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Synthesis of quinone-based heterocycles of broad-spectrum anticancer activity

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Abstract

A synthesis of benzo[e][1,2,4]triazines and 1,2,4-triazolospiro[4,5]deca-2,6,9-trien-8-ones has been developed from reactions of amidrazones with 2-chloro-1,4-benzoquinone in EtOAc containing 0.5 mL of piperidine. This highly regioselective and one-pot process provided rapid access to 1,2,4-triazolospiro[4,5]deca-2,6,9-trien-8-ones (60%–70%) and benzo[e][1,2,4]triazines (11%–18%). On reacting amidrazones with 5-hydroxy-1,4-naphthoquinone in an EtOAc/piperidine mixture, the reaction proceeded to give 5-hydroxy-2-(piperidin-1-yl)naphthalene-1,4-dione. The structures of the isolated products were proved by infrared, NMR (2D-NMR), mass spectra, and elemental analyses in addition to X-ray structure analysis. The reaction mechanisms are discussed. The anticancer screening of selected compounds showed broad-spectrum anticancer activity against most melanoma cancer cell lines, ovarian cancer OVCAR-3, central nervous system cancer SF-295 and U251, non-small cell lung cancer NCI-H23, renal cancer SN12C, and colon cancer HCT-15 and HCT-116. The selected compounds exhibited moderate to weak anticancer activity to other cell lines.

Keywords

1,2,4-triazolospiro[4,5]deca-2,6,9-trien-8-ones, 2-chloro-1,4-benzoquinone, 5-hydroxy-1,4-naphthoquinone, 5-hydroxy-2-(piperidin-1-yl)naphthalene-1,4-dione, amidrazones, benzo[e][1,2,4]triazines

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Introduction

Amidrazones display fungistatic, bacteriostatic, and antimycotic activity.[1] In addition, they have herbicidal[1] and lipoxygenase-1 inhibitory activity.[2] Aly et al.[3] reported that amidrazones reacted with 1,4-benzoquinone or 1,4-naphthoquinone to give, in a few minutes, benzo- and naphtho-1,2,4-triazin-6(4*H*)-ones. The reactions of amidrazones with 2,3,5,6-tetrachloro-1,4-benzoquinone and 2,3-dichloro-1,4-naphthoquinone in dry DMF proceed to give indazole derivatives.[3] The synthesis of various 4-aryl-5-imino-3-phenyl-1*H*-naphtho[2,3-*f*]-1,2,4-triazepine-6,11-diones has also been reported[4] by the reaction between amidrazones and 1,4-dioxo-1,4-dihydronaphthalene-2,3-dicarbonitrile, while 1,4-diphenyl-2-arylamino-2-{[1-phenylmeth-(*Z*)-ylidene]-hydrazino}butane-1,4-diones were obtained from the reaction of amidrazones with 1,4-diphenyl-2-butyne-1,4-dione.[5]

Quinones are widely distributed in the natural world,[6] being found in bacteria, plants, and arthropods and are ubiquitous to living systems. Quinones play pivotal roles in biological functions including oxidative phosphorylation and electron transfer.[7] They also have important roles as electron transfer agents in primary metabolic processes like photosynthesis and respiration which is vital to human life.[7] A large number of chemicals with 1,4-benzoquinone as the basic subunit exhibit prominent pharmacological applications such as antibiotics,[8,9] antitumor,[10–14] antimalarial[13] and anticoagulant compounds.[15] Juglone itself shows

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effective anticancer activity.[16,17] However, 2-chlorocyclohexa-2,5-diene-1,4-dione (CBQ) and 2-chloro-1,4-benzoquinone are common metabolites of polycyclic aromatic hydrocarbons generated through industrial processes. Chlorobenzoquinones have remarkable effects on DNA, and a few studies are available regarding chlorobenzoquinone-induced protein modifications.[18–20] Drugs containing quinone moieties are represented by anthracyclines, anthraquinones, mitomycin C, and streptonigrin.[20] Doxorubicin has been used clinically to treat solid tumors[21] and acute lymphoblastic and myeloblastic leukemia.[22–24] Anti-neoplastic agents such as actinomycin and streptonigrin as well as antibiotics such as mitamycin[25] (Figure 1) and rifamycin[26] have aminoquinone moieties in their structures. 1,4-Dioxo-3-(phenylamino)-1,4-dihydronaphthalene-2-carboxylic acid (Figure 1) shows potent cytostatic effects against both renal and melanoma cancer cell lines. Against renal cell lines, the activity (GI_{50} = 8.38 µM) was nearly as good as that of the anticancer agent, etoposide (GI_{50} = 7.19 µM).

A series of naphthoquinone derivatives showed proteasome inhibitor activity against PI-083 (Figure 1).[27–30] In addition, they also exhibited high selectivity (twofold to fourfold more selective) for cancer cells over normal cells (L-929, IC_{50} = 2.85 µM). The activity of 3-(4-(1-hydroxycyclohexyl)-1*H*-1,2,3-triazol-1-yl)-2,2-dimethyl-2,3-dihydronaphtho[1,2-*b*]furan-4,5-dione against melanoma cells was comparable to that of the initial quinones, nor-ß-lapachone and β-lapachone, and better than that of doxorubicin[31] (Figure 1).

Moreover, 3-amino-1,2,4-benzotriazine-1,4-dioxides have received considerable attention as a class of antitumor agent.[32] Triazoles can easily bind with a variety of enzymes and receptors in biological systems via diverse non-covalent interactions.[33] Therefore, many triazole derivatives serve as medicinal drugs.[34] More specifically, compounds having the 1,2,4-triazole moiety have shown various biological activities, such as antifungal,[35] antimicrobial,[36] antitubercular,[37] anticancer,[38] anticonvulsant,[39] hypoglycemic,[40] anti-inflammatory, and analgesic activities.[41] In this paper, we report a new straightforward reaction of amidrazones with electron-deficient naturally occurring quinones, and the new compounds with a quinone nucleus were screened for their anticancer activity.

