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Original article

Synthesis and primary cytotoxicity evaluation of new 5-benzylidene-2, 4-thiazolidinedione derivatives

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ABSTRACT

In the present work, ten novel derivatives (**3a**–**3j**) of 5-benzylidene-2,4-thiazolidinediones were synthesized and their structures were determined by analytical and spectral (FTIR, ^{1}H NMR, ^{13}C NMR) methods. The newly synthesized compounds were evaluated for their antiproliferative activity at Tata Memorial's Advanced Center for Treatment, Research and Education in Cancer (ACTREC), India, in a panel of 7 cancer cell lines using four concentrations at 10-fold dilutions. Sulforhodamine B (SRB) protein assay was used to estimate cell stability or growth. Though the compounds showed varying degrees of cytotoxicity in the tested cell lines, most marked effect was observed by compound **3e** in MCF7 (breast cancer), K562 (leukemia) and GURAV (nasopharyngeal cancer) cell lines with $\log_{10}$ GI_{50} values of −6.7, −6.72 and −6.73 respectively.

1. Introduction

Thiazolidinediones (TZDs) are a class of insulin sensitizing drugs which include ciglitazone, pioglitazone, troglitazone and rosiglitazone, although troglitazone was removed from the market in 2000 due to hepatotoxicity. Apart from their known antidiabetic activity, the ability of TZDs to contribute to cancer therapy has been evidenced by numerous in vitro and in vivo studies [1–9]. While TZDs are known to stimulate PPAR-γ receptor, they also have multiple PPAR-γ independent effects and the specific role of PPAR-γ activation in the anticancer effects of TZDs is still under investigation. It has been reported that there exists a 3 orders-of-magnitude discrepancy between the concentration required to mediate antitumor effects and that for PPAR-γ activation [10]. Thus, the dose required for anticancer activity of thiazolidinediones would be significantly lower than that required to bring about hypoglycemic activity. We report herein the synthesis and anticancer activity of a novel series of benzylidene thiazolidinedione analogs of general formula (Fig. 1). The TZD ring has been used as scaffold to develop this novel class of anticancer agents, encouraged by the literature report that toxicity of troglitazone is not due to the TZD ring [11–13]. C. C. Yang et al. [14] and J.W. Huang et al. [15] have reported Δ2TG and Δ2CG, the benzylidene analogs of troglitazone and ciglitazone to be more potent than their parent compounds respectively in suppressing cell proliferation in cancer cells. This prompted us to develop TZD analogs with a double bond adjacent to the TZD ring. A carbonyl carbon in the form of amide linkage has been introduced in the linker chain between the lipophilic tail and central aryl portion for a better metabolic profile. We have reported the synthesis and antidiabetic activity of the compound (**3g**) in an earlier work but its anticancer activity has not been described [16].

2. Chemistry

The synthetic route of the compounds is outlined in Scheme 1. A series of 5-benzylidene-2,4-thiazolidinediones (**3a**–**3j**) were synthesized in three steps. 5-(4-hydroxybenzylidene)-2,4-thiazolidinedione (**1**) was prepared by Knoevenagel condensation reaction between 4-hydroxy benzaldehyde and 2,4-TZD. This intermediate was common to all molecules being synthesized. Further, this moiety was condensed with various chloroacetylated heteroaromatic amines or heterocyclic nitrogen containing compounds. The chloroacetylated moieties (**2a**–**2j**) were prepared by acetylation of respective amines or imides with chloroacetyl chloride under basic conditions. The structures of all synthesized compounds were determined by CHN elemental analysis and spectral data (FTIR, ^{1}H NMR and ^{13}C NMR).

In the ^{1}H NMR spectra, the presence of characteristic singlet at δ 7.67 ppm for benzylidene proton provided evidence for formation of (**1**). Formation of the chloroacetylated moieties (**2a**–**2j**) was characterized by a peculiar singlet for $-CH_2$ protons resonating at 4.0–4.8 ppm. In the IR spectra of **3a**–**3j** the CO–NH–CO

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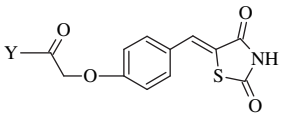


Y=**R- NH** for 3b, 3c, 3e, 3f, 3g, 3i and 3j

Y= **R** for 3a, 3d and 3h

Fig. 1. General formula.

and C=O bands were observed in the region 3460–3446 cm^{-1} and 1735–1670 cm^{-1} respectively. In the ^1H NMR spectra, presence of resonance assigned to the $-CH_2$ protons provides evidence for formation of $-CH_2-O-$ linkage in final thiazolidinedione analogs (**3a**–**3j**). These protons resonated in 4.4–5.0 ppm region as singlet. Momose et al have calculated the value of methine proton in to ^1H NMR be at δ 7.90 in the Z-configurations in good accordance with the observations. Thus the configuration was determined to be Z [17]. Additional spectral characteristics are presented in Section 4.

