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Original article

Synthesis and primary cytotoxicity evaluation of new imidazo[2,1-*b*]thiazole derivatives

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Abstract

A series of arylidenehydrazides (**3a–3i**) were synthesized from [6-(4-bromophenyl)imidazo[2,1-*b*]thiazol-3-yl]acetic acid hydrazide. The structures of new compounds were determined by analytical and spectral (IR, ^{1}H NMR, ^{13}C NMR, EIMS) methods. The synthesized compounds (**3a–3i**) were evaluated in the National Cancer Institute's 3-cell line, one dose in vitro primary cytotoxicity assay. Compounds **3a–3c**, **3h** and **3i** which passed the criteria for activity in this assay were scheduled automatically for evaluation against the full panel of 60 human tumour cell lines at a minimum of five concentrations at 10-fold dilutions. Compounds **3c** demonstrated the most marked effects on a prostate cancer cell line (PC-3, $\log_{10} GI_{50}$ value < -8.00).

Keywords: Imidazo[2,1-*b*]thiazole; Arylidenehydrazide; Cytotoxicity

1. Introduction

Recently much interest has been focused on the chemistry and the biological activity of imidazo[2,1-*b*]thiazoles and their derivatives. The imidazo[2,1-*b*]thiazole derivatives have been reported in the literature as antibacterial [1], antifungal [2], antihelmintic [3,4] and antitumour [5–9] agents. The imidazo[2,1-*b*]thiazole system constitutes the main part of the well-known antihelmintic and immunomodulatory agent levamisole, which is 2,3,5,6-tetrahydro-6-phenylimidazo [2,1-*b*]thiazole. Andreani et al. [10] studied a series of imidazo[2,1-*b*]thiazole guanyl hydrazones which were active against various cancer cell lines. In view of these observations, we planned the synthesis of novel [6-(4-bromophenyl)imidazo[2,1-*b*]thiazol-3-yl]acetic acid arylidenehydrazides to evaluate their primary cytotoxicity.

In this paper we described new arylidenehydrazides bearing imidazo[2,1-*b*]thiazole moiety. The antitumour activity of all new compounds was evaluated on three human tumour cell lines according to the protocols available in the National Cancer Institute (NCI, Bethesda, MD), and the active compounds were tested on the 60 tumour cell lines as well.

2. Chemistry

The synthetic route of the compounds is outlined in Scheme 1. Ethyl 6-(4-bromophenyl)imidazo[2,1-*b*]thiazole-3-acetate (**1**) [11] was refluxed with hydrazine hydrate to obtain [6-(4-bromophenyl)imidazo[2,1-*b*]thiazol-3-yl]acetic acid hydrazide (**2**) [12]. Condensation of **2** with appropriate aromatic aldehydes yielded the corresponding hydrazide hydrazones (**3a–3i**). The structures of the synthesized compounds were confirmed by analytical (Table 1) and spectral data (IR, ^{1}H NMR, ^{13}C NMR, EIMS).

In the IR spectra, the NH, C=O and C=N bands were observed in the 3353–3102 cm^{-1}, 1688–1660 cm^{-1} and 1618–1598 cm^{-1} regions, respectively. In the ^{1}H NMR spectra of hydrazide hydrazones (**3a–3i**), the absence of the NH_2 absorptions of the hydrazide **2** ($\delta = 4.38$ ppm) and the

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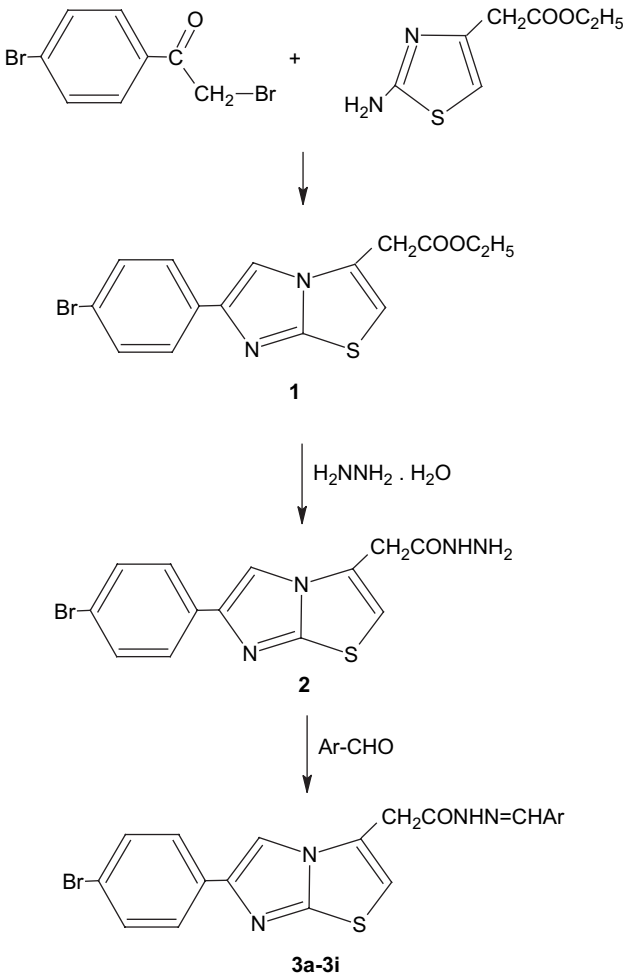

