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Full Paper

Synthesis and Anti-Proliferative Activity of Sulfanyltriazolyl-naphthalenols and Sulfanyltriazolylnaphthalene-1,4-diones

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A series of new sulfanyltriazolylnaphthalenols (**10a–f** and **13a–f**) and sulfanyltriazolylnaphthalene-1,4-diones (**14a–f**) were synthesized and evaluated against a panel of cancer cell lines. Among the tested compounds, **10b** and **10d** showed the best anti-proliferative activity with GI_{50} values ranging from 2.72 to 10 and 3.13 to 13.1 μM, respectively, in several of the tumor cell lines tested. Compound **10d** is highly selective toward leukemia cell lines and can be regarded as a good model for the development of new anti-leukemic agents.

Keywords: Cancer / Leukemia / Quinone / Sulfanyltriazolylnaphthalenols / Synthesis

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Introduction

Cancer is a leading cause of death worldwide. For instance, in European countries, in 2012, there was an estimated 3.45 million new cases of cancer (excluding non-melanoma skin cancer) and 1.75 million deaths due to cancer [1], whereas in the USA, data from 2014 revealed that one in four deaths is caused by cancer [2]. However, despite the dramatically increasing costs of pharmaceutical R&D, the number of new drugs approved each year has greatly diminished during the past decade [3, 4]. As of today, leukemia remains one of the most common types of cancer in children aged 0–19 years [5]. Although survival rates have improved dramatically over the past several decades, leukemia remains the first among the leading causes of death from disease in children and the majority of those who are cured are at risk of short-term and long-term complications of therapy. Despite the fact that drugs such as imatinib have demonstrated great potential based on the initial studies of cellular immunotherapy, the need for new, more effective and selective anticancer agents still remains [4].

Heterocycles are extremely useful scaffolds in the design of novel bioactive compounds [6–10]. Indeed, heterocycles are present in over 60% of the top retailing drugs. Their size and shape, which allows substituent projection along a range of vectors, make them extremely versatile building blocks and they are many times used as amino acid mimetics or, e.g., carboxyl isosteres that offer considerable additional structural variation with which to enhance complementarity to a target protein or nucleic acid [11, 12]. The five-membered 1,3,4-oxadiazole and 1,2,4-triazole scaffolds have been

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successfully used in the design of anticancer compounds [13–21], including anti-leukemic compounds. For instance, 1,3,4-oxadiazole-containing hydroxamates and 2-amino-anilides have been reported as histone deacetylase inhibitors with anti-proliferative effects in several acute myeloid leukemia and U937 cell lines [22]. Other examples include a number of 1,2,4-triazole-based compounds described in literature as human heme oxygenase-1 (HO-1) inhibitors relevant as anti-leukemic agents [23]. It is known that the triazole is one of the key functional groups necessary to block enzyme activity which promotes the formation, growth, and metastasis of different tumors including chronic myelogenous leukemia when up-regulated. 1,2,4-Triazole and imidazole derivatives of L-ascorbic and imino-ascorbic acids were also found to be highly selective for inhibiting the proliferation of human T-cell acute lymphoblastic leukemia cells with IC_{50} values in the low micromolar range [24]. Of note, the 1,2,4-triazole-3-one derivative ganetespib is a highly potent second generation heat shock protein 90 (HSP90) inhibitor with activity against primary acute myeloid leukemia blasts at nanomolar concentrations [25].

Carbohydrazides are useful intermediates for the preparation of 1,3,4-oxadiazolines and 1,2,4-triazoles [26, 27]. We have recently shown that oxadiazoline-substituted naphthalenyl acetates prepared by cyclization of the respective hydrazides with acetic anhydride are potent anti-proliferative agents with moderate selectivity against non-solid (leukemia and melanoma) tumor cell lines [26]. In continuation of our efforts to discover potent anticancer agents, herein we report on the synthesis of a series of new sulfanyltriazolylnaphthalenols and -naphthalene-1,4-diones by reacting carbohydrazides with several aryl isothiocyanates with base-promoted cyclization of the resulting intermediates, and their anti-proliferative activity against a panel of tumor cell lines.

Results and discussion

Chemistry

Synthesis of precursor carbohydrazides

Acid hydrazides are prepared by reacting the corresponding carboxylic esters with hydrazine hydrate [28–30]. We selected three different starting materials for the preparation of carbohydrazide intermediates for the synthesis of the desired set of sulfanyltriazolylnaphthalenols and -naphthalene-1,4-diones, including methyl 1-hydroxy-2-naphthoate **1** and two additional esters bearing a methoxy or hydroxy substituent at position 4 of the naphthalene core, namely compounds **2** and **3** (Fig. 1). The reaction of compounds **1** and **2** with hydrazine hydrate gave the expected carbohydrazides **4** and **5** (Fig. 1A). However, after heating methyl 1,4-dihydroxynaphthalene-2-carboxylate **3** with 40% hydrazine hydrate in ethanol for 2 h, a yellowish brown precipitate was obtained which failed to fulfill the spectral and microanalytical data computed for the expected 1,4-dihydroxynaphthalene-2-carbohydrazide **6**, indicating that the presence of the 4-hydroxy group changed the outcome of this reaction (Fig. 1B). The reaction was repeated under variable reaction conditions (room temperature and heating, as well as performing the reaction in darkness), and the same result was obtained in all cases. Combined structural elucidation analyses revealed that the obtained product in this reaction was 3-amino-1,4-dihydro-1,4-dioxonaphthalene-2-carbohydrazide **7** (Fig. 1B). Infrared (IR) spectrum showed the distinctive absorption band of the carbonyl groups of the naphthoquinone, and the molecular ion peak $[M+H]^+$ of 232.0723, corresponding to the molecular formula $C_{11}H_{10}N_3O_3$, was confirmed by high-resolution mass spectroscopy (HRMS). In addition, the ^{1}H NMR spectrum lacked the two singlets assigned for the two OH protons and instead revealed singlets assigned for the two NH_2 and NH protons at 4.61, 8.61, 10.44, and 10.58 ppm. The ^{13}C NMR spectrum revealed two signals at 180.7 and 181 ppm assigned to the carbonyl groups of the naphthoquinone in addition to the carbonyl group at 167.7 ppm, and the HSQC spectrum showed that the naphthalenyl-C_3 was a quaternary carbon (see the Supporting Information).

Synthesis of sulfanyltriazolylnaphthalenols and sulfanyltriazolylnaphthalene-1,4-diones

We next prepared the hydrazine-1-carbothioamides **8a–c** from carbohydrazide **4** by reaction with various aryl isothiocyanates (Scheme 1).

The hydrazine-1-carbothioamides **8a–c** were then cyclized to the corresponding sulfanyltriazolylnaphthalenols **9a–c** by

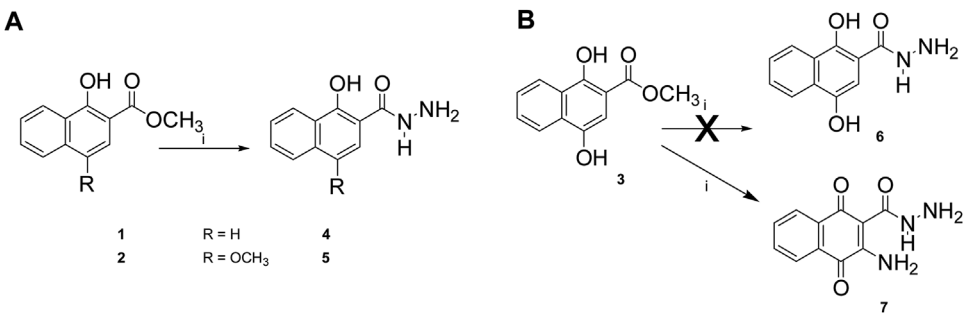

Figure 1. Reagents and conditions: (i) 40% $H_2NNH_2 \bullet H_2O$, EtOH, reflux.

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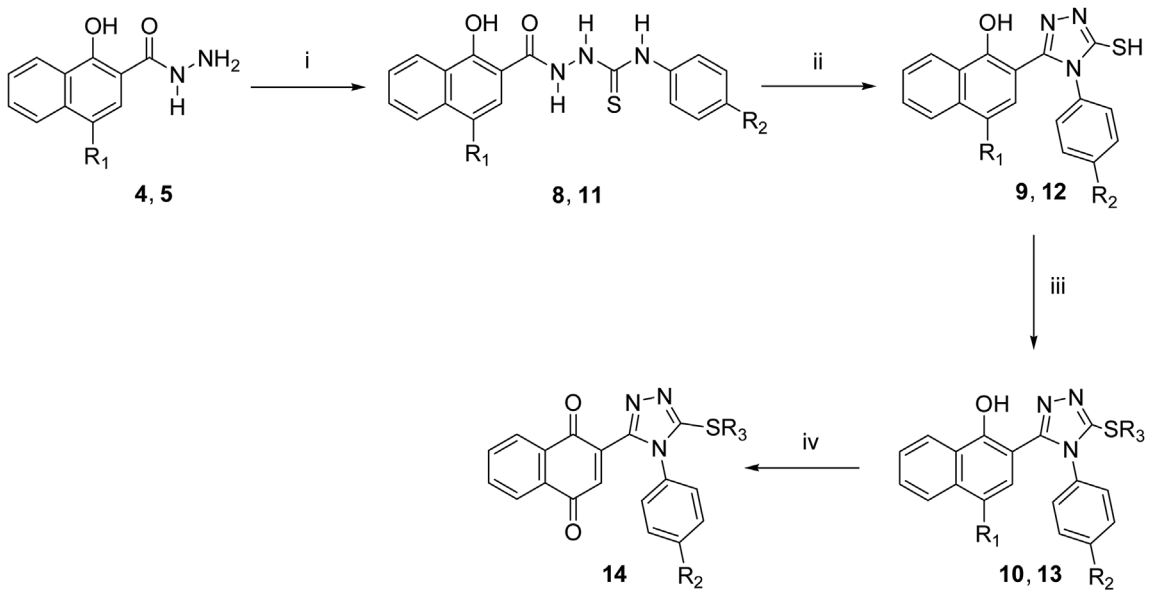


