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Novel 5-(2-hydroxyphenyl)-3-substituted-2,3-dihydro-1,3,4-oxadiazole-2-thione derivatives: Promising anticancer agents

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Abstract—A series of 5-(2-hydroxyphenyl)-3-substituted-2,3-dihydro-1,3,4-oxadiazole-2-thione derivatives was synthesized and 13 of them were selected by the National Cancer Institute (NCI) and evaluated for their in vitro anticancer activity. Seven of the investigated compounds, **3i**, **3j**, **3k**, **3o**, **3p**, **3q**, and **3r**, displayed high anticancer activity in the primary assay. These compounds have been selected for a full anticancer screening against a 60-cell panel assay where they showed non-selective broad spectrum and promising activity against all cancer cell lines. Compounds **3j** and **3k** proved to be the active members in this study compared to 5-fluorouracil and cyclophosphamide as reference drugs, respectively. Compounds **3j** and **3k** were identified as promising lead compounds.

1. Introduction

Of the various human diseases, cancer has proven to be one of the most intractable diseases to which humans are subjected, and as yet no practical and generally effective drugs or methods of control are available. Therefore, identification of novel potent, selective, and less toxic anticancer agents remains one of the most pressing health problems.[1]

Salicylate derivatives are well known for their anti-inflammatory activity[2] and more recently have been discovered to have anticancer effect.[3–5] Furthermore, certain 1,3,4-oxadiazole derivatives and their Mannich bases were reported to possess anti-inflammatory,[6] antitubercular,[7] antifungal,[8] and anticancer activities.[9–12] Therefore, in the present paper we planned to incorporate the salicylate moiety with Mannich bases of 1,3,4-oxadiazole to combine the benefits of their effects to give a compact structure with expected anticancer activity.

2. Results and discussion

2.1. Chemistry

5-(2-Hydroxyphenyl)-3-substituted-2,3-dihydro-1,3,4-oxadiazole-2-thione derivatives **3a**–**v** were prepared according to the procedure depicted in Scheme 1. The precursor 5-(2-hydroxyphenyl)-2,3-dihydro-1,3,4-oxadiazole-2-thione **2** was prepared according to a previously reported method[13] by reaction of salicylic acid hydrazide **1** with CS_2 in KOH. When reacted with formaldehyde and different primary and secondary amines afforded the target compounds **3a**–**v** in good yield. The identities of the compounds obtained were confirmed by elemental analyses, IR, ^{1}H NMR, ^{13}C NMR, and mass spectral data. The IR spectra (KBr) of compounds **3a**–**v** generally showed the characteristic bands corresponding to the hydroxyl group, thione function, in addition to the amine moieties. The ^{1}H NMR spectra of compounds **3a**–**v** displayed a singlet signal corresponding to CH_2 protons, broad exchangeable singlet due to OH proton, in addition to the aromatic protons of hydroxyphenyl moiety. All other aromatic and aliphatic protons were observed at expected regions. Furthermore, the ^{13}C NMR and mass spectral data are in accordance with the expected structures of the obtained compounds (for details of the physical data, see Section 4).

Keywords: Anticancer activity; 1,3,4-Oxadiazoles; Mannich bases; Salicylate analogs.
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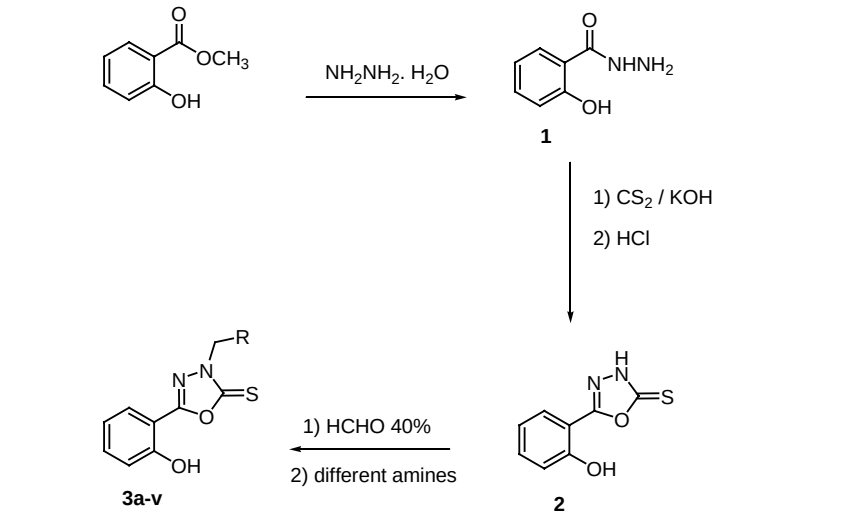

Where; R=

3a = 1-morpholine	**3i** = -NH-C_6H_4(2-Cl)	**3q** = -NH-C_6H_4(4-$COCH_3$)
3b = 1-phenylpiperazine	**3j** = -NH-C_6H_4(3-Cl)	**3r** = -NH-C_6H_3(2-OH-4-COOH)
3c = -NH-C_6H_5	**3k** = -NH-C_6H_4(4-Cl)	**3s** = -N(C_2H_5)-C_6H_5
3d = -NH-C_6H_4(3-CH_3)	**3l** = -NH-C_6H_4(4-Br)	**3t** = -N(C_6H_5)$_2$
3e = -NH-C_6H_4(4-CH_3)	**3m** = -NH-C_6H_4(3-NO_2)	**3u** = -NH-(2-pyridine)
3f = -NH-C_6H_4(2-OCH_3)	**3n** = -NH-C_6H_4(4-NO_2)	**3v** = -NH-(3-pyridine)
3g = -NH-C_6H_4(4-OCH_3)	**3o** = -NH-C_6H_4(2-COOH)	
3h = -NH-C_6H_4(2-OC_2H_5)	**3p** = -NH-C_6H_4(4-COOH)	

Scheme 1. The synthetic pathway of compounds **3a**–**v**.

2.2. Antitumor activity

Out of the synthesized 5-(2-hydroxyphenyl)-3-substituted-2,3-dihydro-1,3,4-oxadiazole-2-thione derivatives, compounds **3a**, **3b**, **3f**, **3g**, **3i**, **3j**, **3k**, **3o**, **3p**, **3q**, **3r**, **3u**, and **3v** were chosen by NCI as prototypes. They were evaluated in the 3-cell line panel consisting of NCI-H460 (lung), MCF7 (breast), and SF-268 (CNS). Primary anticancer assay was performed in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda.[14–16] The compounds were added at a single concentration (10^{-4} M) and the culture was incubated for 48 h. End point determinations were made with a protein binding dye, sulforhodamine B (SRB). Results (Table 1) for each compound were reported as the growth percentage of the treated cells when compared to that of the untreated control cells. All the compounds, which reduced the growth of any one of the cell lines to 32% or less, were selected for further evaluation in the full panel of 60 human tumor cell lines. As shown in Table 1 seven compounds **3i**, **3j**, **3k**, **3o**, **3p**, **3q**, and **3r** of the 13 tested compounds have been selected for a 60-cell panel assay.