Scheme 1. The general synthesis reactions.

presence of new resonances assigned to the −CH═ proton of **3** provided evidence for hydrazone formation. The ^{1}H NMR spectra of **3a**–**3i** revealed the presence of two geometric isomers as concluded from the NH, N═CH and CH_2 protons resonating as double singlets at about δ 11.73–11.30/11.92–11.40, 8.36–7.80/8.55–7.96 and 3.93–3.72/4.33–4.13 ppm, respectively [13]. It is assumed that the N═CH double bond restricted rotation and gave rise to the formation of *E* and *Z* isomers. ^{13}C NMR spectra of **3d**, **3e** and **3h** chosen as prototypes verified the proposed hyrazide–hydrazone structure. EIMS spectra of **3d**, **3e** and **3i** displayed molecular ions which confirmed their molecular weights. Major fragmentation routes involved the breaking of the CO–NH and NH–N bonds of the hydrazide moiety.

3. Cytotoxicity evaluation and discussion

The synthesized compounds were evaluated in the three-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 in 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 for each compound were reported as the percent of growth of the treated cells when compared to the untreated control cells. All the compounds which reduced the growth of any one of the cell lines to 32% or less (negative numbers indicate cell kill) were passed on for evaluation in the full panel of 60 human tumour cell lines (Table 2). The cytotoxic and/or growth inhibitory effects of the compounds **3a**–**3c**, **3h** and **3i** were tested in vitro against the full panel of 60 human tumour cell lines derived from nine neoplastic diseases at 10-fold dilutions of five concentrations ranging from 10^{-4} to 10^{-8} M. The percentage growth was evaluated spectrophotometrically versus controls not treated with test agents. A 48 h continuous drug exposure protocol was followed and an SRB protein assay was used to estimate cell viability or growth. For the compounds, the 50% growth inhibition (GI_{50}) and total growth inhibition (TGI) were obtained for each cell line. The $\log_{10} GI_{50}$ and $\log_{10}$ TGI were then determined, defined as the mean of the $\log_{10}$'s of the individual GI_{50} and TGI values. Negative values indicated the most sensitive cell lines. Compounds having values −4 and <−4 were declared to be active. The cell lines used in the NCI screen were leukemia (L) lines CCRF-CEM, K-562, MOLT-4, RPMI-8226; non-small cell lung cancer (NSCLC) lines A549/ATCC, EKVX, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-H322M, NCI-H460, NCI-H522; colon cancer (CL) lines COLO 205, HCC-2998, HCT-116, HCT-15, HT29, KM12, SW-620; central nervous system cancer (CNSC) lines SF-268, SF-295, SF-539, SNB-19, SNB-75, U251; melanoma (M) lines LOX IMVI, M14, SK-MEL-2,

Table 1
Some physical and analytical data of compounds

Compound	Ar	Yield (%)	M.p. (°C)	Formula (M. wt.)	Analysis, calc./found		
					C	H	N
3a	C_6H_5	91	249–251	$C_{20}H_{15}BrN_4OS$ (439.329)	54.68/55.15	3.44/3.46	12.75/12.77
3b	C_6H_4OH(2-)	95	268–270	$C_{20}H_{15}BrN_4O_2S$ (455.329)	52.76/52.48	3.32/3.00	12.30/12.27
3c	C_6H_4OH(4-)	91	265–266	$C_{20}H_{15}BrN_4O_2S$ (455.329)	52.76/53.03	3.32/3.24	12.30/12.17
3d	$C_6H_4OCH_3$(4-)	92	243–245	$C_{21}H_{17}BrN_4O_2S$ (469.355)	53.74/54.01	3.65/3.52	11.94/11.97
3e	C_6H_4F(4-)	93	253–255	$C_{20}H_{14}BrFN_4OS$ (457.320)	52.53/52.80	3.09/2.66	12.25/12.31
3f	C_6H_4Br(4-)	92	255–256	$C_{20}H_{14}Br_2N_4OS$ (518.226)	46.35/46.75	2.72/2.35	10.81/10.84
3g	$C_6H_4N(CH_3)_2$(4-)	57	262–263	$C_{22}H_{20}BrN_5OS$ (482.397)	54.78/54.14	4.18/3.49	14.52/14.36
3h	$C_6H_3(OCH_3)_2$(2,5-)	95	236–237	$C_{22}H_{19}BrN_4O_3S$ (499.381)	52.91/52.42	3.83/3.45	11.22/11.14
3i	$C_6H_3(Cl)_2$(2,4-)	99	245–247	$C_{20}H_{13}BrCl_2N_4OS$ (508.219)	47.27/47.87	2.58/2.19	11.02/10.61

Table 2
Primary cytotoxicity assay results of **3a–3i**

Compound	Breast (MCF7)	Lung (NCI-H460)	CNS (SF-268)
3a	57	12	65
3b	7	0	50
3c	22	11	42
3d	46	34	56
3e	71	35	84
3f	56	75	107
3g	82	69	77
3h	27	15	82
3i	46	24	94

SK-MEL-28, SK-MEL-5, UACC-257, UACC-62; ovarian cancer (OC) lines IGROV1, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, SK-OV-3; renal cancer (RC) lines 786-0, A498, ACHN, CAKI-1, RXF 393, SN12C, TK-10, UO-31; prostate cancer (PC) lines PC-3, DU-145 and breast cancer (BC) lines MCF7, NCI/ADR-RES, MDA-MB-231/ATCC, HS 578T, MDA-MB-435, BT-549, T-47D (Table 3).