Compound	R_1	R_2	R_3	Compound	R_1	R_2	R_3	Compound	R_1	R_2	R_3
4	H	–	–	**10e**	H	CH_3	CH_3	**13c**	OCH_3	Cl	CH_3
8a	H	H	–	**10f**	H	CH_3	C_2H_5	**13d**	OCH_3	Cl	C_2H_5
8b	H	Cl	–	**5**	OCH_3	–	–	**13e**	OCH_3	CH_3	CH_3
8c	H	CH_3	–	**11a**	OCH_3	H	–	**13f**	OCH_3	CH_3	C_2H_5
9a	H	H	–	**11b**	OCH_3	Cl	–	**14a**	–	H	CH_3
9b	H	Cl	–	**11c**	OCH_3	CH_3	–	**14b**	–	H	C_2H_5
9c	H	CH_3	–	**12a**	OCH_3	H	–	**14c**	–	Cl	CH_3
10a	H	H	CH_3	**12b**	OCH_3	Cl	–	**14d**	–	Cl	C_2H_5
10b	H	H	C_2H_5	**12c**	OCH_3	CH_3	–	**14e**	–	CH_3	CH_3
10c	H	Cl	CH_3	**13a**	OCH_3	H	CH_3	**14f**	–	CH_3	C_2H_5
10d	H	Cl	C_2H_5	**13b**	OCH_3	H	C_2H_5				

Scheme 1. Synthesis of sulfanyltriazolylnaphthalenols and -naphthalene-1,4-diones. Reagents and conditions: (i) RC_6H_4NCS, absolute EtOH, reflux, 2 h (for **8**) or r.t., 24 h (for **11**); (ii) $NaOH/H_2O$, reflux, 4 h; (iii) R_1I, K_2CO_3, dry CH_3COCH_3, r.t., 2 h (for **10**) or r.t., 24 h (for **13**); (iv) CAN, $MeCN/H_2O$, 0°C, 5 min.

heating with aqueous sodium hydroxide and these were alkylated to the corresponding sulfanyltriazolylnaphthalenols **10a–f**, in yields ranging from 63 to 92%. Compound **5** was also converted to the corresponding methoxyhydrazinecarbothioamides **11a–c** by the same method used for compound **4**. Cyclization of compounds **11a–c** with sodium hydroxide gave the corresponding sulfanyltriazolylmethoxynaphthalenols **12a–c** which were alkylated to produce the derivatives **13a–f**. Oxidative demethylation of compounds **13a–f** using ceric ammonium nitrate (CAN) in aqueous acetonitrile produced the sulfanyltriazolylnaphthalene-1,4-diones **14a–f**, in yields ranging from 76 to 97%. The structures of the new compounds **8a–c**, **9a–c**, **10a–f**, **11a–c**, **12a–c**, **13a–f**, and **14a–f** were confirmed by recording their spectral and

analytical data. The structure of compound **13a** was confirmed by 2D NMR experiments namely, COSY, NOESY, HSQC, and HMBC (Supporting Information). COSY and NOESY experiments of compound **13a** showed ^{1}H–^{1}H correlations between the OCH_3 protons signal at 3.25 ppm and naphthalenyl-C_3-H signal at 5.97 ppm, confirming the presence of the methoxy group on the naphthalenyl-C_4 position.

We also attempted to use compound **7** as a precursor for the synthesis of additional heterocycle-bearing naphthalene-1,4-diones **16a** and **16b** (Scheme 2). Thiosemicarbazides **15a** and **15b** were prepared by heating a solution of the carbohydrazide **7** in absolute ethanol, under reflux, with an equimolar amount of the appropriate aryl isothiocyanate.

The structures of compounds **15a** and **15b** were confirmed with complementary structural elucidation techniques. The IR spectra of compounds **15a** and **15b** showed the distinctive absorption for N–C=S thioamide I–IV bands due to their mixed vibrational coupling, besides the absorption bands of NH_2, NH, and C=O groups. ^{1}H NMR spectra revealed the presence of singlets assigned for the NH_2 and three NH protons, in addition to signals assigned for the aromatic protons at their expected chemical shifts. HRMS analysis of compound **15a** showed a molecular ion peak of $[M+H]^+$ at *m/z* 367.0866 consistent with the molecular formula $C_{18}H_{14}N_4O_3S$. However, all attempts to prepare 3-amino-2-(4-aryl-5-sulfanyl-4*H*-1,2,4-triazol-3-yl)-1,4-naphthoquinones **16a** and **16b** from compounds **15a** and **15b** were not successful.

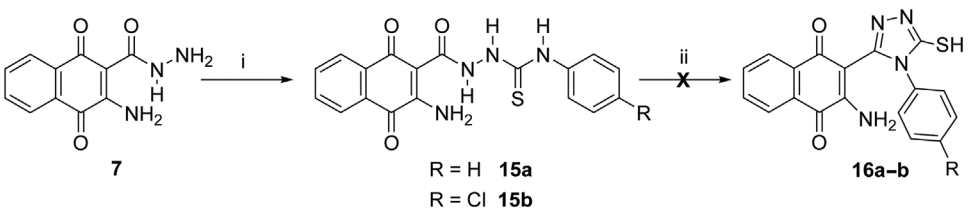
Biology

First-step anti-proliferative activity screening

Eight of the synthesized sulfanyltriazoles namely, compounds **10b**, **10d**, **13a**, **13c**, and **14a–d** were selected by the National Cancer Institute (NCI) *in vitro* disease-oriented human cells screening panel assay to be evaluated for their anti-proliferative activity [31, 32]. All the selected compounds were tested initially at a single concentration of 10 μM in the full NCI 60-cell line panel including leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate and breast cancer cell lines. The data are reported as a mean-graph of the percent growth of treated cells and presented as percentage growth inhibition (GI%) caused by the tested compounds (Supporting Information Table S1). We found that the activity of compounds **13c** and **14a–d** against the various cancer cell lines tested was not significant and that compound **13a** was inactive. In sharp contrast, compounds **10b** and **10d** were active against various cancer cell lines. Compound **10b** exhibited GI% values ranging from 62 to 77% against leukemia, non-small cell lung, colon as well as ovarian cancer cell lines. Compound **10d** showed GI% values ranging from 60 to 84% against leukemia, non-small cell lung, colon, melanoma, ovarian, renal and breast cancer cell lines.

Extended anti-proliferative activity profiling

Compounds **10b** and **10d** were selected for follow-up studies including a five-dose screening assay against a panel of 60 different cancer cell lines, and probing their effects on cell cycle, cell morphology, and the microtubule cytoskeleton. Table 1 shows the results of the testing of the selected compounds against several cancer cells lines, with determination of the respective GI_{50} and LC_{50} values. The GI_{50} value expresses growth inhibitory activity whereas the LC_{50} value is a measure of the cytotoxic activity of the compounds tested. We have found that the GI_{50} values for compound **10b** ranged from 2.72 to 10 μM against non-small cell lung HOP-92, NCI-H460 and NCI-H522, colon HCT-15, CNS SF-268, ovarian IGROV1 and OVCAR-4 in addition to renal TK-10 cancer cell lines, revealing significant anti-proliferative activity. Moderate activity was observed against non-small cell lung HOP-62 and CNS SNB-75 as well as melanoma MALME-3M cancer cell lines with GI_{50} values ranging from 21.5 to 71 μM. Significant anti-proliferative activity was also observed for compound **10d** with GI_{50} values ranging from 3.13 to 13.1 μM in all tumor cell lines tested except for non-small cell lung HOP-62, colon COLO 205 and HT29, CNS SF-268 and SNB-75, ovarian OVCAR-3 and SK-OV-3, renal 786-0 and RXF 393, prostate PC-3 as well as breast HS 578T cancer cell lines where the activity was moderate with GI_{50} values between 22.1 and 85.4 μM. No significant activity was observed against the other cell lines tested. The LC_{50} values of compounds **10b** and **10d** were always higher than 100 μM against all tested tumor cell lines. In this assay, individual panel and full panel mean-graph midpoint values (MG-MID) for the tested compounds represent the average of GI_{50} values of all cell lines in the individual panels or the full panel, respectively. The ratio obtained by dividing compound full panel MG-MID (μM) by its individual panel MG-MID (μM) is considered a measure of compound selectivity. Ratios between 3 and 6 are indicative of moderate selectivity, ratios above 6 of high selectivity toward the corresponding cell line, while compounds meeting neither of these criteria are rated as non-selective [31, 32]. Whereas,



Scheme 2. Attempted synthesis of compounds **16a** and **16b** from the precursor compound **7**. Reagents and conditions: (i) RC_6H_4NCS, absolute EtOH, reflux, 2 h; (ii) $NaOH/H_2O$, reflux, 4 h.

Table 1. GI_{50}, LC_{50}, and MG-MID, in μM, for compounds 10b and 10d.