The cytotoxic and/or growth inhibitory effects of the compounds were tested in vitro against the panel of 60 human tumor cell lines derived from nine neoplastic diseases at 10-fold dilutions of five concentrations ranging from 10^{-4} to 10^{-8} M. The growth percentage was evaluated spectrophotometrically versus controls not treated with test agents. For each tested compound, three response parameters, GI_{50} (50% growth inhibition and signifies the growth inhibitory power of the test agent), TGI (which is the drug concentra-

Table 1. Primary in vitro growth inhibition assay results at 10^{-4} M concentration

Compound	NCI No.	Growth percentage			60-tumor cell line selection
		(Breast) MCF7	(Non-Small Cell Lung) NCI-H460	(CNS) SF-268	
3a	S731983	97	54	93	N
3b	S731984	76	39	93	N
3f	S731993	76	99	97	N
3g	S731994	80	64	91	N
3i	S731988	55	28	102	Y
3j	S731990	44	15	72	Y
3k	S731989	34	10	74	Y
3o	S731991	32	9	72	Y
3p	S731992	39	11	83	Y
3q	S731987	45	19	109	Y
3r	S731995	58	29	109	Y
3u	S731997	69	56	90	N
3v	S731996	104	74	116	N

N, not selected; Y, yes selected.

Table 2. In vitro tumor 50% growth inhibition (GI_{50}, M) of **3i**, **3j**, **3k**, **3o**, **3p**, **3q**, and **3r**

Panel/cell line	**3i** $\log_{10}$ GI_{50}	**3j** $\log_{10}$ GI_{50}	**3k** $\log_{10}$ GI_{50}	**3o** $\log_{10}$ GI_{50}	**3p** $\log_{10}$ GI_{50}	**3q** $\log_{10}$ GI_{50}	**3r** $\log_{10}$ GI_{50}
Leukemia							
CCRF-CEM	−4.70	−5.44	>−4.00	−4.97	−5.13	—	—
HL-60(TB)	—	—	—	−4.19	−4.20	>−4.00	>−4.00
K-562	>−4.00	−4.42	−6.58	−4.15	−4.39	>−4.00	>−4.00
MOLT-4	—	<−8.00	−4.68	−5.00	−5.33	−6.06	−4.58
RPMI-8226	−4.69	−4.82	>−4.00	−5.38	>−4.00	—	—
SR	−4.60	−4.82	−4.80	—		—	—
Non-small cell lung cancer							
A549/ATCC	>−4.00	−4.13	−4.58	−4.29	−4.37	−4.42	>−4.00
EKVX	>−4.00	−4.17	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
HOP-62	>−4.00	−4.04	>−4.00	−4.41	−4.33	>−4.00	>−4.00
HOP-92	−4.79	−5.73	−4.05	−4.52	−4.47	−4.37	>−4.00
NCI-H226	>−4.00	−4.27	−4.85	−4.06	>−4.00	−4.56	−4.68
NCI-H23	>−4.00	−4.64	−6.29	−4.40	−4.29	−4.03	>−4.00
NCI-H322M	>−4.00	−5.26	−5.05	>−4.00	>−4.00	>−4.00	>−4.00
NCI-H460	−4.43	−4.69	−4.59	−4.40	−4.39	4.40	−4.02
NCI-H522	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
Colon cancer							
COLO 205	−4.57	−4.27	−5.15	−4.30	−4.31	−4.36	−4.37
HCC-2998		—	—	−4.47	−4.53	−4.56	>−4.00
HCT-116	−4.53	−4.60	−4.74	−4.59	−4.63	>−4.00	−4.35
HCT-15	−4.75	—	−4.70	−4.61	−4.60	−4.63	−4.49
HT29	−4.68	−4.73	−4.62	−4.46	−4.41	−4.33	>−4.00
KM12	>−4.00	−4.43	−4.93	>−4.00	>−4.00	>−4.00	>−4.00
SW-620	−4.38	−4.57	−4.65	−4.47	−4.41	−4.53	−4.38
CNS cancer							
SF-268	>−4.00	−4.23	−5.04	−4.09	−4.02	>−4.00	>−4.00
SF-295	−4.19	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
SF-539	−4.42	−4.56	−4.62	—	—	—	—
SNB-19	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
U251	−4.50	−5.31	−4.50	−4.32	−4.37	−4.29	−4.11
Melanoma							
LOX IMVI	−4.72			−4.71	−4.70	−4.64	−4.06
M14	>−4.00	−4.45	−4.55	−4.33	−4.27	>−4.00	>−4.00
SK-MEL-2	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.33
SK-MEL-28	—	—	—	>−4.00	>−4.00	>−4.00	>−4.00
SK-MEL-5	>−4.00	−4.05	−5.64	−4.13	>−4.00	−4.25	>−4.00
UACC-257	>−4.00	>−4.00	−4.69	>−4.00	>−4.00	>−4.00	>−4.00
UACC-62	−4.14	−4.38	−6.20	−4.47	−4.47	−4.45	−4.40
Ovarian							
IGR-OV1	>−4.00	−4.09	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
OVCAR-3	−4.05	−4.52	−4.63	>−4.00	>−4.00	>−4.00	>−4.00
OVCAR-5	−4.43	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
OVCAR-8	>−4.00	>−4.00	−4.52	>−4.00	>−4.00	>−4.00	>−4.00
SK-OV-3	>−4.00	>−4.00	>−4.00	—	—	—	—
Renal							
786-0	>−4.00	−4.17	>−4.00	−4.45	−4.40	−4.13	−4.02
A498	>−4.00	−4.22	>−4.00	—	—	—	—
ACHN	−4.77	—	<−8.00	−4.57	−4.56	−4.53	−4.50
CAKI-1	>−4.00	−4.18	−5.43	>−4.00	>−4.00	>−4.00	>−4.00
RXF 393	>−4.00	−4.08	−4.70	−4.16	−4.17	−4.61	>−4.00
SN12C	−4.11	>−4.00	−4.12	−4.25	−4.30	−4.57	−5.05
TK-10	−4.37	−4.88	−5.82	>−4.00	>−4.00	−4.17	>−4.00
UO-31	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
Prostate							
PC-3	−4.00	−4.48	>−4.00	−4.36	−4.22	−4.33	−4.06
Breast cancer							
MCF7	−4.40	−4.42	−5.38	−4.33	−4.31	>−4.00	>−4.00
NCI/ADR-RES	−4.74	−6.43	−4.72	−4.27	−4.20	>−4.00	>−4.00
MDA-MB-231/ATCC	>−4.00	−4.22	>−4.00	−4.77	−4.79	−4.42	−4.41
HS 578T	>−4.00	−4.16	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
MDA-MB-435	>−4.00	−4.11	−5.49	>−4.00	>−4.00	>−4.00	>−4.00

Table 2 (*continued*)

Panel/cell line	**3i** $\log_{10}$ GI_{50}	**3j** $\log_{10}$ GI_{50}	**3k** $\log_{10}$ GI_{50}	**3o** $\log_{10}$ GI_{50}	**3p** $\log_{10}$ GI_{50}	**3q** $\log_{10}$ GI_{50}	**3r** $\log_{10}$ GI_{50}
BT-549	>−4.00	−4.02	−4.21	−4.13	−4.22	−4.25	>−4.00
T-47D	−4.87	−4.54	−4.24	−4.16	>−4.00	−4.07	>−4.00
MG-MID	−4.23	−4.50	−4.68	−4.28	−4.25	−4.22	−4.13
Delta	0.64	3.50	3.32	1.10	1.08	1.83	0.92
Range	0.87	4.00	4.00	1.38	1.33	2.06	1.05

tion resulting in total growth inhibition and signifies the cytostatic effect of the test agent), and LC_{50} (50% lethal concentration and signifies the cytotoxic effect of the test agent), were calculated for each cell line. The $\log_{10}$ GI_{50}, $\log_{10}$ TGI, and $\log_{10}$ LC_{50} were then determined, defined as the mean of the $\log_{10}$'s of the individual GI_{50}, TGI, and LC_{50} values as shown in Tables 2–4, respectively. Negative values indicated the most sensitive cell lines. Compounds having $\log_{10}$ GI_{50} values −4 and <−4 were declared to be active. The panel cell lines used in the NCI screen were leukemia (L) lines, non-small cell lung cancer (NSCLC) lines, colon cancer (CL) lines, central nervous system cancer (CNSC) lines, melanoma (M) lines, ovarian cancer (OC) lines, renal cancer (RC) lines, prostate cancer (PC) lines, and breast cancer (BC) lines. The details of the cell lines used are shown in Table 2.