Among the compounds tested, **3c** demonstrated the most marked effect on a prostate cancer cell line (PC-3, $\log_{10} GI_{50}$ value < -8.00).

Compound **3b** exhibited favorable activity on a leukemia cell line (K-562, $\log_{10} GI_{50}$ value -7.43), non-small cell lung cancer cell lines (NCI-H226, $\log_{10} GI_{50}$ value -5.71; NCI-H322M, $\log_{10} GI_{50}$ value -5.67), a colon cancer cell line (HCT-15, $\log_{10} GI_{50}$ value -6.02), central nervous system cancer cell lines (SNB-19, $\log_{10} GI_{50}$ value -5.17; U251, $\log_{10} GI_{50}$ value -5.44), a melanoma cell line (SK-MEL-5, $\log_{10} GI_{50}$ value -5.53), an ovarian cancer cell line (IGROV1, $\log_{10} GI_{50}$ value -5.98), renal cancer cell lines (ACHN, $\log_{10} GI_{50}$ value -5.80; CAKI-1, $\log_{10} GI_{50}$ value -6.40). On the same cancer cell lines, the $\log_{10} GI_{50}$ values of thioguanine used as anticancer agents were -6.39, -5.39, -5.13, -5.96, -4.10, -5.31, -5.45, -5.32, -5.71, and -6.27, respectively. When these data were examined, it is observed that **3b** was much more active than thioguanine on these cancer cell lines.

Compound **3c** was highly active on a melanoma cell line (UACC-257, $\log_{10} GI_{50}$ value -5.82) and non-small cell lung cancer (NCI-H322M, $\log_{10} GI_{50}$ value -5.36).

Compound **3h** showed high activity on an ovarian cancer cell line (IGROV1, $\log_{10} GI_{50}$ value -5.40).

In addition **3a**, **3c** and **3h** were highly active on a central nervous system cancer cell line (SNB-19). The $\log_{10} GI_{50}$ values of these compounds were -4.36, 4.24, and 4.50, respectively.

4-Hydroxyphenyl substituted compound **3c** was the most active member within the series. The displacement of hydroxy group from 4-position to 2-position on phenyl ring lead to **3b** that showed activity against most of the tested cell lines.

High effectiveness was induced by two methoxy substituents at 2-position and 5-position on phenyl ring (**3h**).

2,4-Dichlorophenyl substituted compound **3i** exhibited no significant activity.

In conclusion, these preliminary results are promising and some of these compounds may be potential candidates for new anticancer agents.

4. Experimental

4.1. Chemistry

Melting points were determined with a Büchi B-540 melting point apparatus (Flawil, Switzerland) in open capillaries and are uncorrected. Elemental analyses were performed on a LECO CHNS 932 elemental analyser (St. Joseph, Michigan). IR spectra were recorded on KBr discs, using a Perkin–Elmer Model 1600 FT-IR spectrophotometer (Norwalk, Connecticut, USA). ^{1}H NMR spectra were obtained on Bruker DPX 400 (400 MHz) spectrophotometer (Rheinstetten, Germany) using DMSO-d_6. EIMS were determined on a VG Zabspec MS (70 eV) mass spectrometer (Manchester, England).

4.2. General procedure for the synthesis of [6-(4-bromophenyl)imidazo[2,1-b]thiazol-3-yl]acetic acid arylidenehydrazides (*3a–3i*)

Compound **2**, 0.005 mol was refluxed with 0.005 mol of the appropriate aromatic aldehyde in 30 ml ethanol for 5 h. The precipitate obtained was purified by washing with hot ethanol.

4.2.1. Compound *3a*

IR (ν cm^{-1}, KBr): 3198, 3149 (N–H), 1683 (C=O), 1598 (hydrazone C=N). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 3.76, 4.18 (2s, 2H, CH_2CO), 6.95 (s, 1H, C_2-H), 7.27–7.30 (m, 3H, ar $C_{3,4,5}$-H), 7.40, 7.42 (2d, 2H, $J = 8.49$, 8.56 Hz, Br–Ph $C_{3,5}$-H), 7.56–7.65 (m, 4H, Br–Ph $C_{2,6}$-H, ar $C_{2,6}$-H), 7.91, 8.08 (2s, 1H, N=CH), 8.13, 8.16 (2s, 1H, C_5-H), 11.51, 11.62 (2s, 1H, CONH). EIMS (70 eV) *m/z* (%): 337 (1.7), 336 (1.9), 335 (2.3), 334 (2.4), 294 (1.4), 292 (3.8), 211 (31.3), 182 (3.4), 180 (2.9), 169 (1.1), 167 (1.7), 125 (1.7), 120 (2.6), 119 (44.4), 114 (8.6), 111 (24.7), 110 (60.4), 104 (16.3), 103 (57.6), 102 (1.4), 100 (4.0), 99 (3.2), 91 (18.2), 90 (15.2), 89 (50.7), 85 (4.2), 84 (3.0), 77 (100), 73 (11.6), 72 (5.6), 65 (54.2), 59 (4.9), 58 (3.0), 51 (35.4), 43 (34.9), 42 (29.3).