Results and discussion

Equimolar amounts of an amidrazone **1a–d** and 2-chloro-1,4-benzoquinone (**2**) were allowed to react in dry EtOAc containing piperidine (0.5 mL) and, after chromatographic separation and recrystallization, the products **3a–d** (60%–70%) and **4a–d** (11%–18%) were isolated (Scheme 1). The reactions proceeded in low yields in the absence of piperidine as shown in Table 1 (Methods **a** and **b**). Method **a** was established using EtOAc, piperidine, reflux for 10–14 h, whereas Method **b** was carried out using refluxing in EtOAc for 20–24 h. We chose amidrazones **1a–d** having aryl groups with either electron-donating or electron-withdrawing substituents on the benzene ring, in order to examine the substituent effect on the reaction. Elemental analyses, infrared (IR), NMR (^{1}H and ^{13}C), and mass spectra were in good agreement with the structures assigned to the products. For example, compound **3b** was identified as 6-chloro-4-(4′-chlorophenyl)-3-phenyl-1,2,4-triazaspiro[4.5]deca-2,6,9-trien-8-one. Mass spectroscopy and elemental analysis indicated the molecular formula of **3b** as $C_{19}H_{13}Cl_2N_3O$, which corresponds to the sum of the molecular weights of the two starting materials with loss of a water molecule. The IR spectrum showed absorptions at υ = 3262 (NH), 1701 (C=O), 1613 (C=N), and 1548 cm^{-1} (C=C). The ^{1}H NMR spectrum revealed a singlet at δ_H = 8.82. ^{13}C NMR confirmed the structure of

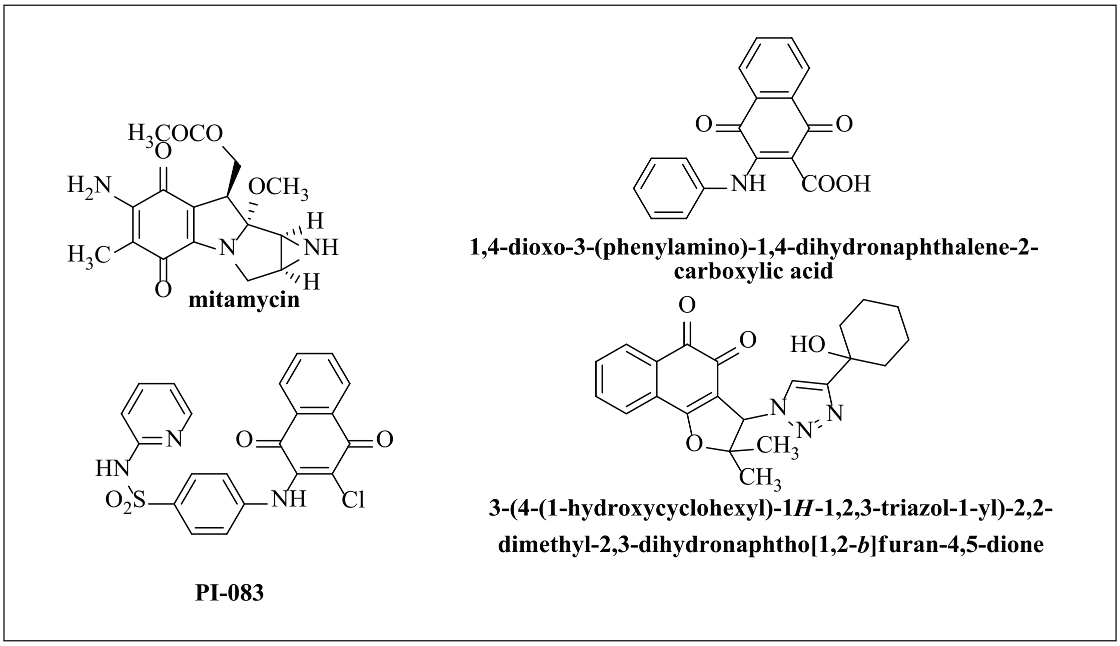
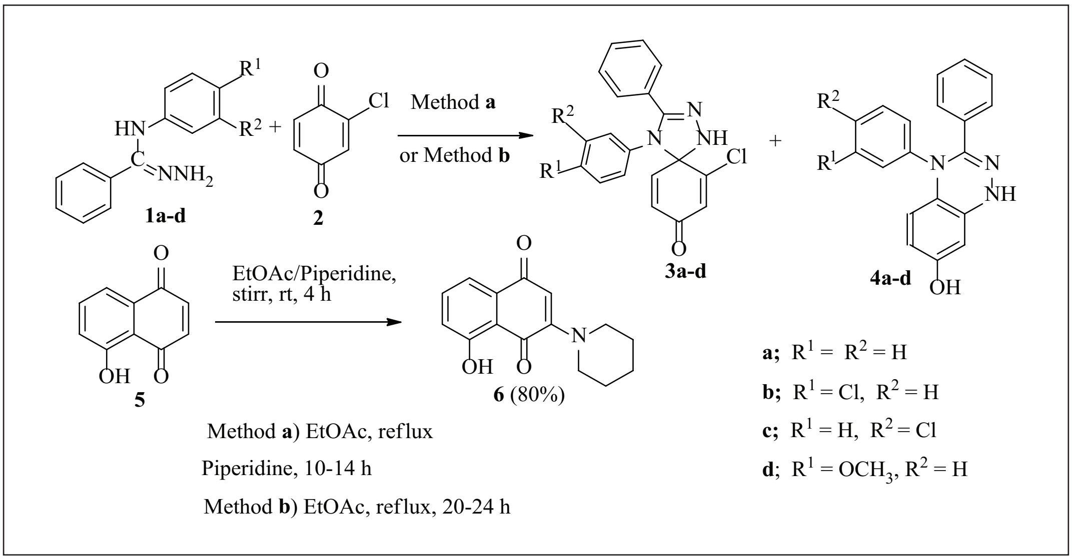


Figure 1. Representative quinone-based anticancer agents.