From Table 2, we can conclude that, all the active compounds in this test showed broad spectrum antitumor activity against the nine tumor subpanels tested. The Mannich bases with chloro substituents on the aromatic amines are the most active compounds (**3i**–**3k**) especially when the chloro atom is in the *meta* or *para* position. Also compounds having free carboxylic acid moiety (**3o**, **3p**, and **3r**) have high activity but are less than that of the chloro substituent. Compounds **3j** and **3k** demonstrated the most marked effects in the National Cancer Institute's 60 human tumor cell line in vitro screen.

Compound **3j** was the most active compound against the following cell lines: CCRF-CEM, MOLT-4, SR leukemia cell lines; EKVX, HOP-92, NCI-H322M, NCI-H460 non-small cell lung cancer lines; HT29 colon cancer cell line; U251 CNS cell line; IGR-OV1 ovarian cancer cell line; A498 renal cancer cell line; PC-3 prostate cancer cell line and NCI/ADR-RES, HS 578T breast cancer cell lines. For example, compound **3j** showed superpotent activity against the leukemia Molt-4 cell line and the breast cancer NCI/ADR-RES cell line with $\log_{10}$ GI_{50} values of <−8.00 (GI_{50} is less than 10 nM) and −6.43 (GI_{50} is 371 nM), respectively. Furthermore, when compared to 5-fluorouracil as a reference drug, compound **3j** showed more potent activity against the leukemia, non-small cell lung cancer, prostate, and breast cancer cell lines (Table 5).

On the other hand, compound **3k** was the most active compound against the following cell lines: K-562 leukemia cell line; A549/ATCC, NCI-H226, NCI-H23 non-small cell lung cancer lines; COLO 205, HCT-116, KM12, SW-620 colon cancer cell line; SF-268, SF-539 CNS cell line; M14, SK-MEL-5, UACC-257, UACC-62 melanoma cell lines; OVCAR-3, OVCAR-8 ovarian cancer cell line; ACHN, CAKI-1, RXF 393, TK-10 renal cancer cell line and MCF7, MDA-MB-435 breast cancer cell lines. For example, compound **3k** showed superpotent activity against the renal ACHN cell line and the leukemia K-562 cell line with $\log_{10}$ GI_{50} values of <−8.00 (GI_{50} is less than 10 nM) and −6.58 (GI_{50} is 263 nM), respectively. Furthermore, compared to cyclophosphamide as reference drug, compound **3k** showed more potent activity against the colon, CNS, melanoma, and ovarian cancer cell lines (Table 6).

As shown in Tables 3 and 4 all the tested compounds showed favorable safety profiles with TGI and LC_{50} values being generally more than 100 μM ($\log_{10}$ TGI and $\log_{10}$ LC_{50} > −4.00).

Although the NCI screening protocol did not conclude of any possible mechanisms for the observed anticancer activity of the test compounds, their activity may be attributed to an antimitotic action. This is due to their structural similarity to the antimitotic 1,3,4-oxadiazole derivatives[11,12] and thus may act through the same mechanism of action.

3. Conclusions

In conclusion, a series of 5-(2-hydroxyphenyl)-3-substituted-2,3-dihydro-1,3,4-oxadiazole-2-thione derivatives was synthesized as potential anticancer agents. Compounds, **3i**, **3j**, **3k**, **3o**, **3p**, **3q**, and **3r**, were selected for a full 60-cell panel screen where they showed non-selective broad spectrum and promising activity against all cancer cell lines. Compounds **3j** and **3k** proved to be the most promising derivatives identified from this series. Combination of the potent anticancer activity of compounds **3j** and **3k** with their less toxicity and the ease of synthesis makes them promising lead compounds for cancer chemotherapy.[17]

4. Experimental

4.1. Chemistry

Melting points were determined with an electrothermal apparatus (Stuart Scientific, England) and are uncorrected. Monitoring the chemical reactions and purity of the compounds was carried out using thin-layer chro-

Table 3. In vitro tumor total growth inhibition (TGI, M) of **3i**, **3j**, **3k**, **3o**, **3p**, **3q**, and **3r**

Panel/cell line	**3i** $\log_{10}$ TGI	**3j** $\log_{10}$ TGI	**3k** $\log_{10}$ TGI	**3o** $\log_{10}$ TGI	**3p** $\log_{10}$ TGI	**3q** $\log_{10}$ TGI	**3r** $\log_{10}$ TGI
Leukemia							
CCRF-CEM	−4.26	−4.56	>−4.00	−4.44	>−4.00	—	—
HL-60(TB)	—	—	—	>−4.00	>−4.00	>−4.00	>−4.00
K-562	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
MOLT-4	—	>−4.00	−4.13	−4.52	−4.57	>−4.00	−4.09
RPMI-8226	−4.22	−4.41	>−4.00	−4.61	>−4.00	—	—
SR	−4.05	−4.31	−4.36	—	−4.30	—	—
Non-small cell lung cancer							
A549/ATCC	>−4.00	>−4.00	−4.00	>−4.00	>−4.00	>−4.00	>−4.00
EKVX	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
HOP-62	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
HOP-92	−4.18	−4.50	>−4.00	−4.02	−4.08	>−4.00	>−4.00
NCI-H226	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.00	>−4.00
NCI-H23	>−4.00	−4.14	−4.19	>−4.00	>−4.00	>−4.00	>−4.00
NCI-H322M	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
NCI-H460	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
NCI-H522	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
Colon cancer							
COLO 205	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
HCC-2998	−4.13	—	—	−4.06	−4.03	−4.18	>−4.00
HCT-116	>−4.00	>−4.00	−4.29	−4.27	−4.24	>−4.00	>−4.00
HCT-15	−4.41	—	−4.34	−4.19	−4.24	−4.31	−4.03
HT29	−4.20	>−4.00	−4.06	>−4.00	>−4.00	>−4.00	>−4.00
KM12	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
SW-620	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
CNS cancer							
SF-268	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
SF-295	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
SF-539	−4.01	>−4.00	>−4.00	—	—	—	—
SNB-19	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
U251	>−4.00	−4.40	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
Melanoma							
LOX IMVI	−4.45		−4.32	−4.41	−4.42	−4.34	>−4.00
M14	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
SK-MEL-2	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
SK-MEL-28	—	—	—	>−4.00	>−4.00	>−4.00	>−4.00
SK-MEL-5	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
UACC-257	>−4.00	>−4.00	−4.32	>−4.00	>−4.00	>−4.00	>−4.00
UACC-62	>−4.00	>−4.00	>−4.00	−4.03	−4.01	>−4.00	>−4.00
Ovarian							
IGR-OV1	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
OVCAR-3	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
OVCAR-5	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
OVCAR-8	>−4.00	>−4.00	−4.05	>−4.00	>−4.00	>−4.00	>−4.00
SK-OV-3	>−4.00	>−4.00	>−4.00	—	—	—	—
Renal							
786-0	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
A498	>−4.00	>−4.00	>−4.00	—	—	—	—
ACHN	−4.41	—		−4.08	−4.14	−4.14	−4.00
CAKI-1	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
RXF 393	>−4.00	>−4.00	−4.31	>−4.00	>−4.00	−4.14	>−4.00
SN12C	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.19	−4.22
TK-10	>−4.00	−4.06	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
UO-31	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
Prostate							
PC-3	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
Breast cancer							
MCF7	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
NCI/ADR-RES	−4.14	−4.40	−4.10	>−4.00	>−4.00	>−4.00	>−4.00
MDA-MB-231/ATCC	>−4.00	>−4.00	>−4.00	−4.32	−4.23	>−4.00	>−4.00
HS 578T	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
MDA-MB-435	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00