4.2.2. Compound *3b*

IR (ν cm^{-1}, KBr): 3139 (N–H), 1685 (C=O), 1618 (hydrazone C=N). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 3.93, 4.31 (2s, 2H, CH_2CO), 6.84, 6.88 (2d, 1H, $J = 7.38$, 7.58 Hz, ar C_5-H), 6.91 (d, 1H, $J = 8.00$ Hz, ar C_3-H), 7.08, 7.11 (2s, 1H, C_2-H), 7.23–7.30 (m, 1H, ar C_4-H), 7.53–7.59 (m, 2H, Br–Ph $C_{3,5}$-H), 7.72–7.81 (m, 3H, Br–Ph $C_{2,6}$-H, ar C_6-H), 8.28, 8.29 (2s, 1H, C_5-H), 8.36, 8.45 (2s, 1H, N=CH), 10.05, 10.97 (2s, 1H, ar C_2-OH), 11.56, 11.92 (2s, 1H, CONH).

Table 3
In vitro tumour cell growth inhibition of **3a–3c**, **3h** and **3i**

Panel/cell line	**3a**		**3b**		**3c**		**3h**		**3i**		Thioguanine	
	Log_{10} GI_{50}	Log_{10} TGI	Log_{10} GI_{50}	Log_{10} TGI	Log_{10} GI_{50}	Log_{10} TGI	Log_{10} GI_{50}	Log_{10} TGI	Log_{10} GI_{50}	Log_{10} TGI	Log_{10} GI_{50}	Log_{10} TGI
Leukemia												
CCRF-CEM	−4.76	>−4.00	−5.74	−5.08	−4.91	−4.10	−5.02	>−4.00	−4.40	>−4.00	−6.81	−4.59
K-562	−	−5.10	−7.43	−5.53	−5.74	−5.15	−5.53	−5.16	−	−	−6.39	−4.15
MOLT-4	−5.17	−4.43	−5.63	−4.97	−5.38	−5.00	−5.46	−5.09	−6.00	−4.58	−6.57	−4.81
RPMI-8226	−5.13	>−4.00	−5.55	−4.68	−5.37	−4.40	−4.99	>−4.00	−4.10	>−4.00	−6.40	−4.68
Non-small cell lung cancer												
A549/ATCC	−4.87	−4.39	−5.21	−4.62	−4.97	−4.47	−4.94	−4.41	−4.77	−4.43	−5.45	−3.98
EKVX	−5.16	−4.28	−5.41	−4.72	−4.94	−4.18	−4.77	−4.24	−4.50	>−4.00	−5.72	−3.91
HOP-62	−4.82	−4.40	−5.41	−4.83	−4.74	−4.27	−4.88	−4.52	−4.78	−4.46	−6.13	−4.54
HOP-92	−4.91	−4.43	−5.68	−4.84	−4.72	−4.16	−4.86	−4.45	−4.55	>−4.00	−6.13	−4.62
NCI-H226	−	−	−5.71	−5.11	−	−	−	−	−4.50	−4.04	−5.39	−4.01
NCI-H23	−4.90	−4.21	−5.47	−4.85	−5.12	−4.49	−4.71	−4.28	−4.59	−4.25	−5.97	−4.64
NCI-H322M	−4.72	−4.03	−5.67	−4.97	−5.36	−4.46	−4.83	−4.21	−4.61	−4.16	−5.13	−3.65
NCI-H460	−4.28	>−4.00	−4.80	>−4.00	−4.24	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−6.17	−4.70
NCI-H522	−4.85	−4.22	−5.28	−4.57	−4.97	−4.46	−4.64	>−4.00	−4.59	>−4.00	−6.04	−5.20
Colon cancer												
COLO 205	−4.18	>−4.00	−5.29	−4.64	−4.36	>−4.00	−4.45	>−4.00	−4.12	>−4.00	−5.77	−5.05
HCC-2998	−4.88	−4.31	−5.46	−4.90	−4.84	−4.53	−5.00	−4.46	−4.70	−4.35	−6.00	−4.74
HCT-116	−5.03	−4.67	−5.49	−4.91	−4.98	−4.62	−5.34	−4.80	−4.77	−4.43	−6.27	−4.79
HCT-15	−4.79	>−4.00	−6.02	−4.76	−4.75	−4.21	−5.13	−4.19	−4.59	−4.14	−5.96	−4.29
HT29	−4.36	>−4.00	−5.22	−4.72	−4.30	>−4.00	−4.79	>−4.00	−4.39	>−4.00	−5.94	−3.98
KM12	−4.58	>−4.00	−5.44	−4.77	−4.75	−4.12	−5.05	>−4.00	−4.42	>−4.00	−5.76	−4.50