	10b (NSC:766073/1)				10d (NSC:766074/1)			
Panel/cell line	**GI_{50}[a)]**	**LC_{50}[b)]**	**MG-MID[c)]**	**SR[d)]**	**GI_{50}**	**LC_{50}**	**MG-MID**	**SR**
Leukemia								
CCRF-CEM	NT[e)]	>100	100	<1	3.58	>100	4.7	7
HL-60(TB)	>100	>100			7.16	>100		
K-562	NT	>100			4.04	>100		
MOLT-4	>100	>100			NT	>100		
RPMI-8226	NT	>100			4.83	>100		
SR	NT	>100			3.86	>100		
Non-small cell lung cancer								
A549/ATCC	NT	>100	26.8	3	4.51	>100	19.9	2
HOP-62	21.5	>100			22.1	>100		
HOP-92	5.00	>100			9.23	>100		
NCI-H226	NT	>100			7.46	>100		
NCI-H23	>100	>100			>100	>100		
NCI-H322M	NT	>100			7.46	>100		
NCI-H460	3.25	>100			3.27	>100		
NCI-H522	4.27	>100			5.15	>100		
Colon cancer								
COLO 205	NT	>100	51.4	1	47.4	>100	39.3	<1
HCC-2998	>100	>100			>100	>100		
HCT-116	NT	>100			4.00	>100		
HCT-15	2.72	>100			3.13	>100		
HT29	NT	>100			16.0	>100		
KM12	NT	>100			>100	>100		
SW-620	NT	>100			4.28	>100		
CNS cancer								
SF-268	8.02	>100	59.7	1	26.1	>100	34.2	1
SF-295	NT	>100			4.24	>100		
SF-539	>100	>100			>100	>100		
SNB-19	ND	>100			5.84	>100		
SNB-75	71.0	>100			65.2	>100		
U251	NT	>100			4.05	>100		
Melanoma								
LOX IMVI	NT	>100	89.8	1	5.52	>100	53	<1
MALME-3M	69.7	>100			>100	>100		
M14	NT	>100			8.11	>100		
MDA-MB-435	NT	>100			NT	>100		
SK-MEL-2	NT	>100			>100	>100		
SK-MEL-28	>100	>100			>100	>100		
SK-MEL-5	NT	>100			6.01	>100		
UACC-257	>100	>100			>100	>100		
UACC-62	NT	>100			4.60	>100		
Ovarian cancer								
IGROV1	7.59	>100	53.7	1	4.42	>100	31.3	1
OVCAR-3	>100	>100			29.4	>100		
OVCAR-4	7.22	>100			5.71	>100		
OVCAR-5	NT	>100			>100	>100		
OVCAR-8	NT	>100			5.50	>100		
NCI/ADR-RES	NT	>100			4.45	>100		
SK-OV-3	>100	>100			69.5	>100		
Renal cancer								
786-0	>100	>100	77.5	<1	60.3	>100	45.4	<1
A498	NT	>100			>100	>100		
ACHN	NT	>100			5.00	>100		
CAKI-1	NT	>100			6.85	>100		

(*Continued*)

Table 1. (*Continued*)

Panel/cell line	10b (NSC:766073/1)				10d (NSC:766074/1)			
	GI_{50}[a)]	LC_{50}[b)]	MG-MID[c)]	SR[d)]	GI_{50}	LC_{50}	MG-MID	SR
RXF 393	>100	>100			42.3	>100		
SN12C	>100	>100			>100	>100		
TK-10	10.0	>100			NT	>100		
UO-31	NT	>100			3.31	>100		
Prostate cancer								
PC-3	NT	>100	100	<1	50.1	>100	31.6	1
DU-145	>100	>100			13.1	>100		
Breast cancer								
MCF7	NT	>100	100	<1	6.66	>100	50.8	<1
MDA-MB-231/ATCC	>100	>100			>100	>100		
HS 578T	>100	>100			85.4	>100		
BT-549	>100	>100			>100	>100		
T-47D	NT	>100			8.96	>100		
MDA-MB-468	NT	>100			3.58	>100		

[a)] GI_{50}: concentration of compounds causing 50% decrease in net cell growth.
[b)] LC_{50}: concentration of compounds causing net 50% loss of initial cells at the end of incubation period.
[c)] MG-MID = panel GI_{50} mean-graph midpoints.
[d)] Selectivity ratio (SR) = full panel MG-MID (μM)/individual panel MG-MID (μM). Full panel MG-MID is 73.2 μM for compound **10b** and 34.5 μM for compound **10d**.
[e)] NT: not tested.

compound **10b** was moderately selective against non-small cell lung cancer cells with a selectivity ratio (SR) value of 3, compound **10d** was found to be highly selective against leukemia cell lines with a high SR value of 7, as depicted in Table 1.

Treatment of A549 cancer cells with compounds **10b** and **10d** at 50 μM did not cause significant changes to cell morphology, with only a number of round non-adherent cells observed after treatment with the compounds (Supporting Information Fig. S1, left panel). We found that these compounds do not accumulate cells in the G2/M phase of the cell cycle and do not inhibit mitosis (Supporting Information Fig. S1, right panel). They also do not seem to have a direct effect on microtubule cytoskeleton (Supporting Information Fig. S2). Among the sulfanyltriazolylnaphthalenols tested, the presence of an ethyl group on the five-membered ring proved beneficial for the anti-proliferative activity, as compounds **10b** and **10d** were more active than the methyl derivatives **10a** and **10c**. The presence of an additional methoxy group on the naphthalene ring in compounds **13a–f** did not improve the overall activity of the compounds neither did the oxidation of the naphthalene core in compounds **14a–f**.

Conclusions

We have synthesized a small set of novel sulfanyltriazolylnaphthalenols and -naphthalene-1,4-diones. The anti-proliferative activity of these compounds against several cancer cell lines and the observed high selectivity of compound **10d** against leukemia cells encourage the design and synthesis of other derivatives bearing similar scaffolds with the aim of developing more effective anti-leukemic agents and establishing their precise mechanisms of action.

Experimental

Chemistry

Melting points were determined in open glass capillaries using a Griffin melting point apparatus or an Electrothermal capillary tube melting point apparatus and are all uncorrected. IR spectra were recorded, using KBr discs, by a Perkin-Elmer 1430 infrared spectrometer, Central Laboratory, Faculty of Pharmacy, Alexandria University, Egypt or by a Vertex 70 (Bruker Optics Inc., MA, USA) Fourier transform infrared spectrometer, Faculty of Pharmacy, University of Helsinki, Finland. Nuclear magnetic resonance spectra were recorded using a Jeol-NMR 400 MHz spectrometer, Faculty of Pharmacy, Assiut University, Egypt or a Varian Mercury Plus 300 MHz spectrometer, Faculty of Pharmacy, University of Helsinki, Finland in $CDCl_3$ or DMSO-d_6 and are reported as δ values (ppm) relative to tetramethylsilane (TMS) as an internal standard. The type of signal was indicated by one of the following letters: s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet. The coupling constants J are quoted in Hertz (Hz). Elemental microanalyses were performed at the microanalytical unit, Faculty of Science, Cairo University or at Robertson Microlit Laboratories Inc., Ledgewood, New Jersey, USA. Reaction progress was monitored by

thin-layer chromatography (TLC) on silica gel (60 GF254, Merck) using glass plates or on Kieselgel 60HF254/Kieselgel 60G TLC plates and the spots were visualized by exposure to iodine vapor or to UV-lamp: at λ 254 nm for few seconds. ESI-MS was performed in positive, high resolution mode, by direct injection using a Synapt G2 HDMS (Waters, MA, USA) instrument.

Compounds **4** and **5** were prepared according to previously reported procedures [26].

The InChI codes of the investigated compounds are provided as Supporting Information.

*3-Amino-1,4-dihydro-1,4-dioxonaphthalene-2-carbohydrazide (**7**)*

A solution of methyl 1,4-dihydroxynaphthalene-2-carboxylate **3** (2.18 g, 10.0 mmol) in ethanol (20 mL) was added to 40% hydrazine hydrate (4 mL). The reaction mixture was then heated under reflux for 2 h and set aside for an overnight for complete precipitation. The formed yellowish brown precipitate was filtered, washed several times with cold 50% ethanol, air dried, and crystallized from ethanol. Yield 1.2 g (52%); mp: 189–190°C with decomposition; IR (ATR, υ cm^{-1}): 3287, 3180 (NH_2, υNH), 1703 (C=O quinone), 1610 (C=O amide), 1572 (δ NH); ^{1}H NMR (300 MHz, DMSO-d_6): 4.61 (s, 2H, NH_2); 7.74–7.79 (m, 1H, naphthalenyl-C_6-H); 7.86–7.92 (m, 1H, naphthalenyl-C_7-H); 8.01 (dd, 1H, J = 7.5, 1.2 Hz, naphthalenyl-C_8-H); 8.08 (dd, 1H, J = 7.8, 1.2 Hz, naphthalenyl-C_5-H); 8.61, 10.44, 10.58 (three s, 3H, NH, and NH_2); ^{13}C NMR (75 MHz, DMSO-d_6): 99.9, 126.4, 126.8, 130.0, 133.1, 133.9, 136.0, 154.6, 167.7, 180.7, 181.0. Anal. calcd. for $C_{11}H_9N_3O_3$: C, 57.14; H, 3.92; N, 18.17. Found: C, 57.25; H, 3.72; N, 17.84; HRMS (ESI): *m/z* calcd. for $C_{11}H_{10}N_3O_3$: 232.0722 $[M+H]^+$; found: 232.0723.

*General method for the synthesis of compounds **8a–c***

A solution of the selected aryl isothiocyanate (10.0 mmol) in absolute ethanol (5 mL) was gradually added to a well-stirred solution of the hydrazide **4** (2.02 g, 10.0 mmol) in absolute ethanol (20 mL). The reaction mixture was heated under reflux for 2 h, concentrated and then set aside for an overnight for complete precipitation. The formed beige precipitates were filtered, washed with cold 50% ethanol, air dried, and recrystallized from ethanol.

