenoy S, Gopalakrishna HN (2001) Synthesis of some new biologically active bis-(thiadiazolotriazines) and bis-(thiadiazolotriazinyl) alkanes. Farmaco 56(12): 899–903

Ibrahim MA, Abdel-Rahman MR, Abdel-Halim MA, Ibrahim SS, Allimony HA (2008) Synthesis and antifungal activity of novel polyheterocyclic compounds containing fused 1,2,4-triazine moiety. ARKIVOC xvi:202–215

Ismail MM (2001) Synthesis and enzymic effect of some novel l,2-dihydro-3-(triazin-5/6-yl)benzo[/i]quinolin-2-one derivatives. Chem Pap 55(4):242–250

Kim S, Salim AA, Swanson SM, Kinghorn AD (2006) Potential of cyclopenta[*b*]benzofurans from *Aglaia* species in cancer chemotherapy. Anti-Cancer Agents Med Chem 6(4):319–345

Kumar R, Sirohi TS, Singh H, Yadav R, Roy RK, Chaudhary A, Pandeya SN (2014) 1,2,4-triazine analogs as novel class of therapeutic agents. Mini Rev Med Chem 14(2):168–207

Magd-El-Din AA, Abd El-All AS, Yosef HA, Abdalla MM (2012) Synthesis of potent antitumor oxo quinazoline, pyrazole and thiazine derivatives. Aust J Basic Appl Sci 6(3):675–685

Makhlouf AA, Maklad YA (2004) Synthesis and analgesic-anti-inflammatory activities of some 1, 2, 4-triazine derivatives. Arzneimittelforschung 54(1):42–49

Makki MSI, Abdel-Rahman RM, Khan KA (2014) fluorine substituted 1,2,4-triazinones as potential anti-HIV-1 and CDK2 inhibitors. J Chem 430573:14

Mullick P, Khan SA, Begum T, Verma S, Kaushik D, Alam O (2009) Synthesis of 1, 2, 4-triazine derivatives a as potential anti-anxiety and anti-inflammatory agents. Acta Pol Pharm Drug Res 66:379–385

Nyffenegger C, Fournet G, Joseph B (2007) Synthesis of 3-amino-5 H-pyrrolo[2,3-e]-1,2,4-triazines by Sonogashira/copper(I)-catalyzed heteroannulation. Tetrahedron Lett 48(29):5069–5072

Paul R, Brockman JA, Hallett WA, Hanifin JW, Tarrant ME, Torley LW, Callahan FM, Fabio PF, Johnson BD (1985) Imidazo[1,5-d][1,2,4]triazines as potential antiasthma agents. J Med Chem 28:1704–1716

Ragab FA, Hussein MM, Hanna MM, Hassan GS, Kenawy SA (1993) Synthesis, anticonvulsant and antimicrobial activities of certain new furochromones. Pharmazie 48(11):808–811

Shawali AS, Gomha SM (2002) Regioselectivity in 1, 5-electrocyclization of N-[as-triazin-3-yl]nitrilimines. Synthesis of s-triazolo[4,3- b]-as-triazin-7(8H)-ones. Tetrahedron 58:8559–8564

Singh S, Husain K, Athar F, Azam A (2005) Synthesis and activity of 3,7-dimethyl-pyrazolo[3,4-e][1,2,4]triazin-4-yl thiosemicarbazide derivatives. Eur J Pharm Sci 25(2–3):255–262

Sztanke K, Fidecka S, Kedzierska E, Karczmarzyk Z, Pihlaja K, Matosiuk D (2005) Antinociceptive activity of new imidazolidine carbonyl derivatives. Part 4. Synthesis and pharmacological activity of 8-aryl-3,4-dioxo-2H,8H-6,7-dihydroimidazo[2,1-c] [1,2,4]triazines. Eurp J Med Chem 40(2):127–134

Yoshida M, Kijima M, Akita M, Beppu T (1990) Potent and specific inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A. J Biol Chem 265:17174–17179