SW-620	−4.73	−4.20	−5.40	−4.76	−4.50	>−4.00	−5.19	−4.31	−4.64	−4.22	−5.81	−3.86
CNS cancer												
SF-268	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−5.94	−3.80
SF-295	−4.92	−4.43	−5.56	−5.03	−4.92	−4.38	−4.91	−4.46	−4.46	>−4.00	−5.99	−3.80
SF-539	−4.79	−4.40	−5.45	−4.93	−4.74	−4.33	−4.95	−4.61	−4.57	−4.20	−5.99	−4.16
SNB-19	−4.36	>−4.00	−5.17	−4.60	−4.24	>−4.00	−4.50	>−4.00	−4.02	>−4.00	−4.10	−3.61
SNB-75	−4.50	−4.05	−4.89	−4.49	>−4.00	>−4.00	−4.62	−4.18	−4.43	>−4.00	−5.84	−4.38
U251	−4.89	−4.49	−5.44	−4.82	−4.67	>−4.00	−4.86	−4.50	−4.69	−4.35	−5.31	−3.62
Melanoma												
LOX IMVI	−4.91	−4.04	−5.46	−4.89	−4.95	−4.58	−4.90	−4.32	−4.67	−4.31	−6.68	−5.04
M14	−4.68	>−4.00	−5.59	−5.11	−4.99	−4.63	−4.82	−4.14	−4.48	>−4.00	−6.23	−4.84
SK-MEL-2	−4.48	>−4.00	−4.68	−4.27	−4.58	−4.02	−4.05	>−4.00	−4.14	>−4.00	−6.03	−4.97
SK-MEL-28	>−4.00	>−4.00	−4.92	−4.62	−4.22	−4.00	−4.38	>−4.00	>−4.00	>−4.00	−5.05	−3.81
SK-MEL-5	−5.25	−4.57	−5.53	−4.97	−5.15	−4.65	−5.05	−4.37	−4.35	>−4.00	−5.45	−4.78
UACC-257	−5.21	−4.50	−5.43	−4.94	−5.82	−5.11	−4.62	−4.01	−4.16	>−4.00	−5.70	−4.01
UACC-62	−4.94	−4.55	−5.68	−5.14	−5.04	−4.63	−4.92	−4.56	−4.63	−4.17	−6.30	−5.34
Ovarian cancer												
IGROV1	−4.95	−4.49	−5.98	−4.94	−4.97	−4.40	−5.40	−4.57	−4.80	−4.34	−5.32	−3.84
OVCAR-3	−4.62	−4.33	−5.49	−4.82	−4.62	−4.31	−4.80	−4.23	−4.46	>−4.00	−6.15	−5.17
OVCAR-4	−4.33	>−4.00	−	−	>−4.00	>−4.00	−4.55	>−4.00	−4.43	>−4.00	−5.82	−4.00
OVCAR-5	−4.51	>−4.00	−5.26	−4.50	−4.56	>−4.00	−4.30	>−4.00	−4.54	−4.17	−6.18	−4.32
OVCAR-8	−5.63	−4.69	−5.64	−4.91	−5.15	−4.59	−4.94	−4.42	−4.73	−4.30	−6.16	−4.22
SK-OV-3	−4.18	>−4.00	−4.89	−4.52	−4.28	>−4.00	−4.24	>−4.00	>−4.00	>−4.00	−6.26	−4.78
Renal cancer												
786-O	−4.56	>−4.00	−5.33	−4.75	−4.58	−4.05	−4.58	>−4.00	−4.59	−4.13	−6.08	−3.97
A498	−	−	−	−	−	−	−	−	−4.53	−4.20	−5.17	−4.02
ACHN	−4.97	−4.59	−5.80	−5.20	−4.83	−4.29	−4.77	−4.46	−4.65	−4.27	−5.71	−3.95
CAKI-1	−4.52	−4.09	−6.40	−5.62	−4.83	−4.31	−4.90	−4.47	−4.44	>−4.00	−6.27	−4.80
RXF 393	−4.84	−4.41	−4.42	−4.20		−4.39	−4.72	−4.17	−4.55	−4.07	−6.12	−5.15
SN12C	−4.88	−4.40	−5.48	−4.80	−4.81	−4.36	−4.68	−4.31	−4.07	>−4.00	−5.90	−3.76
TK-10	−4.85	−4.38	−5.69	−5.06	−5.01	−4.48	−4.79	−4.29	−	−	−5.98	−4.10
UO-31	−4.24	>−4.00	−5.15	>−4.00	−4.58	>−4.00	−4.22	>−4.00	−4.14	>−4.00	−5.83	−4.49
Prostate cancer												
PC-3	−4.69	>−4.00	−5.37	−4.78	<−8.00	−4.63	−4.87	−4.03	−4.52	>−4.00	−5.75	−3.76
DU-145	−4.84	−4.35	−5.54	−4.86	−4.65	−4.06	−4.75	−4.32	−4.58	>−4.00	−6.10	−3.87

(*continued on next page*)

Table 3 (*continued*)