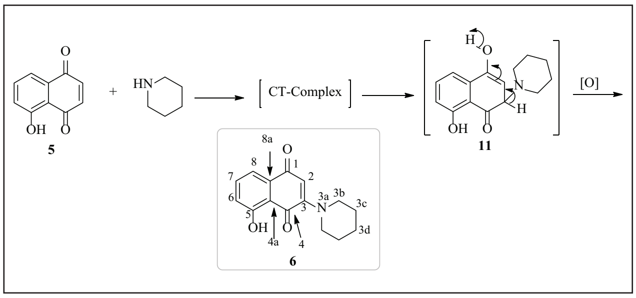


Scheme 1. Reaction between amidrazones **1a–d** with 2-chloro-1,4-benzoquinone (**2**) and synthesis of compound **6**.

Table 1. Methods and corresponding yields of compounds **3a–d** and **4a–d**.

Compound	Yield (%) by Method **a**	Yield (%) by Method **b**
3a/4a	65/15	35/5
3b/4b	60/13	34/2
3c/4c	61/11	35/4
3d/4d	67/18	43/6

Method **a**: EtOAc, piperidine, reflux for 10–14 h; Method **b**: EtOAc, reflux, 20–24 h.



Scheme 2. Plausible mechanism showing the formation of compound **6**.

3b by the appearance of the carbonyl, C=N, and spiro-carbon signals at δ_C 169.2, 161.0, and 78.12 ppm, respectively.

Elemental analysis and mass spectroscopy of compound **4b** showed an elimination of an HCl molecule from the two starting materials. The IR spectrum of **4b** showed the OH absorption at $\upsilon = 3411$, with the NH at $3270\,cm^{-1}$. The ^1H NMR spectrum had two singlets at $\delta_H = 10.05$ (OH) and 7.65 ppm (NH). The aromatic protons resonated as a multiplet at $\delta_H = 7.55–7.40$ ppm. The ^{13}C NMR spectrum showed the Ar–C–OH and C=N carbons at $\delta_C = 156.6$ and 153.5 ppm, respectively (see the experimental). The aforementioned spectroscopic data were in accordance with the absence of the two carbonyl groups with one being converted to a phenolic carbon.

The regioselectivity of the formation of compounds **3a–d** was consistent with the literature previously reported by Aly et al.[4] which described the reactions between amidrazones and 1,4-benzoquinone and/or 1,4-naphthoquinone. Benzoquinone **5** reacted with piperidine to afford initially a charge-transfer (CT) complex (Scheme 2) which gave intermediate **11**. Intermediate **11** was oxidized under the reaction conditions to give **6** (Scheme 2).

The structure of **6** was proved by NMR spectroscopy and has been previously reported.[42,43] Detailed NMR spectra of compound **6** are shown in Table 2. The X-ray structure of **6** is shown in Figure 2.

Biological evaluation

Screening of the in vitro anticancer activity

Among the synthesized compounds, NCI selected compounds **3c** and **6**. The anticancer activity of **3c** and **6** was evaluated according to the protocol of the drug evaluation branch of the National Cancer Institute (NCI), Bethesda, USA, for in vitro anticancer activity (http://www.dtp.nci.nih.gov). The results for each tested compound are reported as the percentage of growth inhibition of the treated cells when compared to the untreated control cells. The compounds were tested against a panel of 60 cancer cell lines, derived from different tumors, including leukemia, melanoma, lung, colon, central nervous system (CNS), ovarian, renal, prostate, and breast cancer. The compounds were incubated with the cells at a concentration of 10 µM for 48 h. The results, in Table 3, were reported for the growth inhibition percent (GI%). Surprisingly, **3c** and **6** exhibited broad-spectrum potent inhibitory effects on most of the Melanoma cell lines especially MDA-MB-435, SK-MEL-5, UASS-62, LOX IMVI, MALME-3M, M14, and UACC-257. In addition, both compounds revealed strong anticancer activity against ovarian cancer cell lines OVCAR-3, CNS cancer cell lines SF-295, SF-539 and U251, non-small cell lung cancer cell lines HOP-62, A549/ATCC and NCI-H23, renal cancer cell lines SN12C and

Table 2. Detailed NMR spectral data of compound **6**.

^{1}H NMR (DMSO-d_6)	^{1}H–^{1}H COSY		Assignment
11.44 (bs; 1H)			OH
7.67 ("t," J = 7.7; 1H)	7.42, 7.21		H-7
7.42 (d, J = 7.4; 1H)	7.67, 7.21		H-8
7.21 (d, J = 8.4; 1H)	7.67, 7.42		H-6
5.97 (s; 1H)	3.46		H-2
3.46 (m; 4H)	5.97, 1.64		H-3b
1.64 (m; 6H)	3.46		H-3c, 3d
^{13}C NMR (DMSO-d_6)	HSQC	HMBC	Assignment
186.73		7.21, 5.97	C-4
181.54		7.42, 5.97	C-1
160.06		11.44, 7.67, 7.42, 7.21	C-5
153.82		5.97, 3.46	C-3
136.48	7.67	7.42	C-7
132.61		7.67, 5.97	C-8a
122.26	7.42	11.44, 7.67, 7.42	C-8
116.97	7.21	11.44, 7.67, 7.42, 7.21	C-6
116.14			C-4a
109.98	5.97		C-2
50.11	3.46	3.46, 1.64	C-3b
25.33	1.64	3.46, 1.64	C-3c
23.70	1.64		C-3d
^{15}N NMR	HSQC	HMBC	Assignment
94.3		5.97, 1.64	N-3a