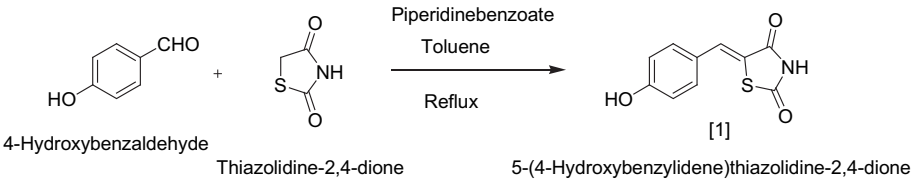
3. Anticancer evaluation and discussion

After successful synthesis of the derivatives of 5-benzylidene-2,4-thiazolidinediones, their anticancer activity was evaluated in vitro on the 7-cell line panel consisting of HOP62 (Lung cancer), PC3 (Prostate cancer), MCF7 (Breast cancer), HEPG2 (Hepatoma), K562 (Leukemia), GURAV (Oral cancer), and KB (Nasopharyngeal cancer). Primary anticancer assay was performed in accordance with the US NCI protocol [18–20].

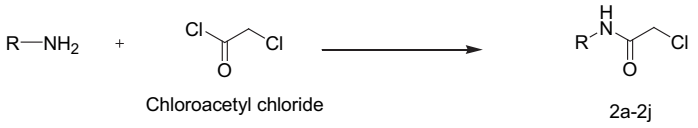
The cytotoxic effects of synthesized compounds were tested in vitro against the panel of cell lines at 10-fold dilutions of four concentrations ranging from 10^{-4} to 10^{-7} M. The percentage growth was evaluated spectrophotometrically versus negative control not treated with test agents and positive control, treated with doxorubicin, a proven anticancer agent. 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 $\log_{10}$ GI_{50} value -4.0 and <-4.0 were declared to be active (Table 1).

Amongst the compounds tested, compound **3e** showed most promising results in the preliminary antiproliferative activity testing. It showed potent cytotoxicity against five of the seven tested cell lines, viz., MCF 7 ($\log_{10}GI_{50}$ − 6.71), PC3 ($\log_{10}GI_{50}$ − 5.60), KB ($\log_{10}GI_{50}$ − 5.65), GURAV ($\log_{10}GI_{50}$ − 6.74) and K562 ($\log_{10}GI_{50}$ − 6.73).

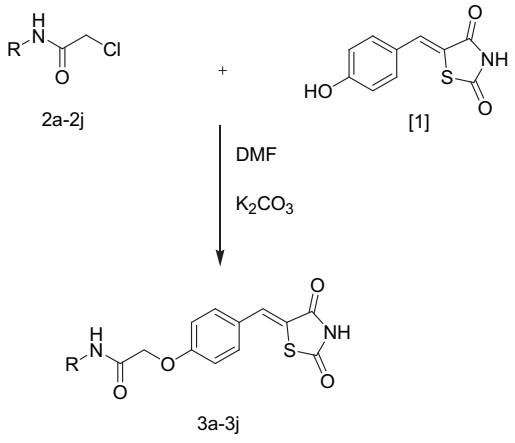
Step 1: Synthesis of 1 (Knoevenagel condensation)



Step 2: Synthesis of 2a-2j (chloroacetylation)



Step 3: Synthesis of 3a-3j (Condensation of 2a-2j and 1)



Scheme 1. General synthetic route for synthesis of **3a**–**3j**.

Table 1
In vitro anticancer activity in 7 human tumor cell lines for **3a**–**3j**.

