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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and antitumor activity of (4-hydroxyphenyl) [5-substituted alkyl/aryl)-2-thioxo-1,3,4-thiadiazol-3-yl]methanone and [(3,4-disubstituted)-1,3-thiazol-2ylidene]-4-hydroxybenzohydrazide

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Abstract To examine new drug leads with potential anticancer activity, some (4-hydroxyphenyl)[5-substituted alkyl/aryl)-2-thioxo-1,3,4-thiadiazol-3-yl]methanone (**4.a–4.c**) and [-(3,4-disubstituted)-1,3-thiazol-2ylidene)]-4-hydroxybenzohydrazide (**6.a–6.d**) were synthesized using appropriate synthetic route. The newly prepared compounds **4.a–4.c** and **6.a–6.d** demonstrated inhibitory effects on the growth of a wide range of cancer cell lines especially on leukemia (HL-60), non-small lung cancer (HOP-92), renal cancer (ACHN) at the range of GI_{50} −4.23 to −7.23.

Keywords Anticancer · Thiadiazole · Thiazoline · Anticancer activity

Introduction

Design and synthesis of novel small molecules which can specifically block some targets in tumor cells are in perspective direction in modern medicinal chemistry. Many synthetic small molecules from different groups of heterocycles with influence on carcinogenesis have been reported and several of them are currently in clinical trials (Prabhakar *et al*., 2006; Hancsh and Leo, 1979). *p*-Hydroxybenzohydrazide with thiazolin and thiadiazole moieties may be a proved perspective scaffolds for design of anticancer drugs. The heterocyclic benzohydrazides constitute an important class of biologically active molecules which have attracted attention of medicinal chemists due to their wide range of pharmacological properties and their potential application as antitumor, antineoplastic, antiviral, and antiinflammatory agents (Xia *et al*., 2007; Vijaya *et al*., 2007). On the other hand, it is well known that thiazoline and thiadiazole derivatives have a great biological relevance; these compounds carry out diverse biological functions, being present in a number of biological systems involving several biochemist reactions of physiological relevance. Although many methods for synthesizing benzohydrazide ring systems have been reported, they continue to receive a great deal attention. Cancer treatment has been a major endeavor of research and development in academia and pharmaceutical industry for the last many years as it is one of the leading causes of death. Many of the available anticancer agents exhibit undesirable side effects such as reduced bioavailability, toxicity, and drug-resistance (Bonde and Gaikwad, 2004; Rolles and Kiraz, 1999). Therefore, the search for novel and selective anticancer agents is urgently required due to problems associated with currently available anticancer drugs (Castro *et al*., 1996, 1998, 2002a, b, 2005a, b, c; Molinari *et al*., 2009; Aguilera *et al*., 2000; Broughton *et al*., 2001; Araya *et al*., 2004).

Chemistry

The chemistry of *p*-hydroxybenzohydrazide is of great interest. They have become drugs of immense importance and having a variety of biological activities such antitumor (Xia *et al*., 2007; Vijaya *et al*., 2007), antianginal (Abadi and Eissa, 2003), antitubercular (Bonde and Gaikwad, 2004; Joshi *et al*., 2008), antihypertensive (Bhandari *et al*., 2009; Raparti *et al*., 2009), MAO enzyme inhibitor

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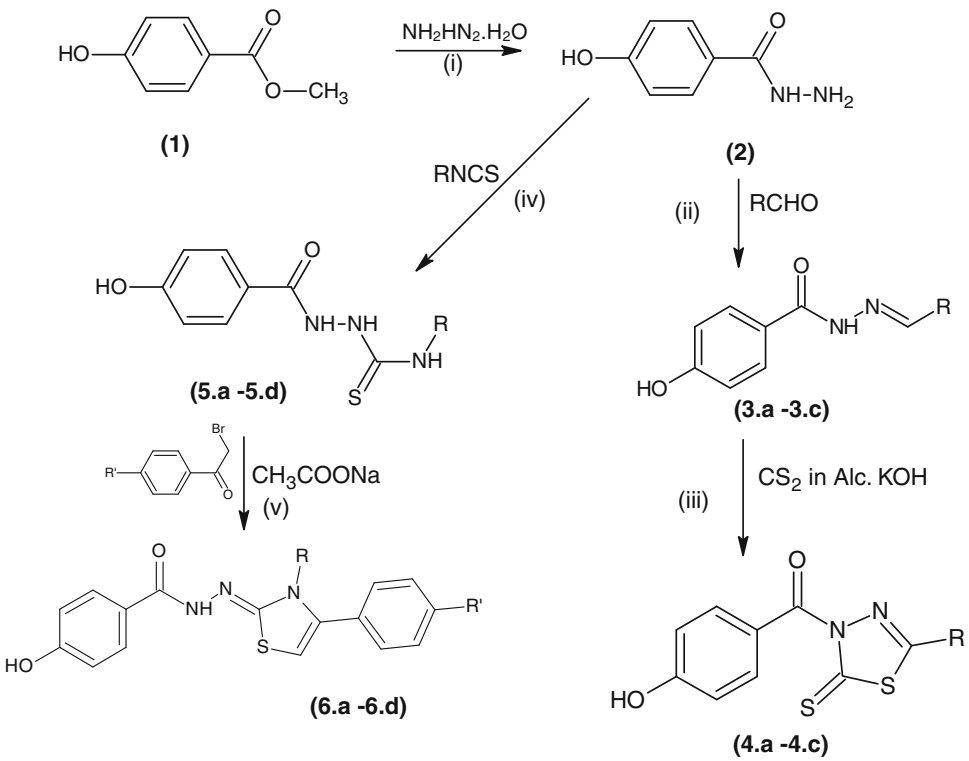
(Ke *et al*., 2008), antibacterial (Joshi *et al*., 2008). Since the *p*-hydroxybenzohydrazide, thiadiazole, and thiazolin (Xia *et al*., 2007; Vijaya *et al*., 2007) moieties were reported for their anticancer properties, the newly synthesized compounds may show similar pharmacological profile to classical anticancer drugs.