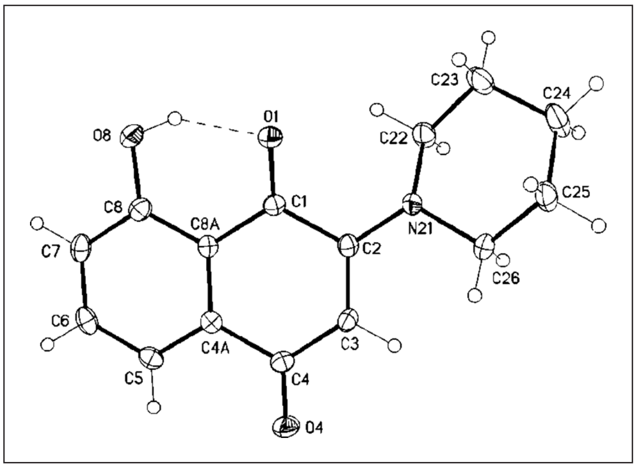


Figure 2. Molecular structure of compound **6** (displacement parameters are drawn at 50% probability level).

UO31, leukemia cell lines HL-60 and K-562, and colon cancer cell lines HCT-15 and HCT-116. Moreover, compounds **3c** and **6** exhibited moderate to weak anticancer activity to other cancer cell lines (Table 2).

In vitro five-dose full NCI 60 cell panel assay

Compounds **6** were selected for five-dose testing against the full panel of 60 human tumor cell lines according to the NCI protocol (http://www.dtp.nci.nih.gov). Compound **6** exhibited noteworthy antiproliferative activity against melanoma cell lines with GI_{50} = 1.38 µM (Table 4) and selectivity ratio = 2.10, against CNS cancer cell lines with GI_{50} = 1.406 and selectivity ratio = 2.07 and against colon cancer cell lines with GI_{50} = 2.06 and selectivity ratio = 1.41 (Figure 3).

Conclusion

Herein, we report reactions of amidrazones with two naturally occurring quinones, namely, 2-chloro-1,4-benzoquinone and 5-hydroxy-1,4-napthalene-1,4-dione (Juglone). Spiro-triazoles and 1,2,4-triazines were obtained from the reactions of amidrazones with the aforementioned quinones. The screening results were encouraging and promising, but further studies of their anticancer activities should be continued.

Experimental

Melting points were determined using open glass capillaries on a Gallenkamp melting point apparatus (Weiss Gallenkamp, Loughborough, UK), and they are uncorrected. The IR spectra were recorded from potassium bromide disks with an FT device, and Minia University NMR spectra were measured on a Bruker AV-400 spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C, and 40.55 MHz for ^{15}N); chemical shifts are expressed in δ (ppm) versus internal tetramethylsilane (TMS) = 0 for ^{1}H and ^{13}C, and external liquid ammonia = 0 for ^{15}N. Coupling constants are stated in Hz. Correlations were established using ^{1}H–^{1}H COSY, and ^{1}H–^{13}C and ^{1}H–^{15}N HSQC and

Table 3. Anticancer activity of compounds **3c** and **6** against 60 cell lines.

Subpanel tumor cell lines	Growth inhibition % (GI%)	
	3c NSC 800747	6 NSC 800746
Leukemia		
CCRF-CEM	16.88	23.47
HL-60(TB)	76.55	70.24
K-562	74.46	71.44
MOLT-4	38.15	28.33
RPMI-8226	35.95	34.49
SR	63.21	61.47
Non-small cell lung cancer		
A549/ATCC	77.69	85.16
EKVX	24.88	42.15
HOP-62	73.49	78.53
HOP-92	0	0
NCI-H226	39.48	48.09
NCI-H23	86.35	90.35
NCI-H322M	10.48	16.10
NCI-H460	35.72	65.89
NCI-H522	48.91	71.31
Colon cancer		
COLO 205	5.55	19.18
HCC-2998	35.59	45.76
HCT-116	73.88	81.88
HCT-15	81.29	83.20
HT29	27.03	55.65
KM12	41.44	52.73
SW-620	47.49	65.15
CNS cancer		
SF-268	42.74	50.61
SF-295	72.19	82.88
SF-539	73.42	74.57
SNB-19	65.17	67.98
SNB-75	66.93	80.11
U251	81.91	88.84
Melanoma		
LOX IMVI	97.91	105.86
MALME-3M	61.23	88.35
M14	71.15	91.19
MDA-MB-435	191.54	192.36
SK-MEL-28	47.35	61.80
SK-MEL-5	170.21	191.49
UACC-257	94.15	96.88
UACC-62	133.07	150.40
Ovarian cancer		
IGROV1	33.27	46.17
OVCAR-3	86.48	88.75
OVCAR-4	56.72	59.20
OVCAR-5	52.37	60.12
OVCAR-8	56.63	37.81
NCI/ADR-RES	54.95	57.77
SK-OV-3	0	8.76
Renal cancer		
786-0	6.56	0
A498	8.66	0
ACHN	38.89	44.79
CAKI-1	15.97	18.44
RXF 393	0	0
SN12C	73.61	92.77
TK-10	0	8.43
UO-31	71.83	77.93
Prostate cancer		
PC-3	60.86	63.96
DU-145	48.07	64.34
Breast cancer		
MCF7	54.57	64.58
MDA-MB-231/ATCC	22.63	24.36
BT-549	66.49	58.90
T-47D	12.85	21.23
MDA-MB-468	52.44	63.77

CNS: central nervous system.