Compound	Disease	Cancer cell line	$Log_{10}GI_{50}$ (μM)	$Log_{10}TGI$ (μM)
3a	Leukemia	K562	>−4.0	>−4.0
	Breast cancer	MCF7	−4.53	>−4.0
	Hepatoma	HEPG2	>−4.0	>−4.0
	NSC lung cancer	HOP62	−6.72	−4.54
	Prostate cancer	PC3	−4.53	>−4.0
	Oral cancer	GURAV	>−4.0	>−4.0
	Nasopharyngeal cancer	KB	>−4.0	>−4.0
3b	Leukemia	K562	>−4.0	>−4.0
	Breast cancer	MCF7	>−4.0	>−4.0
	Hepatoma	HEPG2	>−4.0	>−4.0
	NSC lung cancer	HOP62	−6.73	>−4.0
	Prostate cancer	PC3	>−4.0	>−4.0
	Oral cancer	GURAV	>−4.0	>−4.0
	Nasopharyngeal cancer	KB	>−4.0	>−4.0
3c	Leukemia	K562	−5.65	>−4.0
	Breast cancer	MCF7	>−4.0	>−4.0
	Hepatoma	HEPG2	>−4.0	>−4.0
	NSC lung cancer	HOP62	−4.51	>−4.0
	Prostate cancer	PC3	−5.65	−4.43
	Oral cancer	GURAV	−6.71	−4.49
	Nasopharyngeal cancer	KB	>−4.0	>−4.0
3d	Leukemia	K562	>−4.0	>−4.0
	Breast cancer	MCF7	−4.55	>−4.0
	Hepatoma	HEPG2	>−4.0	>−4.0
	NSC lung cancer	HOP62	−4.53	>−4.0
	Prostate cancer	PC3	>−4.0	>−4.0
	Oral cancer	GURAV	>−4.0	>−4.0
	Nasopharyngeal cancer	KB	>−4.0	>−4.0
3e	Leukemia	K562	−6.72	>−4.0
	Breast cancer	MCF7	−6.71	−4.52
	Hepatoma	HEPG2	>−4.0	>−4.0
	NSC lung cancer	HOP62	>−4.0	>−4.0
	Prostate cancer	PC3	−5.60	>−4.0
	Oral cancer	GURAV	−6.73	−4.52
	Nasopharyngeal cancer	KB	−5.65	>−4.0
3f	Leukemia	K562	>−4.0	>−4.0
	Breast cancer	MCF7	−4.60	>−4.0
	Hepatoma	HEPG2	>−4.0	>−4.0
	NSC lung cancer	HOP62	−6.77	−4.54
	Prostate cancer	PC3	−4.55	>−4.0
	Oral cancer	GURAV	>−4.0	>−4.0
	Nasopharyngeal cancer	KB	>−4.0	>−4.0
3g	Leukemia	K562	>−4.0	>−4.0
	Breast cancer	MCF7	>−4.0	>−4.0
	Hepatoma	HEPG2	>−4.0	>−4.0
	NSC lung cancer	HOP62	>−4.0	>−4.0
	Prostate cancer	PC3	>−4.0	>−4.0
	Oral cancer	GURAV	>−4.0	>−4.0
	Nasopharyngeal cancer	KB	>−4.0	>−4.0
3h	Leukemia	K562	−5.57	>−4.0
	Breast cancer	MCF7	−4.43	>−4.0
	Hepatoma	HEPG2	>−4.0	>−4.0
	NSC lung cancer	HOP62	>−4.0	>−4.0
	Prostate cancer	PC3	>−4.0	>−4.0
	Oral cancer	GURAV	−4.50	>−4.0
	Nasopharyngeal cancer	KB	>−4.0	>−4.0
3i	Leukemia	K562	>−4.0	>−4.0
	Breast cancer	MCF7	−4.53	>−4.0
	Hepatoma	HEPG2	>−4.0	>−4.0
	NSC lung cancer	HOP62	−5.64	>−4.0
	Prostate cancer	PC3	−4.53	>−4.0
	Oral cancer	GURAV	>−4.0	>−4.0
	Nasopharyngeal cancer	KB	>−4.0	>−4.0
3j	Leukemia	K562	−5.56	>−4.0
	Breast cancer	MCF7	−4.40	>−4.0
	Hepatoma	HEPG2	>−4.0	>−4.0
	NSC lung cancer	HOP62	>−4.0	>−4.0
	Prostate cancer	PC3	>−4.0	>−4.0
	Oral cancer	GURAV	−4.41	>−4.0
	Nasopharyngeal cancer	KB	>−4.0	>−4.0
Doxorubicin	Leukemia	K562	−5.59	>−4.0
	Breast cancer	MCF7	−6.88	−5.68
	Hepatoma	HEPG2	<−7.0	−6.87
	NSC lung cancer	HOP62	−6.97	−4.45
	Prostate cancer	PC3	−6.96	−5.68
	Oral cancer	GURAV	−6.97	−6.80
	Nasopharyngeal cancer	KB	<−7.0	−6.85

Compounds **3a**, **3b** and **3f** were highly active in the in vitro screen on NSC lung cancer cell line (HOP62). The $log_{10}GI_{50}$ values of these compounds were −6.72, −6.73 and −6.77 respectively. In addition **3d** ($log_{10}GI_{50}$ value −4.55) and **3i** ($log_{10}GI_{50}$ value −5.64) have also demonstrated marked effects on NSC lung cancer cell line (HOP62).

Compounds **3a** ($log_{10}GI_{50}$ value −4.53), **3d** ($log_{10}GI_{50}$ −5.65), **3e** ($log_{10}GI_{50}$ − 5.60), **3f** ($log_{10}GI_{50}$ value −4.55) and **3i** ($log_{10}GI_{50}$ value − 4.53) exhibited favorable activity on prostate cancer cell line PC3.

In breast cancer cell line MCF7 **3a**, **3d**, **3e**, **3f**, **3h**, **3i** and **3j** were found to be active with $log_{10}GI_{50}$ value −4.53, −4.55, −6.71, −4.60, −4.43, −4.53 and −4.40 respectively.

Compounds **3c** ($log_{10}GI_{50}$ −5.65), **3e** ($log_{10}GI_{50}$ −6.73), **3h** ($log_{10}GI_{50}$ −5.57) and **3j** ($log_{10}GI_{50}$ −5.56) yielded good antiproliferative activity against K562 leukemia cell line. Compound **3e** was found be ten times more potent than positive control, doxorubicin ($log_{10}GI_{50}$ −5.59). Compounds **3c**, **3h** and **3j** showed similar cytotoxicity to that of doxorubicin.