Panel/cell line	**3a**		**3b**		**3c**		**3h**		**3i**		Thioguanine	
	Log_{10} GI_{50}	Log_{10} TGI	Log_{10} GI_{50}	Log_{10} TGI	Log_{10} GI_{50}	Log_{10} TGI	Log_{10} GI_{50}	Log_{10} TGI	Log_{10} GI_{50}	Log_{10} TGI	Log_{10} GI_{50}	Log_{10} TGI
Breast cancer												
MCF7	−4.12	>−4.00	−4.88	>−4.00	−4.20	>−4.00	−4.53	>−4.00	>−4.00	>−4.00	−6.15	−4.37
NCI/ADR-RES	−4.87	−4.17	−5.60	−5.17	−4.67	−4.21	−4.89	−4.45	−4.76	−4.28	–	–
MDA-MB 231/ATCC	−4.89	−4.24	−5.49	−4.77	−4.98	−4.36	−4.79	−4.39	−4.58	>−4.00	−5.71	−3.78
HS 578T	−4.79	−4.29	−4.87	−4.37	−4.63	−4.03	−4.94	−4.34	−4.50	−4.05	−5.13	−3.74
MDA-MB 435	−4.92	−4.29	−5.48	−4.87	−5.20	−4.61	−5.40	−4.79	−4.50	>−4.00	−6.07	−4.49
BT-549	−4.89	−4.31	−5.00	−4.65	−4.87	−4.25	−4.89	−4.47	−4.59	−4.11	−5.44	−3.79
T-47D	−4.43	>−4.00	−5.28	−4.63	−4.50	>−4.00	−4.39	>−4.00	>−4.00	>−4.00	−5.69	−3.97
MG MID	−4.73	−4.24	−5.41	−4.77	−4.83	−4.30	−4.79	−4.28	−4.47	−4.11		
Delta	0.90	0.87	2.02	0.85	3.17	0.85	0.75	0.88	1.53	0.47		
Range	1.63	1.10	3.43	1.62	4.00	1.15	1.53	1.16	2.00	0.58		

*4.2.3. Compound **3c***

IR (ν cm^{-1}, KBr): 3102 (N−H), 1663 (C=O), 1616 (hydrazone C=N). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 3.72, 4.13 (2s, 2H, CH_2CO), 6.66 (d, 2H, $J = 8.64$ Hz, ar $C_{3,5}$-H), 6.93 (s, 1H, C_2-H) 7.36−7.43 (m, 4H, Br−Ph $C_{3,5}$-H, ar $C_{2,6}$-H), 7.59−7.64 (m, 2H, Br−Ph $C_{2,6}$-H), 7.80, 7.96 (2s, 1H, N=CH), 8.12, 8.14 (2s, 1H, C_5-H), 9.76, 9.78 (2s, 1H, ar C_4-OH), 11.30, 11.40 (2s, 1H, CONH).

*4.2.4. Compound **3d***

IR (ν cm^{-1}, KBr): 3208, 3136 (N−H), 1660 (C=O), 1605 (hydrazone C=N). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 3.64 (s, 3H, ar C_4-OCH_3), 3.73, 4.15 (2s, 2H, CH_2CO), 6.83 (d, 2H, $J = 8.75$ Hz, ar $C_{3,5}$-H), 6.94 (s, 1H, C_2-H), 7.40, 7.42 (2d, 2H, $J = 8.58$, 8.57 Hz, ar $C_{2,6}$-H), 7.49, 7.51 (2d, 2H, $J = 8.91$, 9.58 Hz, Br−Ph $C_{3,5}$-H), 7.61, 7.63 (2d, 2H, $J = 8.55$, 8.54 Hz, Br−Ph $C_{2,6}$-H), 7.84, 8.01 (2s, 1H, N=CH), 8.13, 8.15 (2s, 1H, C_5-H), 11.37, 11.48 (2s, 1H, CONH).

^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 32.71, 34.21 (CH_2), 56.16 (ar OCH_3), 109.86, 110.17 (C_5), 111.10, 111.35 (C_2), 115.18 (ar $C_{3,5}$), 120.55, 120.66 (Br−Ph C_4), 127.29 (ar C_1), 127.45, 127.51 (Br−Ph $C_{2,6}$), 127.29, 127.67 (C_3), 129.38, 129.60 (ar $C_{2,6}$), 132.38, 132.43 (Br−Ph $C_{3,5}$), 134.39, 134.50 (Br−Ph C_1), 149.62, 149.71 (C_{7a}), 145.52, 145.66 (C_6), 144.40, 147.78 (N=CH), 161.57, 161.74 (ar C_4), 163.87, 169.65 (CONH). EIMS (70 eV) *m/z* (%): 470 (M + 2, 62.4), 468 (M^+, 61.7), 337 (22.4), 336 (5.1), 335 (23.4), 334 (2.0), 322 (10.8), 321 (60.3), 320 (13.5), 319 (68.1), 294 (62.0), 292 (63.0), 240 (15.9), 211 (42.4), 199 (5.4), 198 (2.7), 182 (4.1), 180 (3.4), 169 (6.8), 167 (4.7), 150 (11.5), 149 (13.5), 134 (19.0), 133 (14.6), 125 (10.2), 120 (9.8), 114 (14.6), 111 (13.2), 110 (5.4), 107 (9.8), 102 (4.7), 101 (9.5), 100 (100), 99 (25.8), 89 (8.1), 85 (21.7), 84 (8.8), 73 (4.1), 72 (6.1), 59 (2.7), 58 (37.3).