Some new (4-hydroxyphenyl)-[5-substituted alkyl/aryl)-2-thioxo-1,3,4-thiadiazol-3-yl]methanones and N′-[-(3,4-disubstituted)-1,3-thiazol-2ylidene)]-4-hydroxybenzo hydrazide were synthesized with the aim of obtaining the new agents which might have more or similar activity profile of existing anticancer drugs (Xia *et al*., 2007; Vijaya *et al*., 2007).

Synthetic pathway depicted in Scheme 1 outlines the chemistry of the present study. *For compounds* **4.a–4.c**: the compound (2), 4-hydroxybenzohydrazide, was synthesized by amination of compound (1) by hydrazine hydrate (80%). The physical and elemental analysis data confirmed the formation of the compound (2). To a solution of compound (2) in ethanol, various aliphatic/aromatic aldehydes were added. The mixture was refluxed and excess of solvent was distilled off to afforded N′-[(substituted alkyl/aryl)methylidene]-4-hydroxybenzohydrazide (**3.a–3.c**). To a solution of compounds (**3.a–3.c**) in ethanol, carbon disulfide in alc. KOH was added to obtain cyclized (4-hydroxyphenyl)-[(5-substituted-alkyl/aryl)-2-thioxo-1,3,4-thiadiazol-3-yl]methanone (**4.a–4.c**). Formation of various imines (Schiff's bases) (**3.a–3.c**) took place by the elimination of water compound. The excess of water was removed. Further, nitrogen in the side chain of N′-[(substituted alkyl/aryl)methylidene]-4-hydroxybenzohydrazide, with its lone pair of electrons, attacks the carbon atom of carbon disulfide to give intermediate, which on intermolecular rearrangement afforded the cyclized products (**4.a–4.c**).

The structures of compounds were confirmed on the basis of elemental analysis and spectral data. The IR spectra showed CN and CO stretching bands at 1569–1500 cm^{-1} and 1682–1682 cm^{-1}. The ^{1}HNMR signal showed downfield signal at δ ppm 4.20–4.30 and δ ppm 11.78–12.00 attributes to substituted NH=CH and CONHN=CH group. Also for compounds (**4.a–4.c**) the structures of the reaction products were confirmed by elemental analysis, IR, ^{1}H NMR and fast atom bambardment mass spctroscopy (FABMS) analyses. IR spectra revealed that the disappearance of CN band at 1569–1500 cm^{-1}. The ^{1}H spectra also lack the signal of CONHN=CH attributed to formation of thidiazole ring.



Scheme 1 Reagents and conditions: (i) EtOH (100 ml), $NH_2{\cdot}NH_2$ (4.5 ml, 99%), 12 h; (ii) EtOH (40 ml), 7 h; (iii) EtOH (20 ml), 6 h; (iv) EtOH, heated at 75–85°C for 12 h; (v) EtOH, heated to 80–90° and cool to 0°

Compd No	R	R'
5,6.a	2,4-Cl- C_6H_4	4-Cl
5,6.b	2,4-OCH_3 C_6H_4	4-Cl
5,6.c	2,6-CH_3 C_6H_4	4-CH_3
5,6.d	2,4-OCH_3 C_6H_4	4-OCH_3

Compd. No	R
3,4.a	2,6 ClC_6H_4
3,4.b	OHC_6H_4
3,4.c	CH3OC6H4

To the compound 2, namely 4-hydroxybenzohydrazide reaction with aryl isothiocyanate in ethanol gives compounds **5.a–5.d**. The structures of the compounds **5.a–5.d** were confirmed on the basis of elemental analysis and spectral data. The IR spectra showed NH and CS stretching bands at 3215–3230 and 1309–1348 cm^{-1}, respectively. The ^1H NMR showed downfield signal at δ 11.6–14.23 attributed to 3-substituted NH. Condensation of product **5.a–5.d** with 4-substituted phenacyl bromides affords compounds **6.a–6.d**. The structure of the compounds **6.a–6.d** was based on previous discussion of the structures of similar compounds (Bonde and Gaikwad, 2004). The structures of the reaction products were confirmed by elemental analysis, IR, ^1H NMR and FABMS analyses. IR spectra revealed that the disappearance of NH band at 3215–3230 cm^{-1}.The ^1H NMR spectra also lacked the NH signals and showed new singlet signal at d 5.8–6.1 attributed to C_5–H of thiazoline ring.

The synthesis of the intermediate and target compounds were performed by the reaction illustrated in Scheme 1.

Synthesis of N′-[-(3,4-disubstituted)-1,3-thiazol-2ylidene)]-4-hydroxybenzo hydrazide and (4-hydroxyphenyl)-[5-substituted alkyl/aryl)-2-thioxo-1,3,4-thiadiazol-3-yl]methanone

In pursuance of our interests for investigating the reactivity of 4-hydroxybenzohydrazide toward electrophile reagents we now extend the scope of this reactivity toward other active reagents.