*2-(1-Hydroxy-2-naphthalenoyl)-N-phenylhydrazinecarbothioamide (**8a**)*

Yield 65%; mp: 181–182°C; IR (ATR, υ cm^{-1}): 3342 (OH), 3136 (δ NH), 1630 (C=O), 1549, 1246, 1171, 931 (N–C=S thioamide I–IV bands, respectively); ^{1}H NMR (300 MHz, DMSO-d_6): 7.15 (t, 1H, J = 7.35 Hz); 7.32 (t, 2H, J = 7.8 Hz); 7.39–7.65 (m, 5H); 7.90 (t, 2H, J = 9.6 Hz); 8.29 (d, 1H, J = 7.8 Hz); 9.82 (s, 1H, OH); 9.97, 10.97, 13.75 (three s, each 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): 106.8, 118.2, 123.4, 123.5, 124.9, 125.5, 126.0, 126.3, 127.9, 128.5, 129.5, 136.4, 139.6, 160.0, 170.9, 181.8. Anal. calcd. for $C_{18}H_{15}N_3O_2S$: C, 64.08; H, 4.48; N, 12.45. Found: C, 63.93; H, 4.29; N, 12.50.

*N-(4-Chlorophenyl)-2-(1-hydroxy-2-naphthoyl)hydrazine-1-carbothioamide (**8b**)*

Yield 65%; mp: 269–270°C with decomposition; IR (ATR, υ cm^{-1}): 3331 (OH), 3159 (υNH), 1630 (C=O), 1520, 1245, 1173, 937 (N–C=S thioamide I–IV bands, respectively); ^{1}H NMR (300 MHz, DMSO-d_6): 7.35–7.68 (m, 7H); 7.89 (d, 1H, J = 9 Hz); 7.92 (d, 1H, J = 9 Hz); 8.29 (d, 1H, J = 9 Hz); 9.93 (s, 1H, OH); 10.01, 10.99, 13.71 (three s, each 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): 108.9, 120.4, 121.3, 121.5, 125.6, 127.1, 127.4, 128.5, 130.1, 130.6, 131.8, 138.6, 140.7, 162.2, 173.1, 184.0. Anal. calcd. for $C_{18}H_{14}ClN_3O_2S$: C, 58.14; H, 3.79; N, 11.30. Found: C, 58.03; H, 3.92; N, 11.26. HRMS (ESI): *m/z* calcd. for $C_{18}H_{15}ClN_3O_2S$: 372.0574 $[M+H]^+$; found: 372.0573.

*2-(1-Hydroxy-2-naphthoyl)-N-(4-tolyl)hydrazine-1-carbothioamide (**8c**)*

Yield 59%; mp: 175–176°C; IR (ATR, υ cm^{-1}): 3329 (OH), 3180 (υNH), 1632 (C=O), 1518, 1248, 1169, 935 (N–C=S thioamide I–IV bands, respectively); ^{1}H NMR (300 MHz, DMSO-d_6): 2.27 (s, 3H, CH_3); 7.12 (d, 2H, J = 9 Hz); 7.30 (d, 2H, J = 9 Hz); 7.40 (d, 1H, J = 9 Hz); 7.54–7.68 (m, 2H); 7.89 (d, 1H, J = 9 Hz); 7.92 (d, 1H, J = 9 Hz); 8.28 (d, 1H, J = 9 Hz); 9.73 (s, 1H, OH); 9.88, 10.93, 13.74 (three s, each 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): 21.0, 106.8, 118.1, 123.4, 123.5, 124.9, 126.0, 126.3, 127.9, 128.9, 129.5, 134.7, 136.4, 137.0, 160.0, 170.9, 181.8. Anal. calcd. for $C_{19}H_{17}N_3O_2S$: C, 64.94; H, 4.88; N, 11.96. Found: C, 64.84; H, 5.12; N, 12.19. HRMS (ESI): *m/z* calcd. for $C_{19}H_{18}N_3O_2S$: 352.1120 $[M+H]^+$; found: 352.1120.

*General method for the synthesis of compounds **9a–c***

A solution of the appropriate hydrazine-1-carbothioamide **8a–c** (1 mmol) in 1 M aqueous solution of sodium hydroxide (20 mL) was heated under reflux for 4 h. The reaction mixture was filtered while hot, cooled to room temperature, acidified with dilute hydrochloric acid to pH 5, and kept in the refrigerator for an overnight. The white precipitates were filtered, washed with water, air dried, and recrystallized from ethanol.

*2-(4-Phenyl-5-sulfanyl-4H-1,2,4-triazol-3-yl)naphthalen-1-ol (**9a**)*

Yield 94%; mp: 269–270°C; IR (KBr, υ cm^{-1}): 3275 (OH), 2914 (SH), 1630 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6): 7.17 (d, 1H, J = 9 Hz); 7.27–7.35 (m, 6H); 7.46–7.55 (m, 2H); 7.78 (dd, 1H, J = 7.5, 3 Hz); 8.15 (dd, 1H, J = 7.5, 3 Hz); 10.12 (s, 1H, OH); 14.05 (s, 1H, SH); ^{13}C NMR (75 MHz, DMSO-d_6): 107.1, 119.1, 123.0, 125.0, 126.0, 127.4, 127.9, 128.1, 128.5, 129.1, 129.2, 134.8, 135.3, 149.7, 152.9, 168.3. Anal. calcd. for $C_{18}H_{13}N_3OS$: C, 67.69; H, 4.10; N, 13.16. Found: C, 67.85; H, 4.02; N, 13.16. HRMS (ESI): *m/z* calcd. for $C_{18}H_{14}N_3OS$: 320.0858 $[M+H]^+$; found: 320.0858.

*2-[4-(4-Chlorophenyl)-5-sulfanyl-4H-1,2,4-triazol-3-yl]-naphthalen-1-ol (**9b**)*

Yield 66%; mp: 261–262°C; IR (KBr, υ cm^{-1}): 3275 (OH), 2933 (SH), 1628 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6): 7.21 (d, 1H, J = 9 Hz); 7.31–7.56 (m, 7H); 7.79 (dd, 1H, J = 6, 3 Hz); 8.14

(dd, 1H, $J = 6$, 3 Hz); 10.10 (s, 1H, OH); 14.09 (s, 1H, SH); ^{13}C NMR (75 MHz, DMSO-d_6): 107.0, 119.3, 123.1, 125.0, 126.1, 127.4, 128.0, 128.2, 129.2, 130.3, 133.72, 133.9, 135.4, 149.6, 152.8, 168.3. Anal. calcd. for $C_{18}H_{12}ClN_3OS$: C, 61.10; H, 3.42; N, 11.88. Found: C, 61.08; H, 3.66; N, 11.75.

*2-[5-Sulfanyl-4-(4-tolyl)-4H-1,2,4-triazol-3-yl]naphthalen-1-ol (**9c**)*
Yield 96%; mp: 261–262°C; IR (ATR, υ cm^{-1}): 3367 (OH), 2935 (SH), 1630 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6): 2.22 (s, 3H, CH_3); 7.10–7.65 (m, 8H); 7.80 (d, 1H, $J = 9.3$ Hz); 8.15 (d, 1H, $J = 8.4$ Hz); 10.16 (s, 1H, OH); 14.05 (s, 1H, SH); ^{13}C NMR (75 MHz, DMSO-d_6): 21.0, 107.2, 119.1, 123.1, 125.0, 126.0, 127.4, 127.9, 128.1, 128.2, 129.7, 132.2, 135.3, 138.8, 149.7, 152.9, 168.4. Anal. calcd. for $C_{19}H_{15}N_3OS$: C, 68.45; H, 4.53; N, 12.60. Found: C, 68.19; H, 4.69; N, 12.61.

*General method for the synthesis of compounds **10a–f***
A mixture of the appropriate sulfanyltriazolylnaphthalenol **9a–c** (1.0 mmol) and anhydrous potassium carbonate (0.14 g, 1.0 mmol) in dry acetone (10 mL) was treated with the appropriate alkyl iodide (1 mmol). The reaction mixture was stirred at room temperature for 2 h, then evaporated almost to dryness under reduced pressure. The reaction mixture was treated with water (30 mL) to remove unreacted potassium carbonate. The yellow precipitates were filtered, washed with water, air dried, and recrystallized from acetone.

*2-[5-(Methylsulfanyl)-4-phenyl-4H-1,2,4-triazol-3-yl]-naphthalen-1-ol (**10a**)*
Yield 77%; mp: 211–212°C; IR (KBr, υ cm^{-1}): 3375 (OH), 1634 (C=N), 1254, 1171 (C–S–C); ^{1}H NMR (300 MHz, $CDCl_3$): 2.97 (s, 3H, SCH_3); 6.61 (d, 1H, $J = 6$ Hz); 6.95 (d, 1H, $J = 9$ Hz); 7.32–7.67 (m, 8H); 8.45–8.51 (m, 1H); 12.70 (s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$): 14.4, 103.1, 117.9, 121.4, 123.7, 125.3, 125.5, 127.0, 127.7, 127.9, 130.4, 130.6, 134.4, 134.5, 153.7, 154.2, 155.7. Anal. calcd. for $C_{19}H_{15}N_3OS$: C, 68.45; H, 4.53; N, 12.60. Found: C, 68.68; H, 4.50; N, 12.28.

*2-[5-(Ethylsulfanyl)-4-phenyl-4H-1,2,4-triazol-3-yl]-naphthalen-1-ol (**10b**)*
Yield 85%; mp: 177–178°C; IR (KBr, υ cm^{-1}): 3058 (OH), 1633 (C=N), 1254, 1156 (C–S–C); ^{1}H NMR (300 MHz, $CDCl_3$): 1.47 (t, 3H, $J = 9$ Hz, SCH_2CH_3); 3.31 (q, 2H, $J = 9$ Hz, SCH_2CH_3); 6.61 (d, 1H, $J = 9$ Hz); 6.95 (d, 1H, $J = 9$ Hz); 7.32–7.67 (m, 8H); 8.47–8.51 (m, 1H); 12.77 (s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$): 15.2, 26.9, 105.0, 118.6, 123.2, 123.3, 125.0, 126.2, 127.8, 128.2, 128.3, 130.5, 130.8, 134.5, 134.7, 152.4, 153.9, 154.3. Anal. calcd. for $C_{20}H_{17}N_3OS$: C, 69.14; H, 4.93; N, 12.09. Found: C, 68.88; H, 4.91; N, 12.03.