*4.2.5. Compound **3e***

IR (ν cm^{-1}, KBr): 3353, 3143 (N−H), 1681 (C=O), 1603 (hydrazone C=N). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 3.75, 4.18 (2s, 2H, CH_2CO), 6.94 (s, 1H, C_2-H), 7.12 (t, 2H, $J = 8.78$ Hz, ar $C_{3,5}$-H), 7.40, 7.42 (2d, 2H, $J = 8.43$, 6.84 Hz, Br−Ph $C_{3,5}$-H), 7.60−7.66 (m, 4H, Br−Ph $C_{2,6}$-H, ar $C_{2,6}$-H), 7.90, 8.08 (2s, 1H, N=CH), 8.13, 8.15 (2s, 1H, C_5-H), 11.51, 11.62 (2s, 1H, CONH). ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 33.17, 34.71 (CH_2), 110.29, 110.55 (C_5), 111.62, 111.85 (C_2), 117.20 (d, $J = 21.9$ Hz, ar $C_{3,5}$), 121.06, 121.16 (Br−Ph C_4), 127.65, 128.03 (C_3), 127.95, 128.00 (Br−Ph $C_{2,6}$), 130.44, 130.64 (2d, $J = 8.5$, 8.8 Hz, ar $C_{2,6}$), 132.04 (d, $J = 2.8$ Hz, ar C_1), 132.84, 132.89 (Br−Ph $C_{3,5}$), 134.87, 134.97 (Br−Ph C_1), 143.93, 147.37 (N=CH), 146.06, 146.21 (C_6), 150.14, 150.22 (C_{7a}), 164.37, 164.48 (2d, $J = 247.7$, 247.0 Hz, ar C_4), 164.66, 170.36 (CONH). EIMS (70 eV) *m/z* (%): 458 (M + 2, 83.8), 456 (M^+, 81.7), 337 (31.1), 336 (7.8), 335 (32.4), 334 (3.4), 322 (15.5), 321 (87.8), 320 (20.9), 319 (100), 294 (85.9), 292 (85.5), 240 (16.2), 211 (78.7), 199 (2.0), 198 (6.1), 182 (5.4), 180 (4.7), 169 (8.1), 167 (6.1), 138 (6.4), 137 (17.6), 125 (6.8), 122 (10.9), 121 (5.4), 114 (4.7), 111 (5.4), 110 (18.9), 108 (13.2), 102 (4.7), 101 (4.1), 100 (6.8), 99 (9.5), 95 (19.2), 89 (3.4), 85 (19.6), 84 (5.4), 73 (4.7), 72 (23.0), 59 (27.7), 58 (6.1).

*4.2.6. Compound **3f***

IR (ν cm^{-1}, KBr): 3133 (N−H), 1681 (C=O), 1604 (hydrazone C=N). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 3.76, 4.18 (2s, 2H, CH_2CO), 6.94 (s, 1H, C_2-H), 7.40, 7.42 (2d, 2H, $J = 8.42$, 6.95 Hz, ar $C_{3,5}$-H), 7.47−7.55 (m, 4H, Br−Ph $C_{3,5}$-H, ar $C_{2,6}$-H), 7.61, 7.63 (2d, 2H, $J = 8.46$, 8.49 Hz, Br−Ph $C_{2,6}$-H), 7.88, 8.05 (2s, 1H, N=CH), 8.12, 8.14 (2s, 1H, C_5-H), 11.57, 11.68 (2s, 1H, CONH). EIMS (70 eV) *m/z* (%): 337 (2.1), 336 (1.8), 335 (1.5), 334 (1.5), 321 (1.5), 319 (1.2), 294 (1.5), 292 (1.2), 240 (1.5), 211 (3.8), 200 (7.7), 199 (8.0), 198 (8.9), 197 (8.9), 185 (2.9), 184 (3.5), 183 (4.4), 182 (4.1), 181 (3.2), 180 (2.7), 170 (2.9), 169 (4.1), 168 (3.9), 167 (3.5), 157 (3.5), 155 (5.0), 125 (7.6), 114 (3.0), 111 (15.5), 102 (1.5), 101 (1.1), 100 (1.6), 99 (4.2), 89 (4.6), 85 (28.2), 76 (2.8), 73 (4.0), 72 (8.0), 59 (15.5), 43 (100).

*4.2.7. Compound **3g***

IR (ν cm^{-1}, KBr): 3151 (N−H), 1672 (C=O), 1600 (hydrazone C=N). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.95

(s, 6H, ar C_4-N(CH_3)$_2$), 3.85, 4.27 (2s, 2H, CH_2CO), 6.71, 6.73 (2d, 2H, $J = 8.85$, 7.60 Hz, ar $C_{3,5}$-H), 7.07 (s, 1H, C_2-H), 7.49–7.58 (m, 4H, Br–Ph $C_{3,5}$-H, ar $C_{2,6}$-H), 7.75, 7.79 (2d, 2H, $J = 8.53$, 8.52 Hz, Br–Ph $C_{2,6}$-H), 7.92, 8.09 (2s, 1H, N=CH), 8.26, 8.28 (2s, 1H, C_5-H), 11.37 (s, 1H, CONH).