Anticancer activity

Newly synthesized compounds were selected by the National Cancer Institute (NCI) Developmental Therapeutic Program (www.dtp.nci.nih.gov) for the in vitro cell line screening to investigate their anticancer activity.

Toxicity assays

Cells were grown in 96-well clear bottom black-well plates and the toxicity of the compounds was measured using the ToxiLight assay kit according to the instructions of the manufacturer. The total adenylate kinase level in each group of treated cells was determined with the 100% ToxiLight lysis reagent.

Results and discussion

The compounds were synthesized and evaluated for their physical, analytical, and spectral data. This selectivity in the scheme is believed to be due to electron density at N_1 and N_2. The latter being richer in electron density is more reactive and provides products of exclusive functionalization at N_2 (Bonde and Gaikwad, 2004).

Antitumor activity

Newly synthesized compounds were selected by the National Cancer Institute (NCI) Developmental Therapeutic Program (www.dtp.nci.nih.gov) for the in vitro cell line screening to investigate their anticancer activity. Anticancer assays were performed according to the US NCI protocol, which was described elsewhere (Kaminsky and Lesyk, 2009; Pati *et al*., 2008). The compounds were first evaluated at one dose primary anticancer assay toward three cell lines (panel consisting of three types of human cancers: breast (MCF7), lung (NCI-H460), and CNS in approximately 60 cell lines (concentration 10^{-5} M). The human tumor cell lines were derived form nine different cancer types: leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate, and breast cancers. As a result five synthesized substances successfully passed pre-screening phase. Only compounds **4.b** and **4.c** were found to be

Table 1 Anticancer screening data in concentration 10^{-5} M

Compound and NSC code	60 Cell lines in assay in 1-dose 10^{-5} M				Active (selected for 5-dose 60 cell lines assay)
	Mean growth	% Range of growth	The most sensitive cell line	Growth % of the most sensitive cell line	
4.a (D-749849)	45.35	17.05–75.94	Colon cancer (HCC-2998)	17.05	Active
4.b (D-749850)	30	−0.61–22.92	Melanoma (LOX IMVI)	−0.61	Inactive
4.c (D-748977)	104.85	68.04–127.24	Renal cancer (CAKI-1)	68.04	Inactive
6.a (D-748947)	56.14	−1.56–99.39	Colon cancer (HCC-2998)	−1.56	Active
6.b (D-749848)	14.70	−1.18–47.86	Ovarian cancer (NCI/ADR-RES)	−1.18	Active
6.c (D-749846)	52.37	5.18–95.66	Melanoma (SK-MEL-5)	5.18	Active
6.d (D-748978)	83.53	58.29–125.51	Melanoma (LOXIMVI)	58.29	Active

inactive in the pre-screening conditions. It is interesting that almost all active substances showed dominant growth inhibition activity against different cancer cell lines were consequently selected for in vitro testing against the full panel of nearly 60 cell lines (Table 1).

The compounds (**4.a**), and (**6.a–6.d**) possessed considerable activity and were selected for further study (five dose testing), whereas compounds **4.b** and **4.c** were tested without preliminary pre-screening stage, in advanced assay against a panel of approximately 60 tumor cell lines at 10-fold dilutions of five concentrations (100, 10, 1, 0.1, and 0.01 mM). The percentage of growth was evaluated spectrophotometrically versus controls not treated with test agents. A 48-h continuous drug exposure protocol followed and SRB (sulforodamine B) protein assay was used to estimate cell viability or growth. Based on the cytotoxicity assays, five antitumor activity dose–response parameters were calculated for experimental agents against each cell line: GI_{50} (molar concentration of the compound that inhibits 50% net cell growth), TGI (molar concentration of the compound leading to total inhibition), and LC_{50} (molar concentration of the compound leading to 50% net cell death). Furthermore, a mean graph midpoints (MG_MID) were calculated for each of the parameters, giving an average activity parameter over all cell lines for tested compounds. For the calculation of the MG_MID, insensitive cell lines are included with the highest concentration tested. The tested compounds showed a broad spectrum of growth inhibition activity against human tumor cells, as well as some distinctive patterns of selectivity. Compounds (**4.a**) (Fig. 1), (**6.a–6.c**) (Fig. 2), and (**6.d**) (Fig. 3) showed the highest cytotoxicity and were active against all tested human tumor cell lines (Table 2).

The tested compounds (**4.a**) and (**6.a–6.d**) showed a broad spectrum of growth inhibition activity against human tumor cells, as well as some distinctive patterns of selectivity (Table 2). These compounds appeared to be the most active against selected individual cell lines with the log GI_{50} varying from −7.23 to −4.93 (Table 3). Selectivity pattern analysis of cell lines by disease origin can definitely affirm selective action of compound (**4.a**) showed remarkable cytotoxic activity on non-small lung cancer (HOP 92) having GI_{50} value at −6.49, colon cancer (HCC-2998) at GI_{50} value −5.31 also showed significant cytotoxic activity on prostate cancer (PC-3) having GI_{50} value −5.48. Compound (**6.a**) showed potent inhibition against leukemia (CCRF-CEM) ($GI_{50} = -6.01$), colon cancer (HCC-2998) ($GI_{50} = -5.31$), ovarian cancer (NCR/ADR-RES) ($GI_{50} = -5.21$); compound (**6.b**) showed potent inhibition of cell lines of non-small cell lung cancer