*2-[4-(4-Chlorophenyl)-5-(methylsulfanyl)-4H-1,2,4-triazol-3-yl]naphthalen-1-ol (**10c**)*
Yield 63%; mp: 229–230°C; IR (KBr, υ cm^{-1}): 3056 (OH), 1634 (C=N), 1269, 1167 (C–S–C); ^{1}H NMR (300 MHz, DMSO-d_6): 2.68 (s, 3H, SCH_3); 6.89 (d, 1H, $J = 8.7$ Hz); 7.26 (d, 1H, $J = 8.7$ Hz); 7.54–7.66 (m, 6H); 7.68 (d, 1H, $J = 6.6$ Hz); 8.27 (d, 1H, $J = 7.2$ Hz); 11.79 (s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6): 14.5, 102.8, 118.1, 121.2, 123.7, 125.3, 125.7, 127.1, 128.0, 129.0, 130.7, 132.9, 134.6, 136.8, 153.6, 154.2, 155.8. Anal. calcd. for $C_{19}H_{14}ClN_3OS$: C, 62.04; C, 3.84; N, 11.42. Found: C, 62.45; H, 3.51; N, 11.38. HRMS (ESI): *m/z* calcd. for $C_{19}H_{15}ClN_3OS$: 368.0624 $[M+H]^+$; found: 368.0624.

*2-[4-(4-Chlorophenyl)-5-(ethylsulfanyl)-4H-1,2,4-triazol-3-yl]naphthalen-1-ol (**10d**)*
Yield 84%; mp: 175–176°C; IR (ATR, υ cm^{-1}): 3059 (OH), 1628 (C=N), 1267, 1153 (C–S–C); ^{1}H NMR (300 MHz, DMSO-d_6): 1.38 (t, 3H, $J = 7.2$ Hz, SCH_2CH_3); 3.21 (q, 2H, $J = 7.2$ Hz, SCH_2CH_3); 6.90 (d, 1H, $J = 8.7$ Hz); 7.26 (d, 1H, $J = 8.4$ Hz); 7.47–7.69 (m, 6H); 7.80 (d, 1H, $J = 6.9$ Hz); 8.27 (d, 1H, $J = 6.6$ Hz); 11.81 (s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6): 15.3, 27.1, 105.3, 118.9, 123.2, 124.0, 125.0, 126.2, 127.9, 128.3, 130.1, 130.5, 133.4, 134.8, 135.4, 152.1, 153.8, 154.0. Anal. calcd. for $C_{20}H_{16}ClN_3OS$: C, 62.90; H, 4.22; N, 11.00. Found: C, 63.13; H, 4.10; N, 10.74. HRMS (ESI): *m/z* calcd. for $C_{20}H_{17}ClN_3OS$: 382.0781 $[M+H]^+$; found: 382.0781.

*2-[5-(Methylsulfanyl)-4-(4-tolyl)-4H-1,2,4-triazol-3-yl]-naphthalen-1-ol (**10e**)*
Yield 77%; mp: 201–202°C; IR (ATR, υ cm^{-1}): 3059 (OH), 1632 (C=N), 1275, 1176 (C–S–C); ^{1}H NMR (300 MHz, $CDCl_3$): 2.51 (s, 3H, C_6H_4-CH_3); 2.73 (s, 3H, SCH_3); 6.68 (d, 1H, $J = 9$ Hz); 6.98 (d, 1H, $J = 9$ Hz); 7.23 (d, 2H, $J = 6$ Hz); 7.39 (d, 2H, $J = 6$ Hz); 7.46–7.52 (m, 2H); 7.63 (dd, 1H, $J = 6$, 3 Hz); 8.47 (dd, 1H, $J = 6$, 3 Hz); 12.87 (s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$): 14.4, 21.4, 103.2, 117.9, 121.4, 123.7, 125.3, 125.5, 127.0, 127.3, 127.8, 131.0, 131.7, 134.5, 141.0, 153.9, 154.3, 155.7. Anal. calcd. for $C_{20}H_{17}N_3OS$: C, 69.14; H, 4.93; N, 12.09. Found: C, 69.38; H, 4.81; N, 11.83. HRMS (ESI): *m/z* calcd. for $C_{20}H_{18}N_3OS$: 348.1171 $[M+H]^+$; found: 348.1171.

*2-[5-(Ethylsulfanyl)-4-(4-tolyl)-4H-1,2,4-triazol-3-yl]-naphthalen-1-ol (**10f**)*
Yield 92%; mp: 179–180°C; IR (ATR, υ cm^{-1}): 3063 (OH), 1636 (C=N), 1254, 1176 (C–S–C); ^{1}H NMR (300 MHz, $CDCl_3$): 1.47 (t, 3H, $J = 6$ Hz, SCH_2CH_3); 2.51 (s, 3H, C_6H_4-CH_3); 3.30 (q, 2H, $J = 6$ Hz, SCH_2CH_3); 6.67 (d, 1H, $J = 9$ Hz); 6.97 (d, 1H, $J = 9$ Hz); 7.23 (d, 2H, $J = 6$ Hz); 7.39 (d, 2H, $J = 6$ Hz); 7.46–7.52 (m, 2H); 7.63 (dd, 1H, $J = 6$, 3 Hz); 8.47 (dd, 1H, $J = 6$, 3 Hz); 12.83 (s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$): 15.2, 21.2, 26.8, 105.0, 118.6, 123.2, 123.2, 125.0, 126.2, 127.8, 127.9, 128.3, 131.0, 131.9, 134.7, 140.6, 152.6, 153.9, 154.4. Anal. calcd. for $C_{21}H_{19}N_3OS$: C, 69.78; H, 5.30; N, 11.63. Found: C, 69.53; H, 5.29; N, 11.59. HRMS (ESI): *m/z* calcd. for $C_{21}H_{20}N_3OS$: 362.1327 $[M+H]^+$; found: 362.1327.

*General method for the synthesis of compounds **11a–c***
A solution of the appropriate aryl isothiocyanate (10.0 mmol) in absolute ethanol (5 mL) was gradually added to a well-stirred solution of hydrazide **5** (2.32 g, 10.0 mmol) in absolute ethanol (100 mL). The reaction mixture was stirred at room

temperature for 24 h. The formed beige precipitates were filtered, washed with cold 50% ethanol, air dried, and recrystallized from ethanol.

*2-(1-Hydroxy-4-methoxy-2-naphthoyl)-N-phenylhydrazine-1-carbothioamide (**11a**)*
Yield 86%; mp: 161–162°C; IR (KBr, υ cm^{-1}): 3254 (OH), 3186 (υNH), 1630 (C=O), 1534, 1298, 1156, 986 (N–C=S thioamide I–IV bands, respectively), 1215, 1028 (C–O–C); ^{1}H NMR (400 MHz, DMSO-d_6): 3.96 (s, 3H, OCH_3); 7.16 (t, 1H, $J = 7.4$ Hz); 7.30–7.37 (m, 3H); 7.40–7.50 (m, 2H); 7.57–7.70 (m, 2H); 8.12 (d, 1H, $J = 8$ Hz); 8.27 (d, 1H, $J = 8$ Hz); 9.88 (s, 1H, OH); 10.00, 11.00, 13.34 (three s, each 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): 56.4, 101.0, 105.7, 121.9, 123.6, 125.5, 125.6, 126.2, 127.0, 128.5, 128.6, 129.2, 139.6, 147.1, 154.3, 170.9, 181.9. Anal. calcd. for $C_{19}H_{17}N_3O_3S$: C, 62.11; H, 4.66; N, 11.44. Found: C, 61.92; H, 4.49; N, 11.30. HRMS (ESI): *m/z* calcd. for $C_{19}H_{18}N_3O_3S$: 368.1069 $[M+H]^+$; found: 368.1069.

*N-(4-Chlorophenyl)-2-(1-hydroxy-4-methoxy-2-naphthoyl)-hydrazine-1-carbothioamide (**11b**)*
Yield 86%; mp: 163–164°C; IR (ATR, υ cm-1): 3255 (OH), 3159 (υNH), 1630 (C=O), 1549, 1294, 1155, 987 (N–C=S thioamide I–IV bands, respectively), 1213, 1030 (C–O–C); ^{1}H NMR (400 MHz, DMSO-d_6): 3.96 (s, 3H, OCH_3); 7.32 (s, 1H); 7.39 (d, 2H, $J = 8.8$ Hz); 7.42–7.55 (m, 2H); 7.62 (t, 1H, $J = 7.4$ Hz); 7.68 (t, 1H, $J = 7.6$ Hz); 8.12 (d, 1H, $J = 8$ Hz); 8.28 (d, 1H, $J = 8$ Hz); 10.00 (s, 1H, OH); 10.01, 11.02, 13.33 (three s, each 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): 56.4, 100.9, 105.6, 121.9, 123.6, 125.6, 127.0, 127.9, 128.4, 128.6, 129.2, 129.6, 138.6, 147.2, 154.3, 170.9, 181.9. Anal. calcd. for $C_{19}H_{16}ClN_3O_3S$: C, 56.79; H, 4.01; N, 10.46. Found: C, 56.97; H, 3.80; N, 10.89.