Table 3 (*continued*)

Panel/cell line	**3i** $\log_{10}$ TGI	**3j** $\log_{10}$ TGI	**3k** $\log_{10}$ TGI	**3o** $\log_{10}$ TGI	**3p** $\log_{10}$ TGI	**3q** $\log_{10}$ TGI	**3r** $\log_{10}$ TGI
BT-549	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
T-47D	−4.13	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
MG-MID	−4.05	−4.06	−4.05	−4.06	−4.04	−4.03	−4.01
Delta	0.40	0.51	0.31	0.55	0.53	0.32	0.21
Range	0.45	0.56	0.36	0.61	0.57	0.34	0.22

matography (TLC) using silica gel 60 GF_{245} precoated sheets and ethanol–$CHCl_3$ (2:1) as eluent and was visualized by a UV-lamp at a wavelength (λ) of 254 nm. Elemental analyses were performed on an 'Analytischer Funktionstest vario EL Fab.-Nr. 11982027' (Germany). IR spectra were recorded as KBr disks on a Shimadzu-470 IR spectrophotometer (Japan). ^{1}H NMR spectra were carried out on Varian EM-360L, 60 MHz, (USA) and JEOL JNM-EX 300, 300 MHz (Japan) using $CDCl_3$ or DMSO-d_6 as solvents relative to TMS as internal standard. ^{13}C NMR spectra were recorded on JEOL JNM-EX 300, 75.45 MHz (Japan), TMS was used as internal standard, and $CDCl_3$ or DMSO-d_6 was used as a solvent. Mass spectra were performed on a 'Finnigan SSQ 7000' instrument (USA).

4.1.1. 5-(2-Hydroxyphenyl)-2,3-dihydro-1,3,4-oxadiazole-2-thione (2). A mixture of salicylic acid hydrazide **1** (7.5 g, 0.05 mol), potassium hydroxide (3 g, 0.05 mol), carbon disulfide (10 mL, 0.17 mol), and ethanol (70 mL) was heated under reflux with stirring until the evolution of hydrogen sulfide ceased (~12 h). Ethanol was distilled off under reduced pressure and the residue was dissolved in water and then acidified with dilute hydrochloric acid (10%). The resulting precipitate was filtered, washed with water, dried, and recrystallized from ethanol. Yield: 8.2 g (84.5%), mp: 133–135 °C (reported 135 °C)[13]. IR (KBr), ν_{max}/cm^{-1}: 3550–3250 (OH, NH), 1630 (C=N), 1590 (C=C), 1437 (C=S), 745; ^{1}H NMR (DMSO-d_6), δ ppm: 5.40–7.00 (br s, 1H, NH, exchangeable), 7.50–7.90 (m, 2H), 8.00–8.70 (m, 2H), 11–12 (br s, 1H, OH, exchangeable).

4.1.2. General procedure for the preparation of 5-(2-hydroxyphenyl)-3-substituted-2,3-dihydro-1,3,4-oxadiazole-2-thione 3a–v. Formalin 40% (1.5 mL, 0.02 mol) was added to a stirred solution of 5-(2-hydroxyphenyl)-2,3-dihydro-1,3,4-oxadiazole-2-thione (**2**) (4 g, 0.02 mol) in absolute ethanol (40 mL). An ethanolic solution (10 mL) of the appropriate amine (0.02 mol) was added portionwise to the reaction mixture, stirred for 3 h at room temperature, and left overnight in a refrigerator. The precipitate formed was filtered, washed with cold ethanol, dried, and crystallized from the suitable solvent.

4.1.2.1. 5-(2-Hydroxyphenyl)-3-(morpholinomethyl)-2,3-dihydro-1,3,4-oxadiazole-2-thione (3a). Recrystallization from ethanol; yield: 92%; mp: 184–186 °C; IR (KBr), ν_{max}/cm^{-1}: 3550–3415 (OH), 1623 (C=N), 1592 (aromatic C=C), 1437 (C=S), 1222 and 1036 (C–O–C), 741; ^{1}H NMR ($CDCl_3$), δ ppm: 3.00 (t, 4H; $J \sim 5.0$, 10.0 Hz), 3.85 (t, 4H; $J \sim 5.0$, 10.0 Hz), 5.25 (s, 2H), 7.25 (d, 1H; $J \sim 8.0$ Hz), 7.66 (m, 2 H), 8.00 (d, 1H; $J \sim 8.0$ Hz), 8.30–8.80 (br s, 1H). Anal. Calcd for $C_{13}H_{15}N_3O_3S$: C, 53.23; H, 5.15; N, 14.32. Found: C,52.88; H, 5.46; N, 14.22.

4.1.2.2. 5-(2-Hydroxyphenyl)-3-[(4-phenylpiperazino)methyl]-2,3-dihydro-1,3,4-oxadiazole-2-thione (3b). Recrystallization from aq ethanol; yield: 95%; mp: 170–172 °C; IR (KBr), ν_{max}/cm^{-1}: 3550–3240 (OH), 1616 (C=N), 1595 (C=C), 1438 (C=S), 1249 and 1186 (C–O–C), 762, 738, 691; ^{1}H NMR (DMSO-d_6), δ ppm: 2.89 (s, 4H), 3.10 (s, 4H), 5.05 (s, 2H), 6.74 (dd, 1H; $J \sim 7.3$ Hz), 6.96 (m, 3H), 7.05 (d, 1H; $J \sim 8.4$ Hz), 7.17 (m, 2H), 7.43 (ddd, 1H; $J \sim 1.5$, 8.4, 8.6 Hz), 7.6 (dd, 1H; $J \sim 1.5$, 7.7 Hz), 10.5 (s, 1H); ^{13}C NMR (DMSO-d_6), δ ppm: 48.3, 49.6, 69.5, 108.9, 115.6, 117, 118.9, 119.4, 128.8, 129.1, 133.6, 150.9, 156.4, 157.9, 177.1. Anal. Calcd for $C_{19}H_{20}N_4O_2S \cdot \frac{1}{2}H_2O$: C, 60.46; H, 5.61; N, 14.84. Found: C, 60.76; H, 5.67; N, 14.88.