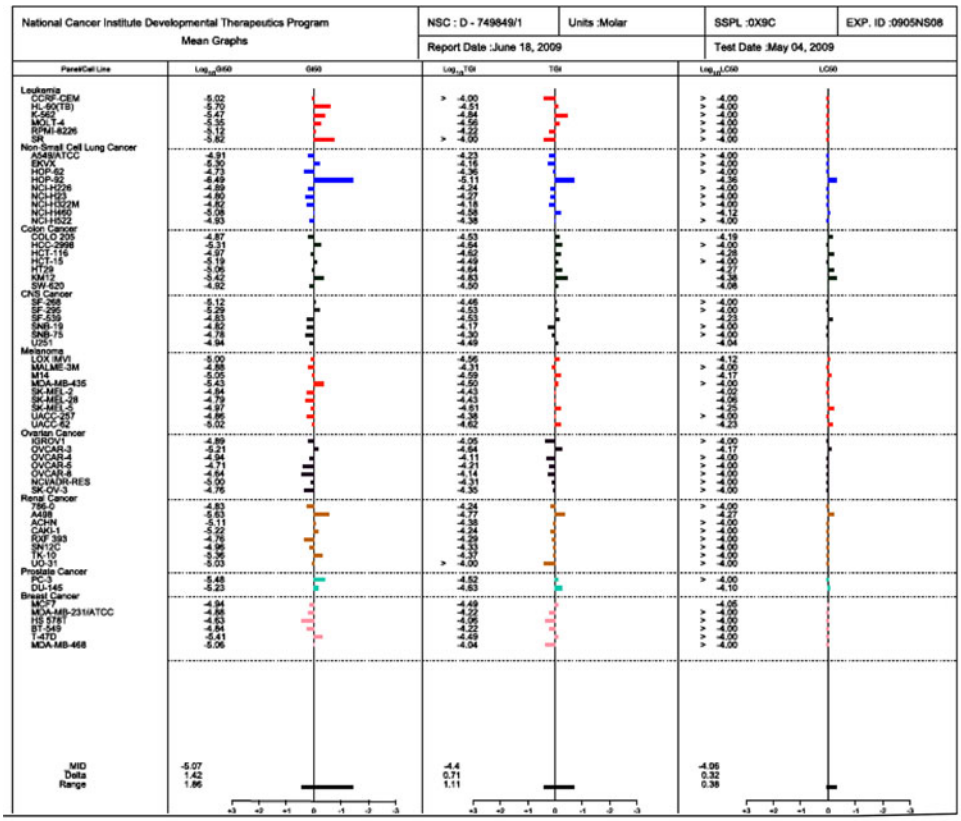


Fig. 1 Mean graph for compound **4.a**

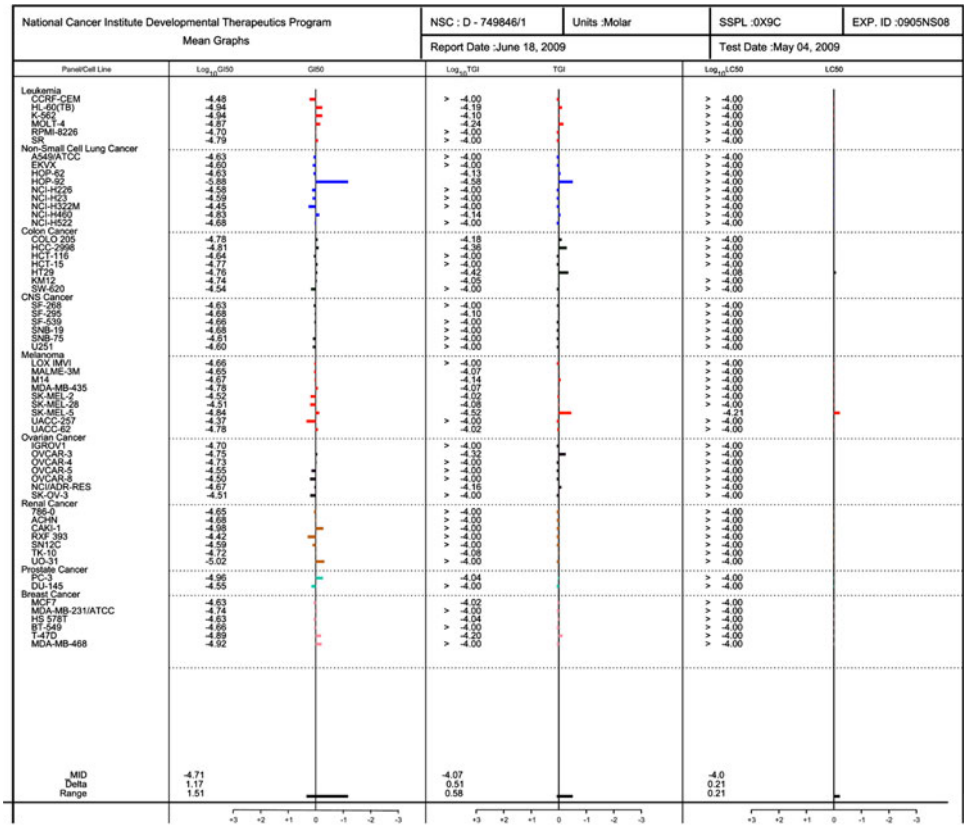


Fig. 2 Mean graph for compound **6.c**

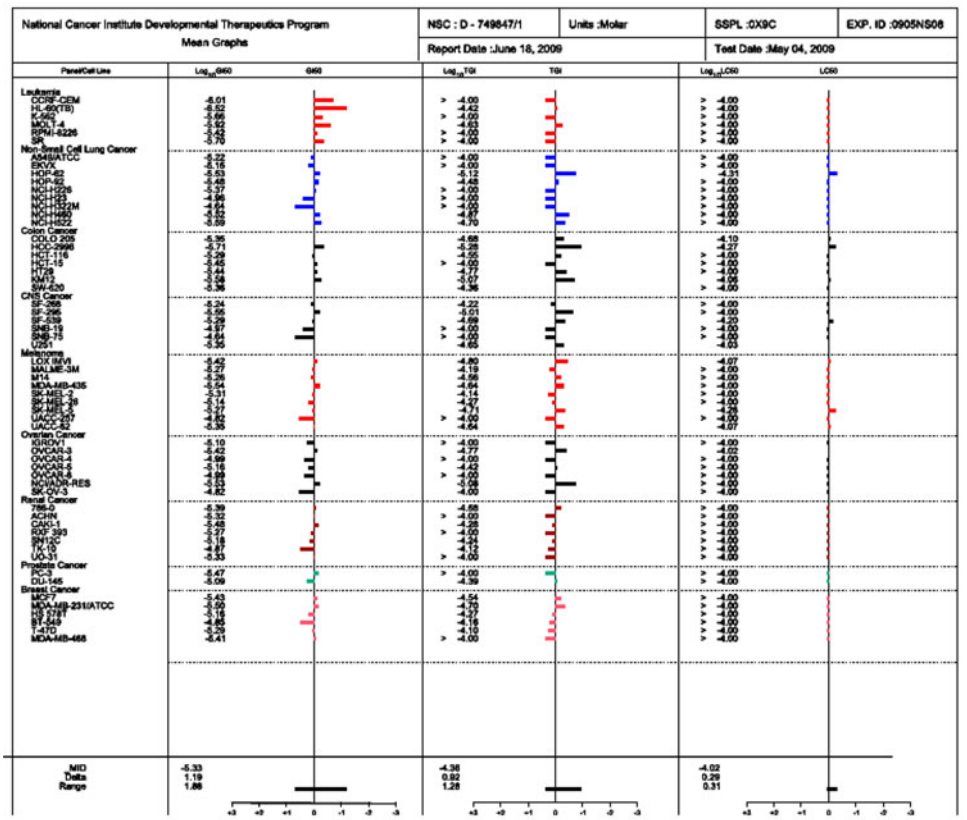


Fig. 3 Mean graph for compound **6.d**

Table 2 Anticancer screening data at dose-dependent assay

NSC code	N[a]	Log GI_{50}			Log TGI			Log LC_{50}		
		N1[b]	Range	MG_MID	N2[b]	Range	MG_MID	N3[b]	Range	MG_MID
(D-749849)	59	59	−4.64 to −6.49	−5.07	56	−4.0 to −4.83	−4.4	19	−4.0 to −4.36	−4.06
(D-749847)	59	59	−4.64 to −6.52	−5.33	37	−4.0 to 5.08	−4.36	09	−4.0 to −4.28	−4.02
(D-749848)	59	58	−4.0 to −4.84	−4.45	08	−4.0 to −4.24	−4.02	00	−4.0 to −4.02	−4.0
(D-749846)	59	59	−4.42 to −5.02	−4.71	25	−4.0 to −4.32	−4.07	03	−4.0 to −4.08	−4.0
(D-748978)	58	58	−4.93 to −7.23	−5.5	58	−4.52 to −5.71	−4.85	52	−4.0 to −4.63	−4.32

[a] Number of human tumor cell lines tested at the 2nd-stage assay

[b] Number of sensitive cell lines, against which the compound possessed considerable growth inhibition according to mentioned parameter (parameters log GI_{50}, log TGI, and log $LC_{50} \geq 4.00$)