*2-(1-Hydroxy-4-methoxy-2-naphthoyl)-N-(4-tolyl)-hydrazine-1-carbothioamide (**11c**)*
Yield 80%; mp: 157–158°C; IR (ATR, υ cm^{-1}): 3244 (OH), 3136 (υNH), 1632 (C=O), 1535, 1296, 1157, 986 (N–C=S thioamide I–IV bands, respectively), 1215, 1029 (C–O–C); ^{1}H NMR (300 MHz, DMSO-d_6): 2.27 (s, 3H, C_6H_4-CH_3); 3.96 (s, 3H, OCH_3); 7.11–7.14 (m, 2H); 7.31–7.40 (m, 3H); 7.58–7.70 (m, 2H); 8.12 (d, 1H, $J = 9$ Hz); 8.27 (d, 1H, $J = 9$ Hz); 9.77 (s, 1H, OH); 9.90, 10.95, 13.33 (three s, each 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): 21.0, 56.4, 101.0, 105.7, 109.9, 121.9, 123.6, 125.6, 127.0, 128.5, 129.0, 129.2, 134.7, 137.0, 140.0, 147.1, 154.2, 171.0. Anal. calcd. for $C_{20}H_{19}N_3O_3S.1/2H_2O$: C, 61.52; H, 5.16; N, 10.76. Found: C, 61.52; H, 4.96; N, 10.76.

*General method for the synthesis of compounds **12a–c***
A solution of the appropriate methoxyhydrazine-1-carbothioamide **11a–c** (1.0 mmol) in 1 M aqueous solution of sodium hydroxide (20 mL) was heated under reflux for 4 h. The reaction mixture was filtered while hot, cooled to room temperature, acidified with dilute hydrochloric acid to pH 5, and kept in the refrigerator for an overnight. The white precipitates formed were filtered, washed with water, air dried, and recrystallized from ethanol.

*2-(4-Phenyl-5-sulfanyl-4H-1,2,4-triazol-3-yl)-4-methoxynaphthalen-1-ol (**12a**)*
Yield 85%; mp: 255–256°C; IR (ATR, υ cm^{-1}): 3107 (OH), 2941 (SH), 1632 (C=N), 1232, 1080 (C–O–C); ^{1}H NMR (300 MHz, DMSO-d_6): 3.64 (s, 3H, OCH_3); 6.56 (s, 1H); 7.31–7.39 (m, 5H); 7.48–7.57 (m, 2H); 7.97–8.02 (m, 1H); 8.11–8.14 (m, 1H); 9.70 (s, 1H, OH); 14.07 (s, 1H, SH); ^{13}C NMR (75 MHz, DMSO-d_6): 55.9, 105.2, 106.0, 121.7, 123.2, 125.9, 126.6, 126.9, 127.6, 128.7, 129.2, 129.3, 135.0, 146.7, 147.7, 149.8, 168.3. Anal. calcd. for $C_{19}H_{15}N_3O_2S$: C, 65.31; H, 4.33; N, 12.03. Found: C, 65.03; H, 4.05; N, 12.20.

*2-[4-(4-Chlorophenyl)-5-sulfanyl-4H-1,2,4-triazol-3-yl]-4-methoxynaphthalen-1-ol (**12b**)*
Yield 90%; mp: 265–266°C; IR (KBr, υ cm^{-1}): 3288 (OH), 2938 (SH), 1632 (C=N), 1230, 1096 (C–O–C); ^{1}H NMR (400 MHz, DMSO-d_6): 3.72 (s, 3H, OCH_3); 6.68 (s, 1H); 7.40–7.57 (m, 6H); 8.02 (d, 1H, $J = 8$ Hz); 8.11 (d, 1H, $J = 8$ Hz); 9.63 (s, 1H, OH); 14.18 (s, 1H, SH); ^{13}C NMR (75 MHz, DMSO-d_6): 56.0, 105.4, 106.1, 121.8, 123.2, 126.0, 126.7, 127.0, 127.6, 129.2, 130.4, 133.8, 133.9, 146.5, 147.8, 149.8, 168.2. Anal. calcd. for $C_{19}H_{14}ClN_3O_2S$: C, 59.45; H, 3.68; N, 10.95. Found: C, 59.18; H, 3.83; N, 10.89. HRMS (ESI): *m/z* calcd. for $C_{19}H_{15}ClN_3O_2S$: 384.0574 $[M+H]^+$; found: 384.0573.

*2-[5-Sulfanyl-4-(4-tolyl)-4H-1,2,4-triazol-3-yl]-4-methoxynaphthalen-1-ol (**12c**)*
Yield 83%; mp: 195–196°C; IR (ATR, υ cm^{-1}): 3296 (OH), 2935 (SH), 1634 (C=N), 1238, 1082 (C–O–C); ^{1}H NMR (300 MHz, DMSO-d_6): 2.23 (s, 3H,C_6H_4-CH_3); 3.67 (s, 3H, OCH_3); 6.60 (s, 1H); 7.15 (d, 2H, $J = 9$ Hz); 7.25 (d, 2H, $J = 9$ Hz); 7.48–7.56 (m, 2H); 7.99–8.02 (m, 1H); 8.10–8.13 (m, 1H); 9.58 (s, 1H, OH); 14.03 (s, 1H, SH); ^{13}C NMR (75 MHz, DMSO-d_6): 21.1, 56.0, 105.3, 106.2, 121.7, 123.2, 125.9, 126.6, 126.9, 127.6, 128.4, 129.7, 132.4, 138.8, 146.6, 147.6, 149.8, 168.4. Anal. calcd. for $C_{20}H_{17}N_3O_2S$: C, 66.10; H, 4.71; N, 11.56. Found: C, 65.87; H, 4.54; N, 11.58.

*General method for the synthesis of compounds **13a–f***
A mixture of the appropriate sulfanyltriazolylmethoxynaphthalenol **12a–c** (1.0 mmol) and anhydrous potassium carbonate (0.14 g, 1.0 mmol) in dry acetone (10 mL) was treated with iodomethane or iodoethane (1.0 mmol) and stirred at room temperature for 24 h. The reaction mixture was evaporated to dryness under reduced pressure, water (30 mL) was added and the yellow precipitates were filtered, washed with water, air dried, and recrystallized from acetonitrile.

*4-Methoxy-2-[5-(methylsulfanyl)-4-phenyl-4H-1,2,4-triazol-3-yl]naphthalen-1-ol (**13a**)*
Yield 62%; mp: 199–200°C; IR (ATR, υ cm^{-1}): 3145 (OH), 1632 (C=N), 1256, 1159 (C–S–C), 1227, 1024 (C–O–C); ^{1}H NMR (300 MHz, $CDCl_3$): 2.75 (s, 3H, SCH_3); 3.25 (s, 3H, OCH_3); 5.97 (s, 1H, naphthalenyl-C_3-H); 7.41–7.45 (m, 2H, phenyl-$C_{2,6}$-H); 7.48–7.54 (m, 2H, naphthalenyl-$C_{6,7}$-H); 7.60–7.69 (m, 3H, phenyl-$C_{3,4,5}$-H); 8.02–8.05 (m, 1H, naphthalenyl-C_8-H);

8.42–8.45 (m, 1H, naphthalenyl-C_5-H); 12.22 (s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$): 14.4, 54.5, 99.3, 101.6, 121.4, 123.5, 126.0, 126.2, 126.8, 127.3, 128.0, 130.3, 130.5, 134.7, 147.2, 149.7, 153.5, 154.2. Anal. calcd. for $C_{20}H_{17}N_3O_2S$: C, 66.10; H, 4.71; N, 11.56. Found: C, 66.38; H, 4.68; N, 11.65. HRMS (ESI): *m/z* calcd. for $C_{20}H_{18}N_3O_2S$: 364.1120 $[M+H]^+$; found: 364.1120.

*2-[5-(Ethylsulfanyl)-4-phenyl-4H-1,2,4-triazol-3-yl]-4-methoxynaphthalen-1-ol (**13b**)*
Yield 75%; mp: 163–164°C; IR (ATR, υ cm^{-1}): 3155 (OH), 1632 (C=N), 1256, 1161 (C–S–C), 1236, 1026 (C–O–C); ^{1}H NMR (300 MHz, $CDCl_3$): 1.47 (t, 3H, $J = 7.2$ Hz, SCH_2CH_3); 3.29 (q, 2H, $J = 7.2$ Hz, SCH_2CH_3); 3.25 (s, 3H, OCH_3); 5.98 (s, 1H); 7.40–7.43 (m, 2H); 7.51–7.54 (m, 2H); 7.60–7.64 (m, 3H); 8.02–8.05 (m, 1H); 8.41–8.45 (m, 1H); 12.20 (s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$): 14.6, 26.8, 54.5, 99.4, 101.6, 121.4, 123.5, 126.0, 126.2, 126.8, 127.3, 128.1, 130.3, 130.4, 134.8, 147.2, 149.8, 152.6, 154.1. Anal. calcd. for $C_{21}H_{19}N_3O_2S$: C, 66.82; H, 5.07; N, 11.13. Found: C, 66.52; H, 5.05; N, 11.12. HRMS (ESI): *m/z* calcd. for $C_{21}H_{20}N_3O_2S$: 378.1276 $[M+H]^+$; found: 378.1276.

*2-[4-(4-Chlorophenyl)-5-(methylsulfanyl)-4H-1,2,4-triazol-3-yl]-4-methoxynaphthalen-1-ol (**13c**)*
Yield 87%; mp: 185–186°C; IR (ATR, υ cm^{-1}): 3109 (OH), 1639 (C=N), 1252, 1169 (C–S–C), 1227, 1024 (C–O–C); ^{1}H NMR (300 MHz, $CDCl_3$): 2.66 (s, 3H, SCH_3); 3.46 (s, 3H, OCH_3); 6.25 (s, 1H); 7.55–7.58 (m, 2H); 7.61–7.63 (m, 2H); 7.67–7.70 (m, 2H); 7.99–8.02 (m, 1H); 8.20–8.23 (m, 1H); 11.35 (s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$): 14.4, 54.5, 99.1, 101.2, 121.5, 123.5, 126.0, 126.3, 126.9, 127.5, 129.3, 130.5, 133.1, 140.4, 147.3, 149.8, 153.2, 154.2. Anal. calcd. for $C_{20}H_{16}ClN_3O_2S$: C, 60.37; H, 4.05; N, 10.56. Found: C, 60.10; H, 4.02; N, 10.83. HRMS (ESI): *m/z* calcd. for $C_{20}H_{17}ClN_3O_2S$: 398.0730 $[M+H]^+$; found: 398.0730.