4.1.2.3. 3-(Anilinomethyl)-5-(2-hydroxyphenyl)-2,3-dihydro-1,3,4-oxadiazole-2-thione (3c). Recrystallization from aq ethanol; yield: 66%; mp: 189–191 °C (reported 145 °C)[13]. IR (KBr), ν_{max}/cm^{-1}: 3550–3235 (OH and NH), 1615 (C=N), 1593 (C=C), 1425 (C=S), 1250 and 1152 (C–O–C), 761, 739, 705; ^{1}H NMR ($CDCl_3$ + DMSO-d_6), δ ppm: 5.18 (s, 1H), 5.52 (d, 2H; $J \sim 7.5$ Hz), 6.92–7.03 (m, 4H), 7.31–7.38 (m, 3H), 7.59–7.69 (m, 2H), 9.19 (br s, 1H). Anal. Calcd for $C_{15}H_{13}N_3O_2S \cdot \frac{1}{2}H_2O$: C, 58.43; H, 4.58; N, 13.63. Found: C, 58.36; H, 3.94; N, 13.57.

4.1.2.4. 5-(2-Hydroxyphenyl)-3-(3-toluidinomethyl)-2,3-dihydro-1,3,4-oxadiazole-2-thione (3d). Recrystallization from aq ethanol; yield: 72%; mp: 167–169 °C; IR (KBr), ν_{max}/cm^{-1}: 3550-3235 (OH and NH), 1615 (C=N), 1595 (C=C), 1419 (C=S), 1250 and 1151 (C–O–C), 758, 747, 691; ^{1}H NMR ($CDCl_3$), δ ppm: 2.22 (s, 3H), 5.88 (s, 2H), 7.12–7.15 (m, 2H), 7.30–7.81 (m, 7H), 8.05 (s, 1H); ^{13}C NMR ($CDCl_3$), δ ppm: 20.4, 59.2, 106.9, 114.2, 117.5, 120.5, 126.9, 129.6, 130, 130.1, 134.4, 141.2, 156.4, 158.8, 174.9. Anal. Calcd for $C_{16}H_{15}N_3O_2S$: C, 61.32; H, 4.82; N, 13.41. Found: C, 61.08; H, 4.54; N, 13.16.

4.1.2.5. 5-(2-Hydroxyphenyl)-3-(4-toluidinomethyl)-2,3-dihydro-1,3,4-oxadiazole-2-thione (3e). Recrystallization from aq ethanol; yield: 72%; mp: 147–149 °C (reported 158 °C).[13] IR (KBr), ν_{max}/cm^{-1}: 3550–3230 (OH and NH), 1616 (C=N), 1595 (C=C), 1427 (C=S), 1252 and 1151 (C–O–C), 805, 753; ^{1}H NMR ($CDCl_3$), δ ppm: 2.30 (s, 3H), 5.70 (s, 2H), 6.80–7.75 (m, 8H), 7.85 (d, 1H; $J \sim 7.0$ Hz), 8.20–8.80 (br s, 1H). Anal. Calcd for $C_{16}H_{15}N_3O_2S$: C, 61.32; H,4.82; N, 13.41. Found: C, 60.95; H, 4.79; N, 13.22.

Table 4. In vitro tumor 50 % lethal conc. (LC_{50}, M) of **3i**, **3j**, **3k**, **3o**, **3p**, **3q**, and **3r**

Panel/cell line	**3i** $\log_{10}$ LC_{50}	**3j** $\log_{10}$ LC_{50}	**3k** $\log_{10}$ LC_{50}	**3o** $\log_{10}$ LC_{50}	**3p** $\log_{10}$ LC_{50}	**3q** $\log_{10}$ LC_{50}	**3r** $\log_{10}$ LC_{50}
Leukemia							
CCRF-CEM	>−4.00	−4.01	>−4.00	>−4.00	>−4.00	—	—
HL-60(TB)	—	—	—	>−4.00	>−4.00	>−4.00	>−4.00
K-562	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
MOLT-4	—	>−4.00	>−4.00	−4.04	>−4.00	>−4.00	>−4.00
RPMI-8226	>−4.00	−4.01	>−4.00	>−4.00	>−4.00	—	—
SR	>−4.00	>−4.00	>−4.00	—	>−4.00	—	—
Non-small cell lung cancer							
A549/ATCC	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
EKVX	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
HOP-62	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
HOP-92	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
NCI-H226	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
NCI-H23	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
NCI-H322M	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
NCI-H460	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
NCI-H522	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
Colon cancer							
COLO 205	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
HCC-2998	>−4.00	—	—	>−4.00	>−4.00	>−4.00	>−4.00
HCT-116	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
HCT-15	>−4.00	—	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
HT29	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
KM12	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
SW-620	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
CNS cancer							
SF-268	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
SF-295	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
SF-539	>−4.00	>−4.00	>−4.00	—	—	—	—
SNB-19	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
U251	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
Melanoma							
LOX IMVI	−4.18	−4.23	>−4.00	−4.11	−4.13	−4.04	>−4.00
M14	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
SK-MEL-2	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
SK-MEL-28	—	—	—	>−4.00	>−4.00	>−4.00	>−4.00
SK-MEL-5	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
UACC-257	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
UACC-62	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
Ovarian							
IGR-OV1	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
OVCAR-3	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
OVCAR-5	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
OVCAR-8	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
SK-OV-3	>−4.00	>−4.00	>−4.00	—	—	—	—
Renal							
786-0	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
A498	>−4.00	>−4.00	>−4.00	—	—	—	>−4.00
ACHN	−4.05	—	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
CAKI-1	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
RXF 393	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
SN12C	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
TK-10	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
UO-31	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
Prostate							
PC-3	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
Breast cancer							
MCF7	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
NCI/ADR-RES	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
MDA-MB-231/ATCC	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
HS 578T	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
MDA-MB-435	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00

Table 4 (*continued*)

Panel/cell line	**3i** $\log_{10}$ LC_{50}	**3j** $\log_{10}$ LC_{50}	**3k** $\log_{10}$ LC_{50}	**3o** $\log_{10}$ LC_{50}	**3p** $\log_{10}$ LC_{50}	**3q** $\log_{10}$ LC_{50}	**3r** $\log_{10}$ LC_{50}
BT-549	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
T-47D	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
MG-MID	−4.01	−4.00	−4.00	−4.00	−4.00	−4.00	−4.00
Delta	0.18	0.22	0.00	0.10	0.13	0.04	0.00
Range	0.18	0.23	0.00	0.11	0.13	0.04	0.00

Table 5. In vitro tumor 50% growth inhibition (GI_{50}, M) of **3j** and 5-fluorouracil

Panel/cell line	**3j** $\log_{10}$ GI_{50}	5-Fluorouracil $\log_{10}$ GI_{50}
Leukemia		
CCRF-CEM	−5.44	−4.53
MOLT-4	<−8.00	−4.86
Non-small cell lung cancer		
EKVX	−4.17	−3.27
HOP-92	−5.73	−4.30
NCI-H322M	−5.26	−4.50
Prostate		
PC-3	−4.48	−4.40
Breast cancer		
NCI/ADR-RES	−6.43	−4.29
HS 578T	−4.16	−3.68