(HOP-92) ($GI_{50} = -4.84$), melanoma (MALME-3M) ($GI_{50} = -4.60$) breast cancer (MCF 7) ($GI_{50} = -4.60$); compound (**6.c**) was found to be a highly active growth inhibitor of the non-small lung cancer (HOP-92) ($GI_{50} = -5.88$), colon cancer cell line (HCC-2998) ($GI_{50} = -4.81$), renal cancer (UO-31) ($GI_{50} = -5.02$), leukemia (HL-60 TB), and melanoma (SKMEL-28) having GI_{50} values in the range of −4.94 to −5.02. Compound (**6.d**) acts on leukemia cell line (K-562) ($GI_{50} = -5.66$) (MOLT-4) ($GI_{50} = -5.96$), non-small lung cancer (HOP-92) ($GI_{50} = -5.79$), melanoma cell lines (SK-MEL-28) ($GI_{50} = -7.02$), renal cancer (ACHN) (−7.23), and breast cell line (HCC-2998) ($GI_{50} = -5.62$).

For structure–activity studies, we choose the aromatic substitutions that are commonly employed in 4-hydroxybenzohydrazide. Thiazoline ring is essential for antitumor activity as compounds **4.a**–**4.c** showed comparatively less activity than compounds **6.a**–**6.d**. The different substituent in compounds **6.a**–**6.d** over the side chain at 3 and 4 positions of thiazol ring exerts significant influence on biological activity. Further, the presence of electron-withdrawing groups, as in **6.a**–**6.d** showed maximum antitumor activity. Literature survey reveals that electrons-withdrawing or donating groups amend the lipophilicity of the test compounds, which in turn alters permeability across the cell membrane.