*2-[4-(4-Chlorophenyl)-5-(ethylsulfanyl)-4H-1,2,4-triazol-3-yl]-4-methoxynaphthalen-1-ol (**13d**)*
Yield 84%; mp: 154–155°C; IR (ATR, υ cm^{-1}): 3097 (OH), 1636 (C=N), 1254, 1161 (C–S–C), 1227, 1022 (C–O–C); ^{1}H NMR (300 MHz, $CDCl_3$): 1.47 (t, 3H, $J = 7.5$ Hz, SCH_2CH_3); 3.32 (q, 2H, $J = 7.5$ Hz, SCH_2CH_3); 3.34 (s, 3H, OCH_3); 5.92 (s, 1H); 7.35 (d, 2H, $J = 8.4$ Hz); 7.51–7.54 (m, 2H); 7.60 (d, 2H, $J = 8.4$ Hz); 8.04–8.06 (m, 1H); 8.41–8.44 (m, 1H); 11.99 (s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$): 14.6, 26.9, 54.6, 99.2, 101.2, 121.4, 123.5, 126.0, 126.3, 126.9, 127.5, 129.4, 130.5, 133.2, 136.7, 147.3, 149.8, 152.4, 154.0. Anal. calcd. for $C_{21}H_{18}ClN_3O_2S$: C, 61.23; H, 4.40; N, 10.20. Found: C, 61.36; H, 4.27; N, 9.97. HRMS (ESI): *m/z* calcd. for $C_{21}H_{19}ClN_3O_2S$: 412.0887 $[M+H]^+$; found: 412.0887.

*4-Methoxy-2-[5-(methylsulfanyl)-4-(4-tolyl)-4H-1,2,4-triazol-3-yl]naphthalen-1-ol (**13e**)*
Yield 58%; mp: 195–196°C; IR (ATR, υ cm^{-1}): 3138 (OH), 1634 (C=N), 1250, 1163 (C–S–C), 1225, 1024 (C–O–C); ^{1}H NMR (300 MHz, DMSO-d_6): 2.39 (s, 3H, C_6H_4-CH_3); 2.65 (s, 3H, SCH_3); 3.33 (s, 3H, OCH_3); 6.17 (s, 1H); 7.41–7.48 (m, 4H); 7.54–7.57 (m, 2H); 7.96–8.00 (m, 1H); 8.21–8.25 (m, 1H); 11.78 (s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6): 14.7, 21.1, 55.0, 101.2, 103.5, 121.7, 123.3, 125.7, 126.4, 126.9, 127.9, 128.2, 131.0, 131.9, 140.9, 147.1, 148.3, 153.4, 154.1. Anal. calcd. for $C_{21}H_{19}N_3O_2S$: C, 66.82; H, 5.07; N, 11.13. Found: C, 66.78; H, 4.82; N, 11.24. HRMS (ESI): *m/z* calcd. for $C_{21}H_{20}N_3O_2S$: 378.1276 $[M+H]^+$; found: 378.1276.

*2-[5-(Ethylsulfanyl)-4-(4-tolyl)-4H-1,2,4-triazol-3-yl]-4-methoxynaphthalen-1-ol (**13f**)*
Yield 75%; mp: 149–150°C; IR (ATR, υ cm^{-1}): 3134 (OH), 1637 (C=N), 1252, 1167 (C–S–C), 1229, 1024 (C–O–C); ^{1}H NMR (300 MHz, DMSO-d_6): 1.35 (t, 3H, $J = 7.3$ Hz, SCH_2CH_3); 2.40 (s, 3H, C_6H_4-CH_3); 3.18 (q, 2H, $J = 7.3$ Hz, SCH_2CH_3); 3.34 (s, 3H, OCH_3); 6.18 (s, 1H); 7.40–7.50 (m, 4H); 7.54–7.58 (m, 2H); 7.98–8.00 (m, 1H); 8.23–8.26 (m, 1H); 11.81 (s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6): 15.2, 21.1, 26.9, 55.0, 101.2, 103.5, 121.6, 123.3, 125.7, 126.4, 126.9, 127.9, 128.3, 131.0, 132.0, 140.8, 147.1, 148.3, 152.4, 154.0. Anal. calcd. for $C_{22}H_{21}N_3O_2S$: C, 67.50; H, 5.41; N, 10.73. Found: C, 67.42; H, 5.38; N, 10.72.

*General method for the synthesis of compounds **14a–f***
A cold solution of ceric ammonium nitrate (1.64 g, 2.99 mmol) in water (3 mL) was added gradually to a stirred ice-cool suspension of sulfanyltriazolyl-methoxynaphthalenols **13a–f** (1.0 mmol) in acetonitrile (20 mL). The reaction mixture was stirred in an ice bath for 5 min, then treated with crushed ice. The yellow precipitates were filtered, washed with water, air dried, and recrystallized from cyclohexane.

*2-[5-(Methylsulfanyl)-4-phenyl-4H-1,2,4-triazol-3-yl]-naphthalene-1,4-dione (**14a**)*
Yield 76%; mp: 177–178°C with decomposition; IR (ATR, υ cm^{-1}): 1666 (C=O), 1622 (C=N), 1254, 1128 (C–S–C); ^{1}H NMR (300 MHz, $CDCl_3$): 2.76 (s, 3H, SCH_3); 7.27–7.29 (m, 2H); 7.40–7.42 (m, 4H); 7.66–7.76 (m, 2H); 7.85 (d, 1H, $J = 7.8$ Hz); 8.07 (d, 1H, $J = 6.9$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): 14.6, 126.3, 126.4, 126.7, 129.7, 129.8, 131.4, 131.7, 133.6, 134.1, 134.2, 136.7, 139.6, 150.2, 155.2, 181.3, 183.9. Anal. calcd. for $C_{19}H_{13}N_3O_2S$: C, 65.69; H, 3.77; N, 12.10. Found: 65.52; H, 3.79; N, 12.00. HRMS (ESI): *m/z* calcd. for $C_{19}H_{14}N_3O_2S$: 348.0807 $[M+H]^+$; found: 348.0807.

*2-[5-(Ethylsulfanyl)-4-phenyl-4H-1,2,4-triazol-3-yl]-naphthalene-1,4-dione (**14b**)*
Yield 86%; mp: 186–187°C with decomposition; IR (ATR, υ cm^{-1}): 1664 (C=O), 1620 (C=N), 1254, 1124 (C–S–C); ^{1}H NMR (300 MHz, $CDCl_3$): 1.46 (t, 3H, $J = 7.2$ Hz, SCH_2CH_3); 3.33 (q, 2H, $J = 7.2$ Hz, SCH_2CH_3); 7.26–7.29 (m, 2H); 7.41–7.42 (m, 4H); 7.65–7.76 (m, 2H); 7.84 (d, 1H, $J = 6.9$ Hz); 8.07 (d, 1H, $J = 6.9$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): 14.6, 26.9, 126.3, 126.4, 126.7, 129.6, 129.6, 131.4, 131.7, 133.7, 134.1, 134.1, 136.8, 139.5, 150.0, 154.5, 181.2, 183.8. Anal. calcd. for $C_{20}H_{15}N_3O_2S$: C, 66.46; H, 4.18; N, 11.63. Found: C, 66.71; H, 4.27; N, 11.38. HRMS (ESI): *m/z* calcd. for $C_{20}H_{16}N_3O_2S$: 362.0963 $[M+H]^+$; found: 362.0963.

*2-[4-(4-Chlorophenyl)-5-(methylsulfanyl)-4H-1,2,4-triazol-3-yl]naphthalene-1,4-dione (**14c**)*
Yield 82%; mp: 301–302°C with decomposition; IR (ATR, υ cm^{-1}): 1661 (C=O), 1622 (C=N), 1251, 1126 (C–S–C); ^{1}H NMR (300 MHz, $CDCl_3$): 2.76 (s, 3H, SCH_3); 7.24 (d, 2H, $J = 8.1$ Hz); 7.40–7.43 (m, 3H); 7.71–7.76 (m, 2H); 7.88 (d, 1H, $J = 7.2$ Hz); 8.08 (d, 1H, $J = 7.2$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): 14.7, 126.4, 126.8, 127.7, 130.0, 131.3, 131.7, 132.1, 134.2, 134.3, 135.9, 136.3, 139.8, 150.1, 155.2, 181.3, 183.7. Anal. calcd. for $C_{19}H_{12}ClN_3O_2S$: C, 59.76; H, 3.17; N, 11.00. Found: C, 59.93; H, 2.91; N, 10.75.