Table 6. In vitro tumor 50% growth inhibition (GI_{50}, M) of **3k** and cyclophosphamide

Panel/cell line	**3k** $\log_{10}$ GI_{50}	Cyclophosphamide $\log_{10}$ GI_{50}
Colon cancer		
COLO 205	−5.15	−3.60
HCT-116	−4.74	−3.60
KM12	−4.93	−3.60
SW-620	−4.65	−3.60
CNS cancer		
SF-539	−4.62	−3.61
Melanoma		
M14	−4.55	−3.60
Ovarian		
OVCAR-8	−4.52	−3.60

4.1.2.6. 5-(2-Hydroxyphenyl)-3-[(2-methoxyanilino)methdihydro-1,3,4-oxadiazole-2-thione (3f). Recrystallization from ethanol; yield: 64%; mp: 148–150 °C; IR (KBr), ν_{max}/cm^{-1}: 3550–3235 (OH and NH), 1620 (C=N), 1596 (C=C), 1420 (C=S), 1250 and 1151 (C–O–C), 739; ^1H NMR (DMSO-d_6), δ ppm: 3.79 (s, 3H), 5.17 (s, 1H), 5.50 (d, 2H; $J \sim 7.2$ Hz), 6.85–6.96 (m, 3H), 6.99–7.03 (m, 2H), 7.38–7.43 (m, 2H), 7.60 (d, 1H; $J \sim 7.9$), 10.45 (s, 1H). Anal. Calcd for $C_{16}H_{15}N_3O_3S$: C, 58.35; H, 4.59; N, 12.76. Found: C, 58.09; H, 5.35; N, 12.74.

4.1.2.7. 5-(2-Hydroxyphenyl)-3-[(4-methoxyanilino)methyl]-2,3-dihydro-1,3,4- oxadiazole-2-thione (3g). Recrystallization from ethanol; yield: 64%; mp: 161–162 °C (reported 125 °C)[13]. IR (KBr), ν_{max}/cm^{-1}: 3550–3245 (OH and NH), 1633 (C=N), 1590 (C=C), 1424 (C=S), 1260 and 1157 (C–O–C), 814, 744; ^1H NMR (DMSO-d_6), δ ppm: 3.62 (s, 3H), 5.50 (s, 3H), 6.74–7.03 (m, 7H), 7.60 (d, 1H; $J \sim 6.2$), 10.47 (s, 1H). Anal. Calcd for $C_{16}H_{15}N_3O_3S$: C, 58.35; H, 4.59; N, 12.76. Found: C, 58.12; H, 4.85; N, 12.80.

4.1.2.8. 3-[(2-Ethoxyanilino)methyl]-5-(2-hydroxyphenyl)-2,3-dihydro-1,3,4-oxadiazole-2-thione (3h). Recrystallization from aq ethanol; yield: 79%; mp: 151–153 °C; IR (KBr), ν_{max}/cm^{-1}: 3550–3240 (OH and NH), 1619 (C=N), 1590 (C=C), 1423 (C=S), 1267&1110 (C–O–C), 765; ^1H NMR ($CDCl_3$), δ ppm: 1.45 (t, 3H; $J \sim 7.0$, 14.0 Hz), 4.06 (q, 2H; $J \sim 7.0$, 14.0 Hz), 5.62 (s, 3H), 6.75 (m, 2H), 6.85 (ddd, 1H; $J \sim 1.1$, 2.0, 7.9 Hz), 6.95–7.10 (m, 3H), 7.43 (ddd,1H; $J \sim 1.7$, 7.0, 7.3 Hz), 7.65 (dd, 1H; $J \sim 1.7$, 7.9 Hz), 8.3 (s, 1H); ^{13}C NMR ($CDCl_3$), δ ppm: 14.8, 58.4, 64, 106.9, 111.2, 111.9, 117.5, 119.7, 120.5, 121.1, 126.9, 133.5, 134.4, 146.7, 156.4, 158.8, 174.8. Anal. Calcd for $C_{17}H_{17}N_3O_3S \cdot \frac{1}{4}H_2O$: C, 58.69; H, 5.07; N, 12.08. Found: C, 58.83; H, 5.15; N, 12.04.

4.1.2.9. 3-[(2-Chloroanilino)methyl]-5-(2-hydroxyphenyl)-2,3-dihydro-1,3,4-oxadiazole-2-thione (3i). Recrystallization from aq ethanol; yield: 79%; mp: 190–191 °C; IR (KBr), ν_{max}/cm^{-1}: 3550-3240 (OH and NH), 1619 (C=N), 1595 (C=C), 1438 (C=S), 1266 and 1097 (C–O–C), 740; ^1H NMR ($CDCl_3$), δ ppm: 5.65 (s, 3H), 6.75 (ddd, 1H; $J \sim 2.2$, 7.9, 8.8 Hz), 6.98–7.09 (m, 2H), 7.16–7.23 (m, 2H), 7.29 (ddd, 1H; $J \sim 0.7$, 2.2, 8.8 Hz), 7.4–7.5 (ddd, 1H; $J \sim 1.7$, 8.4, 8.6 Hz), 7.72 (ddd, 1H; $J \sim 0.4$, 1.7, 7.9 Hz), 8.28 (s, 1H); ^{13}C NMR ($CDCl_3$), δ ppm: 57.9, 106.9, 112.9, 117.6, 120.4, 120.6, 126.9, 127.9, 129.7, 134.5, 140, 156.5, 159, 174.8. MS: 333 [M^+]. Anal. Calcd for $C_{15}H_{12}ClN_3O_2S$: C, 53.98; H 3.62; N, 12.59. Found: C, 53.90; H, 3.61; N, 12.59.

4.1.2.10. 3-[(3-Chloroanilino)methyl]-5-(2-hydroxyphenyl)-2,3-dihydro-1,3,4-oxadiazole-2-thione (3j). Recrystallization from aq ethanol; yield: 72%; mp: 135–137 °C; IR (KBr), ν_{max}/cm^{-1}: 3470–3333 (OH and NH), 1621 (C=N), 1596 (C=C), 1428 (C=S), 1266 and 1093 (C–O–C), 840, 758, 746; ^1H NMR ($CDCl_3$), δ ppm: 5.80 (s, 3H), 6.75–7.15 (m, 2H), 7.15–7.45 (m, 4H), 7.60 (d, 1H; $J \sim 7.0$ Hz), 7.90 (d, 1H; $J \sim 8$ Hz), 8.20–8.75 (br s, 1H). Anal. Calcd for $C_{15}H_{12}ClN_3O_2S$: C, 53.98; H 3.62; N, 12.59. Found: C, 54.06; H, 3.68; N, 12.51.

4.1.2.11. 3-[(4-Chloroanilino)methyl]-5-(2-hydroxyphenyl)-2,3-dihydro-1,3,4-oxadiazole-2-thione (3k). Recrystallization from aq ethanol; yield: 75%; mp: 187–189 °C; IR (KBr), ν_{max}/cm^{-1}: 3550–3235 (OH and NH), 1621 (C=N), 1596 (C=C), 1425 (C=S), 1258 and 1093 (C–O–C), 820, 744; ^1H NMR ($CDCl_3$), δ ppm: 5.80 (s, 3H), 6.75–7.25 (m, 2H), 7.25–7.75 (m,

5H), 7.90 (d, 1H; $J \sim 8$ Hz), 8.50 (s, 1H). Anal. Calcd for $C_{15}H_{12}ClN_3O_2S$: C, 53.98; H 3.62; N, 12.59. Found: C, 53.88; H, 3.36, N, 12.66.