*4.2.8. Compound **3h***

IR (ν cm^{-1}, KBr): 3201 (N–H), 1664 (C=O), 1598 (hydrazone C=N). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 3.71 (s, 3H, ar C_5-OCH_3), 3.80 (s, 3H, ar C_2-OCH_3), 3.88, 4.33 (2s, 2H, CH_2CO), 7.00 (dd, 1H, $J = 9.06$, 2.90 Hz, ar C_4-H), 7.04–7.09 (m, 2H, C_2-H, ar C_3-H), 7.30, 7.40 (2d, 1H, $J = 2.85$, 2.90 Hz, ar C_6-H), 7.55, 7.57 (2d, 2H, $J = 8.44$, 10.01 Hz, Br–Ph $C_{3,5}$-H), 7.76, 7.79 (2d, 2H, $J = 8.37$, 8.41 Hz, Br–Ph $C_{2,6}$-H), 8.27, 8.28 (2s, 1H, C_5-H), 8.36, 8.55 (2s, 1H, N=CH), 11.58, 11.73 (2s, 1H, CONH). ^{13}C NMR (100 MHz, δ, ppm, DMSO-d_6): 32.80, 34.26 (CH_2), 56.29, 56.32 (ar C_5-OCH_3), 57.06, 57.11 (ar C_2-OCH_3), 109.87, 110.10 (C_5), 109.94, 110.56 (ar C_6), 111.12, 111.43 (C_2), 114.13, 114.29 (ar C_3), 117.92, 118.63 (ar C_4), 120.56, 120.67 (Br–Ph C_4), 123.39, 123.55 (ar C_1), 127.15, 127.69 (C_3), 127.44, 127.50 (Br–Ph $C_{2,6}$), 132.36, 132.42 (Br–Ph $C_{3,5}$), 134.39, 134.49 (Br–Ph C_1), 139.98, 143.25 (N=CH), 145.55, 145.68 (C_6), 149.64, 149.72 (C_{7a}), 153.01, 153.14 (ar C_5), 154.09, 154.12 (ar C_2), 163.97, 169.86 (CONH).

*4.2.9. Compound **3i***

IR (ν cm^{-1}, KBr): 3132 (N–H), 1688 (C=O), 1616 (hydrazone C=N). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 3.78, 4.20 (2s, 2H, CH_2CO), 6.94, 6.96 (2s, 1H, C_2-H), 7.34 (dd, 1H, $J = 8.53$, 2.08 Hz, ar C_5-H), 7.39, 7.43 (2d, 2H, $J = 8.55$, 8.49 Hz, Br–Ph $C_{3,5}$-H), 7.57–7.65 (m, 3H, Br–Ph $C_{2,6}$-H, ar C_3-H), 7.79, 7.90 (2d, 1H, $J = 8.58$, 8.58 Hz, ar C_6-H), 8.13, 8.14 (2s, 1H, C_5-H), 8.24, 8.41 (2s, 1H, N=CH), 11.73, 11.84 (2s, 1H, CONH). EIMS (70 eV) *m/z* (%): 512 (M + 6, 7.4), 510 (M + 4, 40.5), 508 (M + 2, 80.4), 506 (M^+, 49.7), 337 (30.4), 336 (6.7), 335 (33.8), 334 (3.4), 322 (19.2), 321 (98.0), 320 (21.6), 319 (100), 294 (90.5), 292 (89.5), 240 (16.2), 211 (77.7), 199 (4.7), 198 (2.0), 192 (0.7), 191 (0.7), 190 (2.0), 189 (2.7), 188 (1.3), 187 (3.4), 182 (4.4), 180 (3.4), 176 (3.4), 175 (3.7), 174 (10.8), 173 (9.1), 172 (9.1), 171 (4.4), 169 (4.7), 167 (3.4), 162 (1.3), 160 (2.7), 158 (2.7), 149 (2.0), 147 (3.4), 145 (5.1), 125 (6.1), 114 (4.7), 111 (6.8), 110 (5.4), 102 (4.7), 101 (7.4), 100 (58.4), 99 (10.1), 89 (4.1), 85 (9.5), 84 (4.7), 73 (4.1), 72 (11.1), 59 (4.7), 58 (14.9).

4.3. In vitro evaluation of anticancer activity

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, 100 μL of cells were inoculated into 96 well microtiter plates 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 trichloroacetic acid (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 dimethyl sulfoxide 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 μg mL^{-1} gentamicin. Additional four, 10-fold or half-log serial dilutions were made to provide a total of five drug concentrations plus control. Aliquots of 100 μL of these different drug dilutions were added to the appropriate microtiter wells already containing 100 μL 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 μL 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 μL) 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). 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 percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as

$[(T_i - T_z)/(C - T_z)] \times 100$ for concentrations for which $T_i \geq T_z$,

$[(T_i - T_z)/T_z] \times 100$ for concentrations for 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 three 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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