HMBC experiments. Mass spectra were recorded on a Finnigan Fab 70 eV, Institute of Organic Chemistry, Karlsruhe University, Karlsruhe, Germany. TLC was performed on analytical Merck 9385 silica aluminum sheets (Kieselgel 60) with Pf_{254} indicator; TLCs were viewed at λ_{max} = 254 nm. Elemental analyses were carried out at National Research Center, Al Doki, Egypt.

Starting materials

Amidrazones **1a–d** were prepared according to reported method by Müller et al.[44]

*Reactions of amidrazones **1a–d** with 2-chloro-1,4-benzoquinone (**2**)*

Equimolar mixtures of amidrazones **1a–d** (1 mmol) and **2** (0.142 g, 1 mmol) in 50 mL of absolute EtOAc and 0.5 mL of piperidine were refluxed for 10–14 h. The reactions were followed by the TLC analysis. The mixtures were then concentrated under reduced pressure, and the resulting solids were separated by preparative PC using toluene: ethyl acetate (2:1). The faster migrating zones gave compounds **4a–d**, followed by products **3a–d**. The products were then recrystallized from the appropriate solvents.

6-Chloro-3,4-diphenyl-1,2,4-triazaspiro[4.5]deca-2,6,9-trien-8-one (**3a**). Red solid (EtOH), yield: 0.218 g (65%), m.p. 115–117 °C. IR (KBr): ν = 3260 (NH), 3136 (Ar–CH), 1702 (C=O), 1614 (C=N), 1599 cm^{-1} (Ar–C=C). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 8.83 (s, 1H, NH), 8.20-8.15 (m, 2H), 7.80-7.75 (m, 2H, Ar–CH), 7.60-6.72 (m, 8H, Ar–CH), 6.60 (d, 1H, *J* = 0.7 Hz, Ar–CH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 169.0 (C=O), 163.0 (C=N), 141.8, 134.0, 130.6 (Ar–C), 130.3, 130.0 (Ar–CH), 129.7, 129.4, 127.9, 126.4 (Ar–2CH), 126.3, 125.5, 122.6 (Ar–CH), 78.1 (Spiro-C). MS: *m/z* (%) = 337 (6), 335.8 (27), 256.0 (42), 225.3 (47), 185 (30), 183.6 (100), 154.0 (58), 149.2 (47), 114.1 (15), 81.0 (39), 77.1 (93). Anal. calcd

Table 4. Results of in vitro five-dose testing of nine human cancer types and selectivity for compound **6**.

Panel	Cell line	GI_{50}			TGI	LC_{50}
		Concentration per cell line	MID[b]	Selectivity ratio		
Leukemia	CCRF-CEM	4.83	4.06	0.715	>100	>100
	HL-60(TB)	2.77			8.03	>100
	K-562	1.60			21.0	>100
	MOLT-4	6.81			>100	>100
	RPMI-8226	3.52			>100	>100
	SR	4.38			7.69	>100
Non-small cell lung cancer	A549/ATCC	1.84	4.08	0.712	5.00	>100
	EKVX	1.41			14.5	>100
	HOP-62	0.693			2.22	5.99
	HOP-92	22.8			54.2	>100
	NCI-H226	1.83			4.53	>100
	NCI-H23	0.46			2.18	8.82
	NCI-H322M	3.96			14.6	40.4
	NCI-H460	2.56			10.2	75.4
	NCI-H522	1.21			3.40	9.60
Colon cancer	COLO 205	1.89	2.06	1.41	3.83	7.76
	HCC-2998	3.83			13.0	57.3
	HCT-116	1.38			5.26	>100
	HCT-15	0.472			2.37	>100
	HT29	2.53			6.21	>100
	KM12	2.65			18.0	>100
	SW-620	1.67			3.64	7.92
CNS cancer	SF-268	1.84	1.406	2.07	14.3	>100
	SF-295	1.34			3.18	7.52
	SF-539	1.26			2.79	6.19
	SNB-19	1.59			10.8	43.5
	SNB-75	1.82			3.44	6.48
	U251	0.587			20.2	>100
Melanoma	LOX IMVI	0.275	1.38	2.10	1.28	5.75
	MALME-3M	1.28			2.58	5.20
	M14	1.30			2.60	5.22
	MDA-MB-435	0.185			0.336	0.609
	SK-MEL-2	4.60			21.5	>100
	SK-MEL-28	1.99			4.38	9.65
	SK-MEL-5	0.272			0.706	2.57
	UACC-257	1.47			2.93	5.84
	UACC-62	1.05			2.26	4.88
Ovarian cancer	IGROV1	3.19	3.225	0.90	17.1	>100
	OVCAR-3	1.08			3.70	18.6
	OVCAR-4	0.698			3.21	13.5
	OVCAR-5	1.79			3.54	>100
	OVCAR-8	2.40			>100	>100
	NCI/ADR-RES	1.62			30.9	>100
	SK-OV-3	11.8			98.0	>100
Renal cancer	786-0	6.13	4.528	0.641	43.0	>100
	A498	5.53			19.6	50.7
	ACHN	3.11			21.5	>100
	CAKI-1	5.71			50.3	>100
	RXF 393	7.36			25.0	74.0
	SN12C	1.47			2.84	5.48
	TK-10	5.46			46.0	>100
	UO-31	1.46			7.67	54.2
Prostate cancer	PC-3	3.19	2.23	1.30	>100	>100
	DU-145	1.27			2.56	5.15

(Continued)

Table 4. (Continued)

Panel	Cell line	GI$_{50}$			TGI	LC$_{50}$
		Concentration per cell line	MID[b]	Selectivity ratio		
Breast cancer	MCF7	1.50	2.443	1.19	3.83	9.74
	MDA-MB-231/ ATCC	2.37			33.9	>100
	HS 578T	2.86			7.63	>100
	BT-549	1.13			2.69	6.36
	T-47D	4.88			37.7	>100
	MDA-MB-468	1.92			9.09	>100

MID[a] = 2.905; selectivity ratio = MID[a]/MID[b].

for $C_{19}H_{14}ClN_3O$ (335.79): C, 67.96; H, 4.20; Cl, 10.56; N, 12.51; found: C, 68.05; H, 4.08; Cl, 10.70; N, 12.60.