In GURAV oral cancer cell line, compounds **3c** ($log_{10}GI_{50}$ −6.71) and **3e** ($log_{10}GI_{50}$ −6.73) showed activity comparable to that of doxorubicin. In addition compounds **3h** ($log_{10}GI_{50}$ −4.50) and **3j** ($log_{10}GI_{50}$ −4.41) exhibited favorable antiproliferative activity in oral cancer.

None of the compounds showed cytotoxicity in HEPG2 liver cancer cell line. This may be attributed to the easily metabolisable amide linkage in the compounds which cleave in the presence of amidases in liver, thus rendering them inactive. 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 Veego melting point apparatus in open capillaries and are uncorrected. IR spectra were recorded as KBr discs, using Shimadzu 8400S FTIR spectrophotometer. ^1H NMR (300 MHz) and ^{13}C NMR (75 Mhz) spectra were recorded on Varian Mercury Plus spectrometer, with DMSO-d_6 as a solvent and TMS as an internal standard. The elemental analysis (C, H, and N) was performed using the Thermo finnigan FLASH EA 1112 analyzer and were within ±0.4% of theoretical values. All reactions as well as column chromatography were followed by TLC using Merk pre-coated silica gel 60 F_{254} plates and spots were visualized by observing in UV cabinet under short UV. All reagents were used as received unless otherwise stated.

4.1.1. Procedure for synthesis of 5-(4-hydroxybenzylidene)-2,4-thiazolidinedione (**1**)

A mixture of *p*-hydroxy benzaldehyde (40 mmol) and 2,4-TZD (40 mmol) with catalytic quantity of piperidinium benzoate was refluxed in toluene with continuous removal of water using Dean-Stark apparatus for 4 h. The reaction mixture was cooled to 25 °C and solid separated was collected by filtration.

Yield 93%, shiny dark yellow crystalline, mp 302–304 °C (Lit. mp-301–303 °C) [21,22]. IR [KBr ν cm^{-1}] 3404 (N–H), 1683 (C=O), 1718 (C=O). ^{1}H NMR [300 MHz, δ, ppm, DMSO-d_6]: 7.67 (s 1H), 7.44 (d, J = 8.4 Hz, 2H), 6.90 (d, J = 8.4 Hz, 2H).

*4.1.2. General procedure for synthesis of **2a–2j***

To heteroaromatic amine or heterocyclic nitrogen containing compound (1.0 mol) was added 1.5 mol of chloroacetyl chloride under cold conditions along with a base in chloroform or dichloromethane. Reaction was stirred overnight at room temperature. Water was added to reaction mixture and organic layer was separated. This was then passed through anhydrous Na_2SO_4. Organic layer was distilled to get crude product (**2a–2j**). The product was further purified by recrystallization by using a suitable solvent.

*4.1.2.1. 2-Chloro-1-(10H-phenothiazin-10-yl) ethanone (**2a**).* Yield 48.1%, white needles, mp 112–114 °C. IR [KBr ν cm^{-1}]: 1693(C=O), 1126,1168(C–N). ^{1}H NMR [300 MHz, δ, ppm, DMSO-d_6]: 7.68–7.70 (d, J = 7.2 Hz, 2H), 7.57–7.60 (d, J = 7.8 Hz, 2H), 7.34–7.45 (m 4H), 4.5 (s 2H).

*4.1.2.2. 2-Chloro-N-(1,5-dimethyl-3oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl) acetamide (**2b**).* Yield 50.14%, white powder, mp 193–194 °C. IR [KBr ν cm^{-1}]: 3188 (N–H), 1639, 1687 (C=O). ^{1}H NMR [300 MHz, δ, ppm, DMSO-d_6]: 9.56 (s 1H), 7.48–7.55 (m 2H), 7.31–7.38 (m 3H), 4.60 (s 2H), 3.1(s 3H), 2.15 (s 3H).

*4.1.2.3. 2-Chloro-N-(5-methylthiazol-2-yl) acetamide (**2c**). Solvent used for recrystallization: Chloroform:* Yield 82%, white needle, mp 191–192 °C. IR [KBr ν cm^{-1}]: 3186 (N–H), 1704 (C=O), 1560 (C=N). ^{1}H NMR [300 MHz, δ, ppm, DMSO-d_6]: 11.0 (bs 1H), 7.15 (s 1H), 4.24 (s 2H), 2.43 (s 3H).

*4.1.2.4. 3-(2-Chloroacetyl)-2-propyl-1,3-diazaspiro[4,4] non-1-en-4-one (**2d**). Solvent used for recrystallization: Benzene:* Yield 42.24%, white needles, mp 120–121 °C. IR [KBr ν cm^{-1}]: 1745, 1639 (C=O), 1539 (C=N). ^{1}H NMR [300 MHz, δ, ppm, DMSO-d_6]: 4.60 (s 2H), 2.18–2.37 (m 4H), 1.73–1.93 (m 7H), 1.25–1.36 (m 3H), 0.9 (t 3H).