On the basis of these results SAR study revealed that,

(1) Anticancer activity of compounds may increase by introducing electron withdrawing group at position 5 of thiadiazol and at position 3 of thiazoline which might imparts its lipophilicity.
(2) Introduction of *p*-OH group enhanced potency, this effect might be due to the linking of *p*-hydroxy group with the receptor.
(3) Linking position of thiadiazole or thiazolin fragment (2 or 4) core did not influence.
(4) Antitumor activity.

Experimental

Synthesis of **2**

A mixture of **1** (1.5 g, 0.02 mol), 85% hydrazine hydrate (4.12 ml, 0.08 mol) was refluxed for 12 h. The excess solvent was removed under reduced pressure and the reaction mixture was cooled at 4−5°C. The solid crystals separated were filtered, washed with cold water, dried, and recrystallized from ethanol. To afford white product (**2**), (1.4 g, mp: 172–173°C).

Yield: 1.32 g (80%). mp 170–171°C (ethanol/water), R_f: 0.62 (acetonitrile; methanol, 1:1), IR (KBr): cm^{-1} 3351 (alcohol O–H and C–O stretching), 3013 (Ar-H stretching), 1622 (C–O stretching), 1185, 1034 (alcohol O–H starching), 832 (benzene 1,4-disubstituted), ^{1}H-NMR (300 MHz, DMSO-*d*6): δ ppm 9.5 (s, 1H, CO–NH), 5.32 (s, 2H, NH_2). Electron emmision mass spctroscopy (EIMS) (*m/z*, 100%):152 ([M + 2], 100%).

Anal. $C_7H_8N_2O_2$: C, 55.26/55.26; H, 5.31/5.30; N, 18.40/18.41.

General procedure for synthesis of **3.a**–**3.c**

A solution of the corresponding compound **2** (1.5 g 10 mmol) in ethanol (40 ml) was refluxed with various aliphatic/aromatic aldehydes (10 mmol) for 3 h. The excess of solvent was removed under reduce pressure. After cooling to room temperature, a white solid appeared. This crude product was filtered, washed with diethyl ether, dried, and recrystallized from rectified sprit.

Data for selective compound

3.a Yield: 1.09 g(73%), mp 156–157°C (rectified sprit), R_f: 0.65 (acetonitrile/methanol), IR (KBr): (m, cm^{-1}) 3276–3390 (Ar-/OH, NH), 1658–1684 (hydrazide –C=O), 1568

Table 3 The most sensitive cancer cell lines to synthesized compounds

NSC code	Cancer type	Most sensitive cell line	Log GI_{50}	Log TGI	Log LC_{50}
(D-749849)	Leukemia	K-562	−5.47	−4.84	>4.00
		HL-60	−5.70	−4.51	>4.00
		SR	−5.82	>−4.00	>4.00
	Non-small lung cancer	HOP-92	−6.49	−5.11	>−4.00
	Colon cancer	HCC-2998	−5.31	−4.64	>−4.00
		HCT-15	−5.19	−4.49	>−4.00
	CNS cancer	SF-295	−5.29	−4.53	>−4.00
	Melanoma	MDA-MB-435	−5.43	−4.50	>−4.00
	Ovarian cancer	OVCAR-3	−5.21	−4.64	−4.17
	Renal cancer	A 498	−5.63	−4.77	−4.27
	Prostate cancer	PC-3	−5.48	−4.52	−4.10
	Breast cancer	T-47D	−5.41	−4.49	>−4.00
(D-749847)	Leukemia	CCRF-CEM	−6.01	>−4.00	>−4.00
		HL-60 (TB)	−6.52	−4.42	>−4.00
	Non-small lung cancer	HOP-92	−5.53	−5.12	−4.31
	Colon cancer	NCI-H522	−5.59	−4.70	>−4.00
		HCC-2998	−5.71	−5.28	−4.27
	CNS cancer	KW12	−5.58	−5.07	−4.06
		SF-295	−5.55	−5.01	>−4.00
	Melanoma	MDA-MB-435	−5.54	−4.64	>−4.00
	Ovarian cancer	NCI/ADR-RES	−5.53	−5.08	>−4.00
	Renal cancer	CAKI-1	−5.48	−4.28	>−4.00
	Prostate cancer	PC-3	−5.47	>−4.00	>−4.00
	Breast cancer	MDA-MB-468	−5.41	>−4.00	>−4.00
(D-749848)	Leukemia	HL-60 (TB)	−4.75	−4.09	>−4.00
	Non-small lung cancer	HOP-92	−4.84	−4.19	>−4.00
	Colon cancer	HCT-116	−4.56	>−4.00	>−4.00
	CNS cancer	SF-295	−4.56	>−4.00	>−4.00
	Melanoma	MALME-3M	−4.60	−4.04	>−4.00
	Ovarian cancer	OVCAR-3	−4.57	−4.08	>−4.00
	Renal cancer	CAKI-1	−4.64	>−4.00	>−4.00
	Prostate cancer	PC-3	−4.74	>−4.00	>−4.00
	Breast cancer	MCF7	−4.60	>−4.00	>−4.00
(D-749846)	Leukemia	HL-60 (TB)	−4.94	−4.19	>−4.00
	Non-small lung cancer	HOP-92	−5.88	−4.58	>−4.00
	Colon cancer	HCC-2998	−4.81	−4.36	>−4.00
	CNS cancer	SF-295	−4.68	−4.10	>−4.00
	Melanoma	SK-MEL-28	−4.84	−4.52	−4.21
	Ovarian cancer	OVCAR	−4.75	−4.32	>−4.00
	Renal cancer	UO-31	−5.02	>−4.00	>−4.00
	Prostate cancer	PC-3	−4.96	−4.04	>−4.00
	Breast cancer	T-47D	−4.89	−4.20	>−4.00