6-Chloro-4-(4′-chlorophenyl)-3-phenyl-1,2,4-triazaspiro[4.5]deca-2,6,9-trien-8-one (**3b**). Red solid (EtOH), yield: 0.222 g (60%), m.p. 204–206 °C. IR (KBr): ν = 3262 (NH), 3135 (Ar–CH), 1701 (C=O), 1613 (C=N), 1548 cm^{-1} (Ar–C=C). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 8.82 (s, 1H, NH), 8.40-8.35 (m, 2H, Ar–CH), 8.25-8.22 (m, 2H, Ar–CH), 7.62-7.52 (m, 7H, Ar–CH), 7.42 (d, 1H, J = 0.8 Hz, Ar–CH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 169.2 (C=O), 161.0 (C=N), 141.17, 138.49, 132.48, 130.49 (Ar–C), 130.22 (Ar–CH), 129.57, 129.46, 129.12, 128.89 (Ar–2CH), 125.72, 125.65, 125.19 (Ar–CH), 78.12 (Spiro-C). MS: m/z (%) = 370.6 (63), 372.6 (42), 336.0 (14), 296.1 (18), 294.1 (59), 261.1 (6), 259.1 (26), 236.0 (36), 225.3 (47), 185.6 (29), 154.0 (59), 149.2 (47), 114.2 (4), 112.2 (14), 81.1 (39), 77.1 (93). Anal. calcd for $C_{19}H_{13}Cl_2N_3O$ (370.23): C, 61.64; H, 3.54; N, 11.35; Cl, 19.15; found: C, 61.52; H, 3.43; Cl, 19.03; N, 11.13.

6-Chloro-4-(3′-chlorophenyl)-3-phenyl-1,2,4-triazaspiro[4.5]deca-2,6,9-trien-8-one (**3c**). Red solid (CH_3OH), yield: 0226 g (61%), m.p. 174–176 °C. IR (KBr): ν = 3269 (NH), 3130 (Ar–CH), 1705 (C=O), 1616 (C=N), 1548 cm^{-1} (Ar–C=C). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 8.73 (s, 1H, NH), 8.35 (dd, 2H, J = 1.2, 0.8 Hz, Ar–CH), 8.13-7.95 (m, 2H, Ar–CH), 7.53-7.35 (m, 7H, Ar–CH), 7.33 (d, 1H, J = 0.7 Hz, Ar–CH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 169.7 (C=O), 161.8 (C=N), 141.14, 140.7, 135.8, 132.59 (Ar–C), 131.4 (Ar–CH), 131.17, 130.96, 130.45, 129.58 (Ar–2CH), 126.28, 125.22, 124.34 (Ar–CH), 78.23 (Spiro-C). MS: m/z (%) = 372.2 (16), 370.2 (53), 336.0 (15), 296.1 (20). 294.1 (62), 261.1 (9), 259.1 (27), 236.0 (37), 225.3 (47), 185.0 (100), 154.0 (60), 149.2 (47), 114.0 (7), 112.0 (26), 81.1 (39), 77.1 (70). Anal. calcd for $C_{19}H_{13}Cl_2N_3O$ (370.23): C, 61.64; H, 3.54; Cl, 19.15; N, 11.35; found: C, 61.72; H, 3.60; Cl, 19.25; N, 11.20.

6-Chloro-3-phenyl-4(4′-methoxyphenyl)-1,2,4-triazaspiro[4.5]deca-2,6,9-trien-8-one (**3d**). Red solid (CH_3CN) 245 mg (67%), m.p. 254–256 C. IR (KBr): ν = 3260 (NH), 3140 (Ar–CH), 1700 (C=O), 1610 (C=N), 1550 (C=C), 1110 cm^{-1} (OCH_3). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 8.90 (s, 1H, NH), 8.40 (dd, 2H, J = 1.2, 0.7 Hz, Ar–CH), 8.25-8.20 (m, 3H, Ar–CH), 7.52-7.45 (m, 5H, Ar–CH), 6.60 (dd, 2H, J = 0.8 Hz, Ar–CH), 3.90 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 169.2 (C=O), 161.0 (C=N), 150.1 (Ar–C–OCH_3), 138.2, 132.0, 130.3 (Ar–C), 129.2 (Ar–CH), 128.1, 127.5, 127.1, 126.8 (Ar–2CH), 125.7, 123.6, 122.2 (Ar–CH), 78.1 (Spiro-C), 57.6 (OCH_3). MS: m/z (%) = 367 (13), 365.0 (40), 2960 (20), 184.6 (19), 154.0 (60), 121.0 (100), 77.0 (60). Anal. calcd for $C_{20}H_{16}ClN_3O_2$ (365.81): C, 65.67; H, 4.41; N, 11.49; Cl, 9.69; found: C, 65.72; H, 4.43; N, 11.30; Cl, 9.60.

3,4-Diphenyl-1,4-dihydrobenzo[e][1,2,4]triazin-6-ol (**4a**). White solid (CH_3CN), yield: 0.045 g (15%), m.p. 302–304 °C. IR (KBr): ν = 3424 (OH), 3264 (NH), 1631 (C=N), 1590 cm^{-1} (Ar–C=C). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 10.12 (s, 1H, OH), 8.54 (s, 1H, NH), 7.48-7.13 (m, 13H, Ar–CH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 158.2 (Ar–C–OH), 154.0 (C=N), 132.0, 130.8, 130.0, 129.0 (Ar–C), 128.6, 128.4, 127.8, 127.4, 127.0 (Ar–2CH), 126.9, 126.5, 126.0 (Ar–CH). MS: m/z (%) = 301.34 (60), 225.25 (33), 156.26 (59), 133.15 (100), 77.05 (58). Anal. calcd for $C_{19}H_{15}N_3O$ (301.34): C, 75.73; H, 5.02; N, 13.94; found: C, 75.61; H, 5.11; N, 14.02.