*4.1.2.5. 2-Chloro-N-[3-(trifluoromethyl) phenyl] acetamide (**2e**).* Yield 67.0%, white crystalline, mp 129–130 °C. IR [KBr ν cm^{-1}]: 3305 (N–H), 1683 (C=O), 1070,1124 (C–F). ^{1}H NMR [300 MHz, δ, ppm, DMSO-d_6]: 10.05 (s 1H), 8.2 (s 1H), 7.95 (d 1H), 7.30–7.44 (m 2H), 4.38 (s 2H).

*4.1.2.6. N-(Benzo[d]thiazol-2-yl)-2-chloroacetamide (**2f**). Solvent used for recrystallization: Chloroform:* Yield 74%, white crystals, mp 143–144 °C. IR [KBr ν cm^{-1}]: 3181 (N–H), 1660 (C=O). ^{1}H NMR [300 MHz, δ, ppm, DMSO-d_6]: 12.79 (s 1H), 8.01 (d, J = 6.0 Hz, 1H), 7.77 (d, J = 8.1 Hz, 1H), 7.46 (t 1H), 7.35 (t 1H), 4.22 (s 2H).

*4.1.2.7. 2-Chloro-N-(5-nitrothiazol-2yl)acetamide (**2g**).* Yield 82%, yellow crystals, mp 146–147 °C. IR [KBr ν cm^{-1}]: 1687 (C=O), 1350 (C–N). ^{1}H NMR [300 MHz, δ, ppm, DMSO-d_6]: 10.07 (bs 1H), 8.36 (s 1H), 4.14 (s 2H).

*4.1.2.8. 2-Chloro-N-(1,1-dioxo-1,2-benzothiazole-3-one-2-yl)-acetamide (**2h**). Solvent used for recrystallization: Acetonitrile:* Ethanol (1:1). Yield 42%, white needles, mp 145–146 °C. IR [KBr ν cm^{-1}]: 1747 (C=O), 1315, 1363 (O=S=O). ^{1}H NMR [300 MHz, δ, ppm, DMSO-d_6]: 8.19 (m 1H), 7.8–8.07 (m 3H), 4.8(s 2H).

*4.1.2.9. 2-Chloro-N-(pyridine-2-yl) acetamide (**2i**).* Yield 69%, brown sticky mass, mp 178–180 °C. IR [KBr ν cm^{-1}]: 3141(N–H), 1770 (C=O), 1650 (C=N). This product was directly taken to next step without recording ^{1}H NMR.

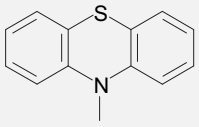
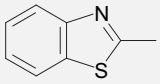
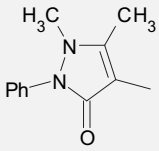
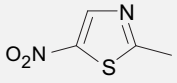
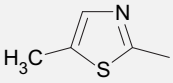
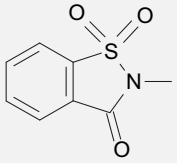
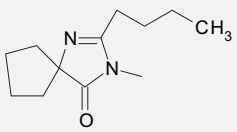
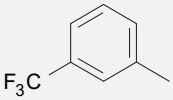
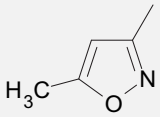
*4.1.2.10. 2-Chloro-N-(5-methylisoxazol-3-yl) acetamide (**2j**). Solvent used for recrystallization: Ethanol:* Yield 67%, white needles, mp 190–191 °C. IR [KBr ν cm^{-1}]: 3222 (N–H), 1708 (C=O), 1573 (C=N), 1190 (C–O). ^{1}H NMR [300 MHz, δ, ppm, DMSO-d_6]: 10.45 (s 1H), 6.61 (s 1H), 4.03 (s 2H), 2.36–2–37 (m 3H).

*4.1.3. General procedure for synthesis of **3a–3j***

5-(4-hydroxybenzylidene)-2,4-thiazolidenedione (**1**) (1.0 mol) along with 1.5 mol of potassium carbonate was taken in dimethyl formamide (DMF) and added to this solution of chloroacetylated product (**2a–2h**) (1.5 mol) in DMF. Reaction mixture was stirred at room temperature till the completion of reaction and monitored by TLC. After completion of reaction water was added to precipitate product (**3a–3j**). If precipitate was not formed the product was extracted in organic layer. All products were purified by column chromatography (Table 2).