Table 3 continued

NSC code	Cancer type	Most sensitive cell line	Log GI_{50}	Log TGI	Log LC_{50}
(D-749878)[a]	Leukemia	K-562	−5.66	−5.04	−4.44
		MOLT-4	−5.96	−4.82	−4.19
		RPMI-8226	−5.61	−4.64	>−4.00
	Non-small lung cancer	HOP-92	−5.79	−4.80	−4.35
		NCI-H522	−5.66	−5.05	−4.42
	CNS cancer	SF-298	−5.62	−5.11	−4.56
		SF-295	−5.61	−4.87	−4.36
	Melanoma	SK-MEL-28	−7.02	−5.71	−5.20
	Ovarian Cancer	NCI/ADR-RES	−5.73	−5.29	−4.65
	Renal cancer	ACHN	−7.23	−5.36	−4.65
	Prostate cancer	PC-3	−5.58	−4.83	−4.33
	Breast cancer	MDA-MB-468	−5.62	−4.92	−4.20

[a] Data of repeated assay

(C-Ar stretching), 1610 (–C=N). ^{1}H NMR (DMSO-*d*6) δ ppm 1.21 (t, 3H, OCH_3–C_6H_4), 5.27 (s, 1H, Ar-OH), 8.22 (s, 1H, CH–N)/11.78 (d, 1H, CONHN=CH). EIMS (*m/z*, 100%): 270 ([M + 2], 100%).

Anal. $C_{15}H_{14}N_2O_3$: C 66.61/66.66; H 5.22/5.22; N 10.30/10.36.

General procedure for synthesis of compounds (**4.a–4.c**)

To a mixture of corresponding compound **3** (0.01 mol) in ethanol (50 ml) a solution of potassium hydroxide (0.01 mol) in ethanol (10 ml) was added followed by carbon disulfide (20 ml). The reaction mixture was heated under refluxed for 6 h. It was concentrated and poured into crush ice. The resultant solid obtain was filtered, dried, and recrystallized using the mixture of DMF and water (1:1).

4.a Yield: 2.5 g (81%), mp 237–238°C (DMF/water), R_f: 0.76 (acetonitrile/methanol, 1:1), IR (KBr): cm^{-1} 1191–1240 (C=S stretching), 1670-1730 (–C=O), 1577 (C-Ar stretching), 1092–1100 (Ar-Cl stretching), ^{1}H NMR (DMSO-*d*6) δ 2.23 (s, Ar-Cl), 6.88–7.78 (m, 4H, Ar-H_1), 7.40 (s, 3H, Ar-H_2). EIMS (*m/z*, 100%): 383 ([M + 2], 100%). Anal. $C_{15}H_8Cl_2N_2OS_2$: C, 46.97/47.01; H, 2.10/2.00; N, 18.45/18.50.

4.b Yield: 1.9 g (79%), mp 218–219°C (DMF/water), R_f: 0.77 (acetonitrile/methanol, 1:1), IR (KBr): 1191–1240 (C=S stretching), 1670–1730 (–C=O), 3600–3400 (Ar-OH), 1577 (C-Ar stretching), ^{1}H NMR (DMSO-*d*6) δ 5.35 (s, 1H, Ar-OH), 6.88–7.78 (m, 4H, Ar-H), 7.01–7.85 (m, 4H, Ar-H). EIMS (*m/z*, 100%): 330 ([M + 2], 100%). Anal. $C_{15}H_{10}N_2O_3S_2$: C, 54.51/54.53; H, 3.02/3.05; N, 8.39/8.48.

4.c Yield: 1.9 g (74%), mp 202–203°C(DMF/water), R_f: 0.67 (acetonitrile/methanol, 1:1), IR (KBr): 1191–1240 (C=S stretching), 1670–1730 (–C=O), 3600–3400 (Ar-OH), 1577 (C-Ar stretching), ^{1}H NMR (DMSO-*d*6) δ 1.2–1.31 (s, 3H,OCH_3), 5.26 (s, 1H, Ar-OH), 6.88–7.78 (m, 4H, Ar-H_1), 7.10–7.71 (m, 4H, Ar-H_2). EIMS (*m/z*, 100%): 343 ([M + 2], 100%). Anal. $C_{16}H_{12}N_2O_3S_2$: C, 55.72/55.80; H, 3.50/3.51; N, 8.10/8.13.

General procedure for synthesis of compounds from **5.a–5.d**

To a solution of 2 (0.01 mol) in ethanol (50 ml), various aliphatic/aromatic isothiocyanate (0.01 mol) were added. The reaction mixture was refluxed for 12 h. Excess solvent was removed under vacuum. The residue was washed with diethyl ether and recrystallized using methanol.