4-(4′-Chlorophenyl)-3-phenyl-1,4-dihydrobenzo[e][1,2,4]triazin-6-ol (**4b**). White solid (CH_3CN), yield: 0.044 g (13%), m.p. 264–266 °C. IR (KBr): ν = 3411 (OH), 3270 (NH), 1629 (C=N), 1593 cm^{-1} (C=C). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 10.05 (s, 1H, OH), 8.70 (s, 1H, NH), 7.55-7.40 (m, 12H, Ar–CH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 156.6 (Ar–C–OH), 153.5 (C=N), 134.2, 133.7, 130.2, 129.7, 128.5 (Ar–C), 128.3, 128.1, 127.8, 127.2, 127.0 (Ar–2CH), 126.9, 126.8 (Ar–CH). MS: m/z (%) = 337.1 (15), 335.1 (45), 261.7 (19), 259.1 (61), 225.3 (100), 149.2 (58), 133.2 (33), 114.6 (19), 112.6 (53), 77.1 (92). Anal. calcd for $C_{19}H_{14}ClN_3O$ (335.79): C, 67.96; H, 4.20; Cl, 10.56; N, 12.51; found: C, 68.05; H, 4.08; Cl, 10.70; N, 12.77.

4-(3′-Chlorophenyl)-3-phenyl-1,4-dihydrobenzo[e][1,2,4]triazin-6-ol (**4c**). White solid (CH_3CN), yield: 0.037 g (11%), m.p. 262–264 °C. IR (KBr): ν = 3424 (OH), 3264 (NH), 1631 (C=N), 1584 cm^{-1} (C=C). ^{1}H NMR (400 MHz,

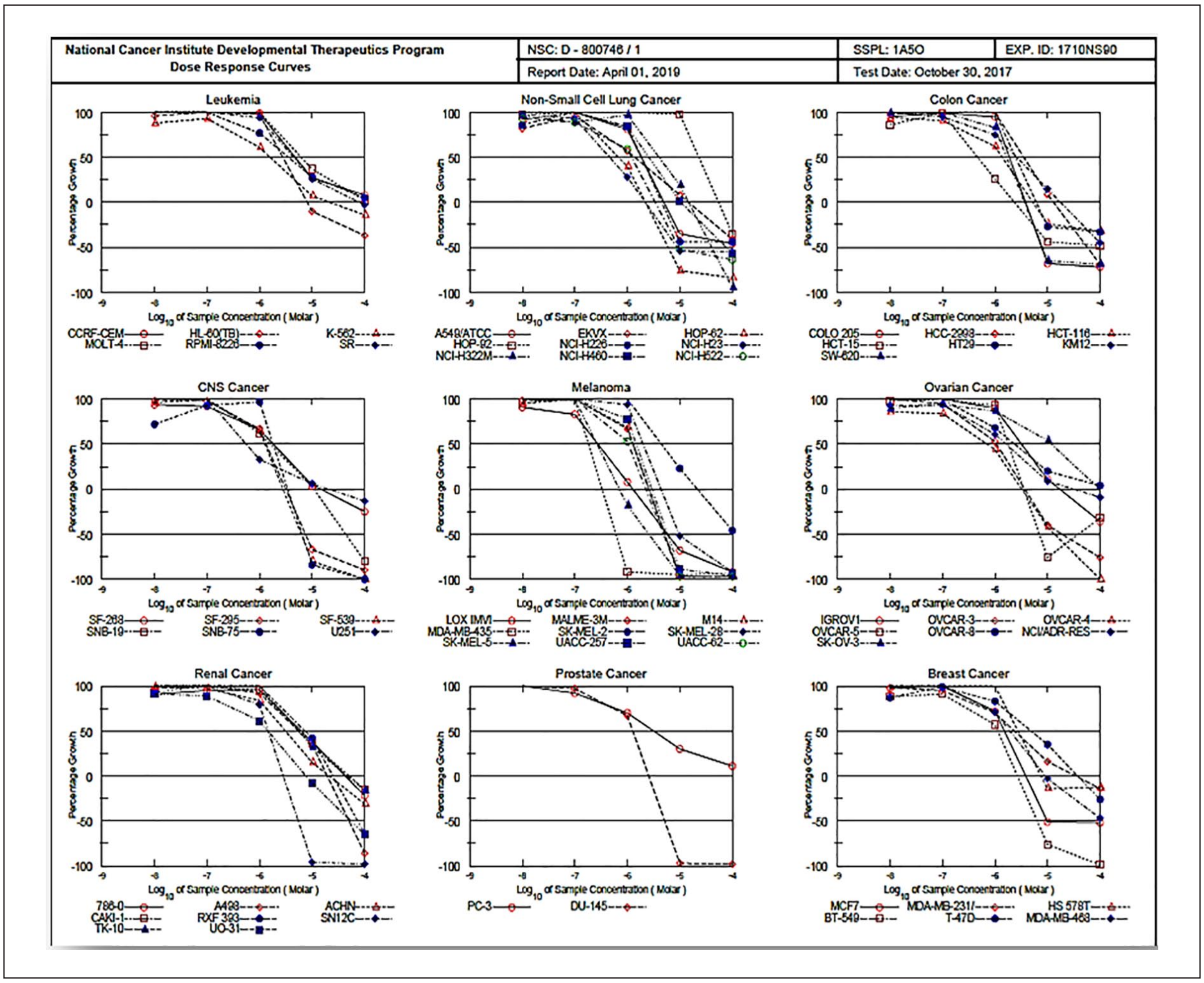


Figure 3. Five-dose diagram of compound **6**.