*4.1.3.1. 5-[4-Oxo-2-(10H-phenothiazin-10-yl) benzylidene] thiazolidine-2,4-dione (**3a**). Eluent used for column chromatography: Chloroform:* Yield 59.8%, pale yellow powder, mp 219–220 °C. IR [KBr ν cm^{-1}]: 3199 (N–H), 1735 (C=O), 1681 (C=O), 1147 (C–O). ^{1}H NMR (300 MHz, δ, ppm, DMSO-d_6): 12.51 (bs 1H, –NH), 7.32–7.80 (m 11H, aromatic protons and benzylidene proton), 6.9 (d, J = 9.0 Hz, 2H, aromatic protons), 5.01 (s 2H, $-CH_2-$). ^{13}C NMR

Table 2
R groups for **3a–3j**.

Compound	R	Compound	R
3a		**3f**	
3b		**3g**	
3c		**3h**	
3d		**3i**	
3e		**3j**	

(75 MHz, DMSO-d_6, δ): 167.4 (C=O), 166.9 (C=O), 165.1, 164.4 (C=O), 160.3, 159.3, 137.4, 137.2, 134.1, 132.7, 132.1, 131.8, 131.6, 128.2, 127.6, 127.1, 126.9, 126.0, 123.7, 120.7, 116.5, 116.1, 115.3, 65.7. Calc. for $C_{24}H_{16}N_2O_4S_2$, %: C, 62.59; H, 3.50; N, 6.08. Found, %: C, 62.28; H, 3.56; N, 5.71.

*4.1.3.2. N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-2-[4-[(2,4-dioxo thiazolidin-5-ylidene) methyl] phenoxy] acetamide (**3b**). Eluent used for column chromatography: Hexane:* Ethyl acetate (3:2). Yield 44.5%, pale yellow powder, mp 224–225 °C. IR [KBr ν cm^{-1}]: 3261 (N–H), 1735 (C=O), 1679 (C=O), 1149, 1095 (C–O). ^{1}H NMR (300 MHz, δ, ppm, DMSO-d_6): 10.4 (s 1H, –CO–NH–CO–), 9.6 (s 1H, NH), 7.9 (s 1H, benzylidene proton), 7.5 (m 4H, aromatic protons), 7.35 (m 3H, aromatic protons), 6.95 (d, J = 8.7 Hz, 2H, aromatic protons), 4.45 (s 2H, $-CH_2-$), 3.04 (s 3H, $CH_3-N<$), 2.1 (s 3H, $-CH_3$). ^{13}C NMR (75 MHz, DMSO-d_6, δ): 167.3 (C=O), 165.5 (C=O), 164.8 (C=O), 161.5 (C=O), 160.3, 152.1, 134.9, 133.9, 132.7, 129.1, 126.4, 123.8, 123.7, 116.5, 106.6, 66.8, 35.9, 11.2. Calc. for $C_{23}H_{20}N_4O_5S$, %: C, 59.47; H, 4.34; N, 12.06. Found, %: C, 59.37; H, 4.52; N, 12.64.

*4.1.3.3. 2-[4-[(2,4-Dioxothiazolidin-5-ylidene) methyl] phenoxy]-N-(5-methylthiazol-2-yl) acetamide (**3c**). Eluent used for column chromatography: Benzene:* Methanol (10:0.25). Yield 48.0%, pale yellow powder, mp 233–235 °C. IR [KBr ν cm^{-1}]: 3321 (N–H), 1790 (C=O), 1635 (C=O), 1589 (C=N), 1153,1045 (C–O). ^{1}H NMR (300 MHz, δ, ppm, DMSO-d_6): 12.4 (bs 1H, –NH), 10.40 (s 1H, –CO–NH–CO–), 7.9 (s 1H, benzylidene proton), 7.52 (d, J = 9.0 Hz, 2H, aromatic protons), 7.16 (s 1H, thiazolyl proton), 6.94 (d, J = 8.4 Hz, 2H, aromatic protons), 4.88 (s 2H, $-CH_2-$), 2.4 (s 3H, $-CH_3$). ^{13}C NMR (75 MHz, DMSO-d_6, δ): 169.2 (C=O), 167.4 (C=O), 166.8 (C=O), 165.7, 160.3, 157.7, 140.1, 134.2, 132.8, 123.8, 120.0, 116.5, 65.7, 11.1. Calc. for $C_{16}H_{13}N_3O_4S_2$, %: C, 51.19; H, 3.49; N, 11.19. Found, %: C, 50.85; H, 3.75; N, 11.15.

*4.1.3.4. 5-[4-[2-(2-Butyl-4-oxo-1,3-diazaspiro[4,4]non-1-en-3-yl)-2-oxoethoxy] benzylidene] thiazolidine-2,4-dione (**3d**). Eluent used for column chromatography: Hexane:* Ethyl acetate (2:1). Yield 97.0%, pale yellow powder, mp 236–237 °C. IR [KBr ν cm^{-1}]: 3406 (N–H), 1724 (C=O), 1677 (C=O), 1155 (C–O). ^{1}H NMR (300 MHz, δ, ppm, DMSO-d_6): 10.83 (s 1H, –CO–NH–CO–), 7.88 (s 1H, benzylidene proton), 7.5 (d, J = 8.4 Hz, 2H, aromatic protons), 6.95 (d, J = 8.4 Hz, 2H, aromatic protons), 4.73 (s 2H, $-CH_2-$), 1.2–1.82 (m 17H, aliphatic and alicyclic protons). ^{13}C NMR (75 MHz, DMSO-d_6, δ): 174.5 (C=O), 172.7 (C=O), 168.0 (C=O), 167.1 (C=O), 165.3, 160.3, 134.1, 132.8, 123.8, 116.5, 116.2, 66.5, 45.7, 35.8, 34.6, 27.1, 23.8, 21.8, 13.8. Calc. for $C_{23}H_{25}N_3O_5S$, %: C, 60.64; H, 5.53; N, 9.22. Found, %: C, 60.47; H, 5.56; N, 9.60.