4.1.2.12. 3-[(4-Bromoanilino)methyl]-5-(2-hydroxyphenyl)-2,3-dihydro-1,3,4-oxadiazole-2-thione (3l). Recrystallization from ethanol; yield: 80%; mp: 192–194 °C (reported 160 °C)[13]; IR (KBr), ν_{max}/cm^{-1}: 3545–3235 (OH and NH), 1621 (C=N), 1593 (C=C), 1424 (C=S), 1260 and 1123 (C–O–C), 815, 740; ^1H NMR (DMSO-d_6), δ ppm: 5.7 (s, 3H), 6.75–7.25 (m, 2H), 7.25–7.75 (m, 5H), 7.90 (d, 1H; $J \sim 8$ Hz), 8.50 (s, 1H). Anal. Calcd for $C_{15}H_{12}BrN_3O_2S$: C, 47.63; H, 3.20; N, 11.11. Found: C, 47.97; H 3.07; N, 10.90.

4.1.2.13. 5-(2-Hydroxyphenyl)-3-[(3-nitroanilino)methyl]-2,3-dihydro-1,3,4-oxadiazole-2-thione (3m). Recrystallization from ethanol; yield: 80%; mp: 214–216 °C (reported 200 °C)[13]. IR (KBr), ν_{max}/cm^{-1}: 3550–3245 (OH and NH), 1621 (C=N), 1595 (C=C), 1525 and 1386 (NO_2), 1427 (C=S), 1249 and 1103 (C–O–C), 850, 755, 749,; ^1H NMR (DMSO-d_6), δ ppm: 5.90 (d, 2H; $J \sim 8.0$ Hz), 7.00–8.10 (m, 8H), 8.20 (d, 1H; $J \sim 8.0$ Hz), 11.00 (s, 1H). Anal. Calcd for, $C_{15}H_{12}N_4O_4S$: C, 52.32; H, 3.51; N, 16.27. Found: C, 52.02; H, 3.57; N, 16.32.

4.1.2.14. 5-(2-Hydroxyphenyl)-3-[(4-nitroanilino)methyl]-2,3-dihydro-1,3,4-oxadiazole-2-thione (3n). Recrystallization from ethanol; yield: 79%; mp: 209–210 °C (reported 210 °C).[13] IR (KBr), ν_{max}/cm^{-1}: 3545–3245 (OH and NH), 1620 (C=N), 1592 (C=C), 1524 and 1343 (NO_2), 1427 (C=S), 1261 and 1088 (C–O–C), 864, 755; ^1H NMR (DMSO-d_6), δ ppm: 5.75 (d, 2H; $J \sim 8.0$ Hz), 7.00 (d, 1H; $J \sim 8.0$ Hz), 7.20 (d, 1H; $J \sim 8.0$ Hz), 7.40–7.70 (m, 4H), 7.85 (d, 1H; $J \sim 8.0$ Hz), 8.00 (m, 2H), 10.80 (s, 1H). Anal. calcd. for, $C_{15}H_{12}N_4O_4S$: C, 52.32; H, 3.51; N, 16.27. Found: C, 52.04; H, 3.52; N, 16.43.

4.1.2.15. 2-([5-(2-Hydroxyphenyl)-2-thioxo-2,3-dihydro-1,3,4-oxadiazol-3-yl]methyl amino)benzoic acid (3o). Recrystallization from ethanol; yield: 82%; mp: 202–204 °C; IR (KBr), ν_{max}/cm^{-1}: 3550–3255 (OH, NH and COOH), 1673 (C=O), 1622 (C=N), 1595 (C=C), 1424 (C=S), 1263 and 1176 (C–O–C), 756;^1H NMR (DMSO-d_6), δ ppm: 5.66 (d, 2H; $J \sim 7.2$ Hz), 6.73 (dd, 1H; $J \sim 1.0$, 7.6 Hz), 6.90 (dd, 1H; $J \sim 7.3$, 7.5), 7.00 (dd, 1H; $J \sim 4.7$, 8.3 Hz), 7.22 (d, 1H; $J \sim 8.4$ Hz), 7.32-7.45 (m, 2H), 7.62 (dd, 1H; $J \sim 7.9$, 9.5 Hz), 7.84 (d, 1H; $J \sim 7.9$ Hz), 8.86 (t, 1H; $J \sim 7.2$, 14.5 Hz), 10.50 (s, 1H), 12.94 (br s, 1H). Anal. Calcd for $C_{16}H_{13}N_3O_4S$: C, 55.97; H, 3.82; N, 12.24. Found: C, 55.69; H, 4.32; N, 12.12.

4.1.2.16. 4-([5-(2-Hydroxyphenyl)-2-thioxo-2,3-dihydro-1,3,4-oxadiazol-3-yl]methyl amino)benzoic acid (3p). Recrystallization from ethanol; yield: 80%; mp: 213–215 °C (reported 212 °C)[13]. IR (KBr), ν_{max}/cm^{-1}: 3550–3245 (OH, NH and COOH), 1674 (C=O), 1613 (C=N), 1606 (C=C), 1431 (C=S), 1269 and 1185 (C–O–C), 856, 739; ^1H NMR (DMSO-d_6), δ ppm: 5.75 (d, 2H; $J \sim 8$ Hz), 6.8–7.35 (m, 4H), 7.30–7.90 (m, 2H), 7.90–8.30 (m, 4H), 10.50 (s, 1H). Anal. Calcd for $C_{16}H_{13}N_3O_4S$: C, 55.97; H, 3.82; N, 12.24. Found: C, 55.58; H, 3.70; N, 12.24.

4.1.2.17. 1-[4-([5-(2-Hydroxyphenyl)-2-thioxo-2,3-dihydro-1,3,4-oxadiazol-3-yl]methyl amino)phenyl]-1-ethanone (3q). Recrystallization from aq ethanol; yield: 82%; mp: 206–208 °C; IR (KBr), ν_{max}/cm^{-1}: 3550–3227 (OH and NH), 1653 (C=O), 1618 (C=N), 1594 (C=C), 1425 (C=S), 1237 and 1181 (C–O–C), 821, 758; ^1H NMR (DMSO-d_6), δ ppm: 2.55 (s, 3H), 5.70 (d, 2H; $J \sim 7$), 6.80–7.40 (m, 4H), 7.40–8.35 (m, 5H), 10.80 (s, 1H). Anal. Calcd for $C_{17}H_{15}N_3O_3S \cdot \frac{1}{2}H_2O$: C, 58.27; H, 4.60; N,12.04. Found: C, 58.34; H, 4.53; N, 12.01.