$CDCl_3$): $\delta_H = 10.22$ (s, 1H, OH), 7.94 (s, 1H, NH), 7.48-7.30 (m, 12H, Ar–CH). ^{13}C NMR (100 MHz, DMSO-d_6): $\delta_C = 158.2$ (Ar–C–OH), 154.2 (C=N), 134.3, 133.9, 132.1, 130.3, 129.8 (Ar–C), 128.7, 128.2, 127.9, 127.3, 127.1 (Ar–2CH), 126.8, 126.5 (Ar–CH). MS: *m/z* (%) = 337.1 (15), 335.1 (45), 261.7 (28), 259.1 (60), 225.3 (100), 190.0 (8), 149.2 (59), 133.2 (22), 114.6 (15), 112.6 (44), 77.1 (80). Anal. calcd for $C_{19}H_{14}ClN_3O$ (335.79): C, 67.96; H, 4.20; Cl, 10.56; N, 12.51; found: C, 68.05; H, Cl, 10.65; 4.08; N, 12.72.

4-(4′-Methoxyphenyl)-3-phenyl-1,4-dihydrobenzo[e][1,2,4]triazin-6-ol (**4d**). White solid (CH_3CN), yield: 0.060 g (18%), m.p. 210–212 °C. IR (KBr): ν = 3410 (OH), 3250 (NH), 1620 (C=N), 1590 cm^{-1} (C=C). ^1H NMR (400 MHz, DMSO-d_6): $\delta_H = 10.00$ (s, 1H, OH), 8.80 (s, 1H, NH), 7.60 (dd, 2H, *J* = 1.2, 0.7 Hz), 7.30-7.10 (m, 8H, Ar–CH), 6.60 (dd, 2H, *J* = 1.2, 0.7 Hz), 3.90 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6): $\delta_C = 157.8$ (Ar–C–OH), 152.0 (Ar–OCH_3), 151.2 (C=N), 135.0, 130.2, 129.4, 128.6 (Ar–C), 127.6, 126.4, 125.8, 125.2, 122.0 (Ar–2CH), 121.0, 120.8 (Ar–CH), 52.0 (OCH_3). MS: *m/z* (%) = 331.1 (30), 225.0 (100), 149.2 (40), 133.2 (30), 77.1 (90). Anal. calcd for $C_{20}H_{17}N_3O_2$ (331.37): C, 72.49; H, 5.17; N, 12.68; found: C, 73.00; H, 5.00; N, 12.82.

*Reaction of piperidine with 5-hydroxy-1,4-naphthaquinone (**5**)*

An equimolar mixture of **5** (0.161 g, 1 mmol) and piperidine (0.085 g, 1 mmol) in 100 mL of absolute EtOAc was stirred at room temperature for 4 h. Blue precipitate was formed, which by time (1 h) dissolved and a green precipitate of compound **6** was formed. The green precipitate was then dissolved in acetone and purified on applying to preparative PC using toluene: ethyl acetate (10:1). The formed product **6** was then recrystallized from EtOH.

*8-Hydroxy-2-(piperidin-1-yl)naphthalene-1,4-dione (**6**) [42 and 43].* Green solid (EtOH), yield: 0.206–0.219 g (80%–85%), m.p. 159–160 °C (rep. 158 °C). IR (KBr): ν = 3440 (OH), 1700 (C=O), 1590 cm^{-1} (C=C). NMR (see Table 2). MS: *m/z* (%) = 257 (M^+, 100). Anal. calcd for $C_{15}H_{15}NO_3$ (257.28): C, 70.02; H, 5.88; N, 5.44; found: C, 70.20; H, 5.98; N, 5.60.

*Crystal structure determinations of **6***

The single-crystal X-ray diffraction study was carried out on a Bruker D8 Venture diffractometer with Photon100 detector at 123(2) K using Mo-Kα radiation (λ = 0.71073 Å).

Dual space methods (SHELXT)[45] were used for structure solution, and refinement was carried out using SHELXL-2014 (full-matrix least squares on F^2).[46] Hydrogen atoms were localized by difference electron density determination and refined using a riding model (H(O) free). A semi-empirical absorption correction and an extinction correction were applied. **6**: red crystals, $C_{15}H_{15}NO_3$, $M_r = 257.28$, crystal size 0.236 mm × 0.24 mm × 0.16 mm, monoclinic, space group $P2_1/c$ (no. 14), $a = 10.1353(5)$ Å, $b = 12.0295(5)$ Å, $c = 11.1132(7)$ Å, $\beta = 116.045(2)°$, $V = 1217.35(11)$ Å^3, $Z = 4$, $\rho = 1.404$ Mg/m^{-3}, μ(Mo-Kα) = 0.10 mm^{-1}, $F(000) = 544$, $2\theta_{max} = 55.0°$, 32,736 reflections, of which 2810 were independent ($R_{int} = 0.031$), 176 parameters, 1 restraint, $R_1 = 0.038$ (for 2480 $I > 2\sigma(I)$), $wR_2 = 0.101$ (all data), $S = 1.03$, largest difference peak/hole = 0.37/−0.19 e Å^{-3}.

CCDC 1968210 (**6**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

NCI screening assay

The methodology of the NCI procedure for primary anticancer assay is detailed on their site (http://www.dtp.nci.nih.gov), but briefly, the protocol was performed using the 60 human tumor cell lines panel derived from nine different neoplastic diseases. NCI-60 testing is performed in two parts: first, a single concentration is tested in all 60 cell lines at a single dose of 10^{-5} M or 15 µg/mL in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda, USA. If the results obtained meet selection criteria, then the compound is tested again in all 60 cell lines in 5 × 10 folds of dilution.

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Supplemental material

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