*4.1.3.5. 2-[4-[(2,4-Dioxothiazolidin-5-ylidene) methyl] phenoxy]-N-[3-(trifluromethyl) phenyl] acetamide (**3e**). Eluent used for column chromatography: Hexane:* Ethyl acetate (3:2). Yield 97%, pale yellow power, mp 235–236 °C. IR [KBr ν cm^{-1}]: 3317 (N–H), 1731 (C=O), 1674 (C=O), 1146, 1176 (C–O). ^{1}H NMR (300 MHz, δ, ppm, DMSO-d_6): 10.75 (s 1H, –CO–NH–CO–), 10.4 (s 1H, –NH–), 8.05 (s 1H, aromatic proton), 7.9 (s 1H, benzylidene proton), 7.42–7.75 (m 5H, aromatic protons), 6.9 (d, J = 9.0 Hz, 2H, aromatic protons), 4.5 (s 2H, $-CH_2-$). ^{13}C NMR (75 MHz, DMSO-d_6, δ): 167.3 (C=O), 165.5 (C=O), 164.7 (C=O), 160.3, 139.1, 134.1, 132.8, 130.2, 129.8, 129.4, 125.8, 123.8, 122.8, 122.2, 120.0, 116.5, 116.3, 115.2, 66.2. Calc. for $C_{19}H_{13}F_3N_2O_4S$, %: C, 54.03; H, 3.10; N, 6.63. Found, %: C, 53.83; H, 2.96; N, 6.32.

*4.1.3.6. N-(Benzo[d]thiazol-2-yl)-2-[4-[(2,4-dioxothiazolidin-5-ylidene) methyl] phenoxy] acetamide (**3f**). Eluent used for column chromatography: Chloroform:* Ethanol (10:0.3). Yield 51%, yellow powder, mp 280–281 °C. IR [KBr ν cm^{-1}]: 3419(N–H), 1731 (C=O), 1674 (C=O), 1172,1089 (C–O). ^{1}H NMR (300 MHz, δ, ppm, DMSO-d_6): 12.8 (s 1H, –CO–NH–CO–), 10.4 (s 1H, –NH–), 7.94 (d, J = 7.8 Hz, 1H, aromatic proton), 7.9 (s 1H, benzylidene proton), 7.77 (d, J = 7.5 Hz, 1H, aromatic proton), 7.56 (d, J = 8.4 Hz, 2H, aromatic protons), 7.42–7.48 (m 1H, aromatic proton), 7.30–7.35 (m 1H, aromatic proton), 6.95 (d 2H, aromatic protons), 4.68 (s 2H, $-CH_2-$). ^{13}C NMR (75 MHz, DMSO-d_6, δ): 172.1, 167.3 (C=O), 165.7 (C=O), 165.4 (C=O), 160.4, 157.5, 148.5, 132.8, 131.5, 126.3, 126.1, 123.8, 123.6, 121.8, 121.7, 120.6, 116.5, 116.2, 61.2. Calc. for $C_{19}H_{13}N_3O_4S_2$, %: C, 55.46; H, 3.18; N, 10.21. Found, %: C, 55.81; H, 3.42; N, 9.90.

*4.1.3.7. 2-[4-[(2,4-Dioxothiazolidin-5-ylidene) methyl] phenoxy]-N-(5-nitrothiazol-2-yl) acetamide (**3g**). Solvent used for recrystallization: Ethanol:* Yield 50%, yellow shiny powder, mp 254–256 °C. IR [KBr ν cm^{-1}]: 1716 (C=O), 1664 (C=O), 1348 (C–N), 1286, 1176 (C–O). ^{1}H NMR (300 MHz, δ, ppm, DMSO-d_6): 13.59 (bs 1H, –CO–NH–CO–), 10.44 (s 1H, –NH–), 8.78 (s 1H, tiazolyl proton), 7.9 (s 1H, benzylidene proton), 7.53 (d, J = 8.4 Hz, 2H, aromatic protons), 6.95 (d, J = 8.7 Hz, 2H, aromatic protons), 4.7 (s 2H, $-CH_2-$). ^{13}C NMR (75 MHz, DMSO-d_6, δ): 172.8 (C=O), 167.4 (C=O), 165.7 (C=O), 160.2, 145.8, 136.7, 133.7, 132.7, 127.4, 123.9, 116.8, 116.5, 64.2.Calc. for $C_{15}H_{10}N_4O_6S_2$, %: C, 44.33; H, 2.48; N, 13.79. Found, %: C, 43.95; H, 2.42; N, 13.48.