4.1.2.18. 2-Hydroxy-4-([5-(2-hydroxyphenyl)-2-thioxo-2,3-dihydro-1,3,4-oxadiazol-3-yl]methylamino)benzoic acid (3r). Recrystallization from ethanol; yield: 84%; mp: 197–199 °C; IR (KBr), ν_{max}/cm^{-1}: 3550–3125 (OH, NH and COOH), 1644 (C=O), 1620 (C=N), 1591 (C=C), 1428 (C=S), 1251 and 1101 (C–O–C), 859, 773, 692; ^1H NMR (DMSO-d_6), δ ppm: 5.46 (s, 2H), 6.42–6.45 (m, 2H), 6.95 (dd, 1H; $J \sim 7.3$, 7.7 Hz), 7.03 (d, 1H; $J \sim 7.9$ Hz), 7.43 (ddd, 1H; $J \sim 1.7$, 7.3, 8.8 Hz), 7.52 (d, 1H; $J \sim 9.2$ Hz), 7.62 (dd, 1H; $J \sim 1.7$, 7.9 Hz), 7.90 (t, 1H; $J \sim 7.0$, 14 Hz), 10.50 (s, 1H), 11.40 (s, 1H), 13.10 (br s, 1H); ^{13}C NMR (DMSO-d_6), δ ppm: 56.8, 98.5, 102.5, 105.8, 108.7, 117, 119.4, 129.1, 131.3, 133.8, 152.3, 156.4, 158.2, 163.3, 171.9, 175.1. Anal. Calcd for $C_{16}H_{13}N_3O_5S$: C, 53.48; H, 3.65; N, 11.69. Found: C, 53.22; H, 3.65; N, 11.45.

4.1.2.19. 3-[(Ethylanilino)methyl]-5-(2-hydroxyphenyl)-2,3-dihydro-1,3,4-oxadiazole-2-thione (3s). Recrystallization from aq ethanol; yield: 85%; mp: 152–154 °C; IR (KBr), ν_{max}/cm_{-1}: 3550–3300 (OH), 1632 (C=N), 1596 (C=C), 1423 (C=S), 1251 and 1095 (C–O–C), 755, 732, 695; ^1H NMR ($CDCl_3$), δ ppm: 1.30 (t, 3H), 3.70 (q, 2H), 5.80 (s, 2H), 6.75–7.60 (m, 8H), 7.70 (d, 1H; $J \sim 9.0$ Hz), 7.90 (d, 1H; $J \sim 8$ Hz). Anal. Calcd for $C_{17}H_{17}N_3O_2S \cdot \frac{1}{2}H_2O$: C, 57.61; H, 5.69; N, 11.86. Found: C, 57.93; H, 5.68; N, 12.02.

4.1.2.20. 3-[(Diphenylamino)methyl]-5-(2-hydroxyphenyl)-2,3-dihydro-1,3,4-oxadiazole-2-thione (3t). Recrystallization from ethanol; yield: 70%; mp: 191–193 °C; IR (KBr), ν_{max}/cm^{-1}: 3550–3260 (OH), 1617 (C=N), 1595 (C=C), 1420 (C=S), 1230 and 1115 (C–O–C), 750, 725, 691; ^1H NMR ($CDCl_3$), δ ppm: 1.30 (t, 3H), 3.70 (q, 2H), 5.80 (s, 2H), 6.75–7.60 (m, 8H), 7.70 (d, 1H; $J \sim 9.0$ Hz), 7.90 (d, 1H; $J \sim 8$ Hz); ^{13}C NMR ($CDCl_3$), δ ppm: 64.8, 108.6, 116.6, 116.9, 119.2, 121.3, 122.6, 129.1, 133.5, 145.6, 156.4, 158.2, 175.2. MS: 375 [M^+]. Anal. Calcd for $C_{21}H_{17}N_3O_2S$: C, 67.18; H, 4.56; N, 11.19. Found: C, 66.92; H, 5.03; N, 11.16.

4.1.2.21. 5-(2-Hydroxyphenyl)-3-[(2-pyridylamino)methyl]-2,3-dihydro-1,3,4-oxadiazole-2-thione (3u). Recrystallization from ethanol; yield: 81%; mp: 207–209 °C; IR (KBr), ν_{max}/cm^{-1}: 3550–3245 (OH and

NH), 1620 (C=N), 1590 (C=C), 1422 (C=S), 1268 and 1097 (C–O–C), 760, 750, 740; ^{1}H NMR (DMSO-d_6), δ ppm: 5.51 (d, 2H; $J \sim 7.3$ Hz), 6.93 (t, 1H; $J \sim 7.2$, 15.2 Hz), 7.02 (d, 1H; $J \sim 8.3$ Hz), 7.15 (dd, 1H; $J \sim 3.7$, 8.4 Hz), 7.29 (1H; $J \sim 1.3$, 8.4 Hz), 7.36–7.44 (m, 2H), 7.60 (dd, 1H; $J \sim 1.5$, 7.9 Hz), 7.89 (d, 1H; $J \sim 4.2$ Hz), 8.23 (s, 1H), 10.53 (s, 1H); ^{13}C NMR (DMSO-d_6), δ ppm: 57.3, 108.8, 117.1, 119.3, 119.5, 123.7, 129.1, 133.8, 135.7, 138.9, 141.9, 156.4, 158.3, 175.2. Anal. Calcd for $C_{14}H_{12}N_4O_2S$: C, 55.99; H, 4.03; N, 18.65. Found: C, 55.93; H, 4.03; N, 18.35.

4.1.2.22. 5-(2-Hydroxyphenyl)-3-[(3-pyridylamino)methyl]-2,3-dihydro-1,3,4-oxadiazole-2-thione (3v). Recrystallization from ethanol; yield: 81%; mp: 204–205 °C; IR (KBr), ν_{max}/cm^{-1}: 3545–3240 (OH and NH), 1608 (C=N), 1572 (C=C), 1438 (C=S), 1268 and 1102 (C–O–C), 803, 745, 727; ^{1}H NMR (DMSO-d_6), δ ppm: 4.10–5.00 (br s, 1H), 5.80 (d, 2H), 7.00–8.3.0 (m, 8H), 8.50 (s, 1H). Anal. Calcd for $C_{14}H_{12}N_4O_2S$: C, 55.99; H, 4.03; N, 18.65. Found: C, 55.57; H, 3.95; N, 18.34.

4.2. In vitro cytotoxicity screening

The human tumour 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 mL at plating densities ranging from 5000 to 40,000 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 drugs. After 24 h, two plates of each cell line were fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (T_z). Experimental drugs were solubilized in dimethylsulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 mg ml^{-1} gentamicin. Additional four-, 10-fold or 1/2 log serial dilutions were made to provide a total of five drug concentrations plus control. Aliquots of 100 mL of these different drug dilutions were added to the appropriate microtiter wells already containing 100 mL of medium, resulting in the required final drug concentrations. Following drug 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 mL of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min 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 mL) 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 of 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 mL of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [time zero (T_z), control growth (C), and test growth in the presence of drug at the five concentration levels (T_i)], the growth percentage was calculated at each of the drug concentration levels. Percentage GI was calculated as:

$$[(T_i - T_z)/(C - T_z)] \times 100 \text{ for concentrations of which } T_i \geqslant T_z,$$

$$[(T_i - T_z)/T_z] \times 100 \text{ for concentrations of which } T_i < T_z.$$

Three dose–response parameters were calculated for each experimental agent. GI_{50} was calculated from $[(T_i - T_z)/(C - T_z)] \times 100 = 50$, which was the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in TGI was calculated from $T_i = T_z$. Values were calculated for each of these parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the value for that parameter was expressed as greater or less than the maximum or minimum concentration tested.

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