5.d Yield: 0.8 g (56%), mp 189–190°C (methanol), R_f: 0.66 (acetonitrile/methanol, 1:1), IR (KBr): 3215, 3230 (NH), 1731 (C=O stretching), 1315 (C=S stretching) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.33(s, 1H, Ar-OH), 6.88–7.82 (m, 8H, ArH), 7.78 (s, 1H, CONH), 10.40 (s, 1H, NH), 11.81 (s, 1H, NH-Ar). EIMS (*m/z*, 100%): 322 ([M + 2], 100%). Anal. $C_{14}H_{12}N_4O_4S$: C, 50.59/50.60; H, 3.64/3.64; N, 16.83/16.86.

General procedure for synthesis of compounds (**6.a–6.d**)

The mixture of the thiosemicarbazide (0.01 mol) (**3.a–3.i**) appropriate phenacyl bromide (0.01 mol) and sodium acetate (0.2 mol) in ethanol (50 ml) was refluxed for 7 h. The mixture was cooled, diluted with enough water to develop turbidity, and left overnight to obtain the product. The product was filtered, dried, and recrystallized using aqueous ethanol.

6.a: Yield: 2.6 g (76%), mp 194–195°C (ethanol/water), R_f: 0.72 (acetonitrile/methanol, 1:1), IR (KBr) cm^{-1}: 3224

(NH), 1739 (C=O stretching), 1573, 1485, 1056,(thiazoline), 3003 (Ar-H stretching); ^{1}H NMR ($CDCl_3$): δ 5.33 (s, 1H, Ar-OH), 5.97 (s, 1H, thiazoline), 7.09–8.01 (m, 11H, Ar-H), 7.76 (s, 1H, CO-NH). EIMS (*m/z*, 100%): 490 ([M + 2], 100%). Anal. $C_{22}H_{14}Cl_3N_3O_2S$: C, 53.86/53.84; H, 2.87/2.88; N, 8.55/8.56.

6.b Yield: 1.9 g (56%), mp 233–234°C (ethanol/water), R_f: 0.65 (acetonitrile/methanol, 1:1), IR (KBr) cm^{-1}: 3234 (NH), 1740 (C=O stretching), 1572, 1480, 1052 (thiazoline), 3003 (Ar-H stretching); ^{1}H NMR ($CDCl_3$): δ 3.26 (s, 1H, OCH_3–C_6H_4), 5.32 (s, 1H, Ar-OH), 5.97 (s, 1H, thiazoline), 6.89–8.01 (m, 11H, Ar-H), 7.76 (s, 1H, CO-NH). EIMS (*m/z*, 100%): 488 ([M + 2], 100%). Anal. $C_{24}H_{20}ClN_3O_4S$: C, 59.72/59.81; H, 4.12/4.18; N, 8.63/8.72.

6.c Yield: 1.8 g (61%), mp 221–222°C (ethanol/water), R_f: 0.63 (acetonitrile/methanol, 1:1), IR (KBr) cm^{-1}: 3217 (NH), 1740 (C=O stretching), 1562, 1481, 1061 (thiazoline), 3018 (Ar-H stretching); ^{1}H NMR ($CDCl_3$): δ 2.32 (s, 1H, Ar-CH_3), 2.22 (s, 6H, CH_3), 5.30 (s, 1H, Ar-OH), 6.22 (s, 1H, thiazoline), 6.86–7.81 (m, 11H, Ar-H), 7.80 (s, 1H, CO–NH). EIMS (*m/z*, 100%): 429 ([M + 2], 100%). Anal. $C_{25}H_{23}N_3O_2S$: C, 68.90/69.91; H, 5.41/5.40; N, 9.77/9.78.

6.d Yield: 2.2 g (76%), mp 197–198°C (ethanol/water), R_f: 0.72 (acetonitrile/methanol, 1:1), IR (KBr) cm^{-1}: 1040 (Ar-F stretching), 3218 (NH), 1732 (C=O stretching), 1571, 1484, 1063 (thiazoline), 3009 (Ar-H stretching), 865 (Ar-F stretching); ^{1}H NMR ($CDCl_3$): δ 3.26 (s, 1H, OCH_3–C_6H_5), 5.12 (s, 1H, Ar-OH), 6.88 (s, 1H, thiazoline), 6.88–8.01 (m, 12H, Ar-H), 7.30 (s, 1H, CO–NH). EIMS (*m/z*, 100%): 435 ([M + 2], 100%). Anal. $C_{23}H_{18}FN_3O_3S$: C, 63.43/63.44; H, 4.15/4.17; N, 9.64/9.65.

Anticancer activity

Primary anticancer assay was performed at human tumor cell lines panel derived from nine neoplastic diseases, in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda. The cytotoxic and/or growth inhibitory effects of the most active selected compounds were tested in vitro against the full panel of about 60 human tumor cell lines at 10-fold dilutions of five concentrations ranging from 10^{-4}–10^{-8} to 10^{-8} M. A 48-h continuous drug-exposure protocol was followed and an SRB protein assay was used to estimate cell viability or growth. Using the seven absorbance measurements [time zero (T_z), control growth in the absence of drug (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 $T_i > / = T_z$

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

Five dose response parameters are calculated for each experimental agent. Growth inhibition of 50 % (GI_{50}) is calculated from $[(T_i - T_z)/(C - T_z)] \times 100 = 50$, which is 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 total growth inhibition (TGI) is calculated from $T_i = T_z$. The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from $[(T_i - T_z)/T_z] \times 100 = -50$. Values are calculated for each of these three parameters if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter is expressed as greater or less than the maximum or minimum concentration tested.

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