*2-[4-(4-Chlorophenyl)-5-(ethylsulfanyl)-4H-1,2,4-triazol-3-yl]naphthalene-1,4-dione (**14d**)*
Yield 97%; mp: 135–136°C with decomposition; IR (ATR, υ cm^{-1}): 1668 (C=O), 1616 (C=N), 1250, 1124 (C–S–C); ^{1}H NMR (300 MHz, $CDCl_3$): 1.45 (t, 3H, $J = 7.2$ Hz, SCH_2CH_3); 3.33 (q, 2H, $J = 7.2$ Hz, SCH_2CH_3); 7.22 (d, 2H, $J = 8.4$ Hz); 7.38–7.42 (m, 3H); 7.70–7.77 (m, 2H); 7.87 (d, 1H, $J = 7.2$ Hz); 8.07 (d, 1H, $J = 6.9$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): 14.6, 27.0, 126.4, 126.8, 127.8, 129.9, 131.3, 131.7, 132.2, 134.2, 134.3, 135.8, 136.4, 139.8, 150.0, 154.5, 181.3, 183.7. Anal. calcd. for $C_{20}H_{14}ClN_3O_2S$: C, 60.68; H, 3.56; N, 10.61. Found: C, 60.75; H, 3.28; N, 10.37.

*2-[5-(Methylsulfanyl)-4-(4-tolyl)-4H-1,2,4-triazol-3-yl]-naphthalene-1,4-dione (**14e**)*
Yield 78%; mp: 189–190°C with decomposition; IR (ATR, υ cm^{-1}): 1666 (C=O), 1636 (C=N), 1254, 1126 (C–S–C); ^{1}H NMR (300 MHz, $CDCl_3$): 2.35 (s, 3H, C_6H_4-CH_3); 2.75 (s, 3H, SCH_3); 7.15 (d, 2H, $J = 8.4$ Hz); 7.22 (d, 2H, $J = 8.4$ Hz); 7.26 (s, 1H); 7.66–7.76 (m, 2H); 7.89 (d, 1H, $J = 7.5$ Hz); 8.06 (d, 1H, $J = 7.2$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): 14.6, 21.1, 126.1, 126.3, 126.8, 130.3, 130.9, 131.5, 131.8, 134.1, 134.1, 136.7, 139.5, 140.0, 150.3, 155.2, 181.3, 183.8. Anal. calcd. for $C_{20}H_{15}N_3O_2S$: C, 66.46; H, 4.18; N, 11.63. Found: C, 66.75; H, 3.93; N, 11.44.

*2-[5-(Ethylsulfanyl)-4-(4-tolyl)-4H-1,2,4-triazol-3-yl]-naphthalene-1,4-dione (**14f**)*
Yield 86%; mp: 188–189°C with decomposition; IR (ATR, υ cm^{-1}): 1666 (C=O), 1614 (C=N), 1250, 1126 (C–S–C); ^{1}H NMR (300 MHz, $CDCl_3$): 1.44 (t, 3H, $J = 7.2$ Hz, SCH_2CH_3); 2.35 (s, 3H, C_6H_4-CH_3); 3.32 (q, 2H, $J = 7.2$ Hz, SCH_2CH_3); 7.14 (d, 2H, $J = 8.4$ Hz); 7.21 (d, 2H, $J = 8.4$ Hz); 7.26 (s, 1H); 7.66–7.76 (m, 2H); 7.88 (d, 1H, $J = 7.8$ Hz); 8.06 (d, 1H, $J = 6.6$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): 14.6, 21.1, 26.9, 126.2, 126.3, 126.8, 130.2, 131.0, 131.5, 131.7, 134.1, 134.1, 136.9, 139.5, 139.9, 150.0, 154.6, 181.3, 183.8. Anal. calcd. for $C_{21}H_{17}N_3O_2S$: C, 67.18; H, 4.56; N, 11.19. Found: C, 67.08; H, 4.37; N, 10.89. HRMS (ESI): *m/z* calcd. for $C_{21}H_{18}N_3O_2S$: 376.1120 $[M+H]^+$; found: 376.1120.

*General method for the synthesis of compounds **15a** and **15b***
A solution of 3-amino-1,4-dihydro-1,4-dioxonaphthalene-2-carbohydrazide **7** (2.31 g, 10.0 mmol) in absolute ethanol (50 mL) was treated gradually with a solution of the appropriate aryl isothiocyanate in absolute ethanol (30 mL). The reaction mixture was heated under reflux while stirring for 2 h then set aside to attain room temperature and the formed yellow precipitates were filtered, washed with cold 50% ethanol, air dried, and recrystallized from ethanol.

*2-(3-Amino-1,4-dioxo-1,4-dihydronaphthalene-2-carbonyl)-N-phenylhydrazine-1-carbothioamide (**15a**)*
Yield 69%; mp: 224–225°C. IR (ATR, υ cm^{-1}): 3302, 3177 (NH_2, υNH), 1688 (C=O quinone), 1634 (C=O amide), 1528, 1288, 1115, 999 (N–C=S thioamide I–IV bands, respectively). ^{1}H NMR (300 Mz, DMSO-d_6): (NH_2, under DMSO); 7.11 (d, 1H, $J = 7.2$ Hz, phenyl-C_4-H); 7.31 (t, 2H, $J = 7.8$ Hz, phenyl-$C_{2,6}$-H); 7.53 (t, 2H, $J = 7.8$ Hz, phenyl-$C_{3,5}$-H); 7.78 (t, 1H, $J = 7.2$ Hz, naphthalenyl-C_6-H); 7.90 (t, 1H, $J = 7.05$ Hz, naphthalenyl-C_7-H); 8.07 (d, 1H, $J = 7.5$ Hz, naphthalenyl-C_8-H); 8.12 (d, 1H, $J = 7.5$ Hz, naphthalenyl-C_5-H); 8.84, 9.82, 10.30 (three s, each 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 99.7, 117.5, 124.9, 126.3, 126.6, 127.0, 128.7, 129.4, 130.0, 133.3, 133.8, 136.2, 139.6, 155.0, 180.4, 181.0. Anal. calcd. for $C_{18}H_{14}N_4O_3S$: C, 59.01; H, 3.85; N, 15.29. Found: C, 58.75; H, 3.62; N, 15.10. HRMS (ESI): *m/z* calcd. for $C_{18}H_{15}N_4O_3S$: 367.0865 $[M+H]^+$; found: 367.0866.

*2-(3-Amino-1,4-dioxo-1,4-dihydronaphthalene-2-carbonyl)-N-(4-chlorophenyl)hydrazine-1-carbothioamide (**15b**)*
Yield 56%; mp: 243–244°C. IR (ATR, υ cm^{-1}): 3356, 3231 (NH_2, υNH), 1690 (C=O quinone), 1647 (C=O amide), 1541, 1290, 1157, 997 (N–C=S thioamide I–IV bands, respectively). ^{1}H NMR (300 MHz, DMSO-d_6): (NH_2, under DMSO); 7.35 (d, 2H, $J = 9$ Hz); 7.57 (d, 2H, $J = 8.4$ Hz); 7.75–7.80 (m, 1H); 7.88–7.93 (m, 1H); 8.01–8.04 (m, 1H); 8.11–8.13 (m, 1H); 8.85, 9.86, 10.30 (three s, each 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 109.9, 119.0, 126.4, 126.5, 126.6, 127.0, 128.5, 129.3, 130.0, 133.3, 133.8, 136.2, 138.6, 155.0, 180.3, 181.0. Anal. calcd. for $C_{18}H_{13}ClN_4O_3S$: C, 53.94; H, 3.27; N, 13.98. Found: C, 54.22; H, 3.01; N, 13.76. HRMS (ESI): *m/z* calcd. for $C_{18}H_{14}ClN_4O_3S$: 401.0475 $[M+H]^+$; found: 401.0476.

Biology

Preliminary in vitro one-dose antitumor screening
Eight compounds, **10b**, **10d**, **13a**, **13c**, and **14a–d**, were selected by the NCI *in vitro* disease-oriented human cells screening panel assay to be evaluated for their *in vitro* anticancer activity. Primary *in vitro* one-dose anticancer assay was performed using the full NCI 60-cell line panel in accordance with the current protocol of the Drug Evaluation Branch, NCI, Bethesda, Maryland, USA [31, 32]. These cell lines were incubated with one concentration (10 μM) for each tested compound. A 48-h continuous compound exposure protocol was used and a sulforhodamine B (SRB) protein assay was employed to estimate cell viability or growth (Supporting Information Table S1). Two derivatives **10b** and **10d** were selected from this primary anticancer assay and consequently carried over to the five-dose screen against a panel of about 60 different cancer cell lines.

Full in vitro five-dose antitumor assay
A total of 60 cell lines of nine cancer subpanels including leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate and breast cancer cell lines were utilized. The human cancer cell lines of the cancer screening panel were grown in RPMI-1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells were inoculated into 96-well microtiter plates in 100 μL at plating densities ranging from 5000 to 40000 cells/well depending on the doubling time of individual cell lines. 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 compounds (0.01–100 μM for each tested compound). After 24 h, two plates of each cell line were fixed *in situ* with trichloroacetic acid (TCA), to represent a measurement of the cell population for each cell line at the time of compound addition. Experimental 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 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. Additional four, 10-fold or 1/2 log serial dilutions were made to provide a total of five compound concentrations plus control. Aliquots of 100 μL of these different compound dilutions were added to the appropriate microtiter wells already containing 100 μL of medium resulting in the required final compound concentrations. Doses start at 100 μM and go down in 1 log increments. Following compound addition, the plates were incubated for an additional 48 h at 37°C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 μL of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 1 h at 4°C. The supernatant was discarded and the plates were washed five times with tap water and air dried. 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 was 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 (final concentration, 16% TCA) [31, 32].

Cell culture
Human A549 non-small lung carcinoma cells were cultured in RPMI 1640 supplemented with 10% FCS, glutamine, and antibiotics as previously described [33].

Indirect immunofluorescence and cell cycle
Progression through the cell cycle analysis was assessed by flow cytometry DNA determination with propidium iodide. A549 cells were incubated for 20 h with drug vehicle or serial concentrations of **10b** and **10d** and with taxol as a control. Cells were fixed, treated with RNase, and stained with propidium iodide as previously described [34]. Analysis was performed in a Coulter Epics XL flow cytometer.

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