*4.1.3.8. 2-[2-[4-[(2,4-Dioxothiazolidin-5-ylidene) methyl] phenoxy] acetyl]-1,2-benzothiazol-3(2H)-one-1,1-dioxide (**3h**). Eluent used for column chromatography: Hexane:* Ethyl acetate (2:1).Yield 40%, pale yellow powder, mp 246–248 °C. IR [KBr ν cm^{-1}]: 1695, 1743 (C=O), 1319 (O=S=O), 1226, 1172 (C–O). ^{1}H NMR (300 MHz, δ, ppm, DMSO-d_6): 10.45 (s 1H, –CO–NH–CO–), 7.93 (s 1H, benzylidene proton), 7.68 (d, J = 9.0 Hz, 2H, aromatic protons), 7.53 (d, J = 8.4 Hz, 2H, aromatic protons), 7.34 (d, J = 8.7 Hz, 2H, aromatic protons), 6.94 (d, J = 8.4 Hz, 2H, aromatic protons), 4.70 (s 2H, $-CH_2-$). ^{13}C NMR (75 MHz, DMSO-d_6, δ): 168.1 (C=O), 167.3 (C=O), 167.0 (C=O), 165.1, 160.5, 151.0, 134.9, 132.9, 132.4, 131.5, 131.3, 130.7, 124.0, 123.6, 122.5, 116.5, 115.6, 62.8. Calc. for $C_{19}H_{12}N_2O_7S_2$, %: C, 51.35; H, 2.72; N, 6.30. Found, %: C, 51.10; H, 2.57; N, 6.64.

*4.1.3.9. 2-[4-[(2,4-Dioxothiazolidin-5-ylidene) methyl] phenoxy] -N-(pyridine-2-yl) acetamide (**3i**). Eluent used for column chromatography: Hexane:* Ethyl acetate (2:1).Yield 32.1%, yellow powder, mp 177–178 °C. IR [KBr ν cm^{-1}]: 3465 (N–H), 1735 (C=O), 1677 (C=O), 1141,1085 (C–O). ^{1}H NMR (300 MHz, δ, ppm, DMSO-d_6): 10.97 (s 1H, –CO–NH–CO–), 10.41(s 1H, –NH–), 8.34 (d, J = 4.2 Hz, 1H, aromatic protons), 7.69–7.98 (m 1H, aromatic protons), 7.88 (s 1H, benzylidene proton), 7.76–7.87 (m 1H, aromatic proton), 7.52 (d, J = 8.7 Hz, 2H, aromatic protons), 7.11–7.15 (m 1H, aromatic proton), 6.94 (d, J = 8.4 Hz, 2H, aromatic protons), 4.57 (s 2H, $-CH_2-$). ^{13}C NMR (75 MHz, DMSO-d_6, δ): 167.3 (C=O), 165.5 (C=O), 164.8 (C=O), 160.3, 151.4, 148.1, 138.4, 134.1, 132.7, 123.8, 119.8, 116.5, 116.3, 113.5, 66.9. Calc. for $C_{17}H_{13}N_3O_4S$, %: C, 57.46; H, 3.69; N, 11.82. Found, %: C, 57.84; H, 4.00; N, 11.60.

*4.1.3.10. 2-[4-[(2,4-Dioxothiazolidin-5-ylidene) methyl] phenoxy]-N-(5-methylisoxazol-3-yl) acetamide (**3j**). Eluent used for column chromatography: Hexane:* Ethyl acetate (3:2).Yield 76%, pale yellow powder, mp 245–246 °C. IR [KBr ν cm^{-1}]: 3483 (N–H), 1739 (C=O), 1689 (C=O), 1587 (C=N), 1143,1087 (C–O). ^{1}H NMR (300 MHz, δ, ppm, DMSO-d_6): 11.4 (s 1H, –CO–NH–CO–), 10.4 (s 1H, –NH–), 7.88 (s 1H, benzylidene proton), 7.52 (d, J = 8.7 Hz, 2H, aromatic protons), 6.93 (d, J = 8.4 Hz, 2H, aromatic protons), 6.56 (s 1H, isoxazolyl proton), 4.51 (s 2H, $-CH_2-$), 2.37 (s 3H, $-CH_3$). ^{13}C NMR

(75 MHz, DMSO-d_6, δ): 169.9, 167.3 (C=O), 165.4 (C=O), 164.5 (C=O), 160.3, 157.6, 134.1, 132.8, 123.8, 116.5, 116.3, 96.1, 63.6, 12.1. Calc. for $C_{16}H_{13}N_3O_5S$, %: C, 53.48; H, 3.65; N, 11.69. Found, %: C, 53.65; H, 3.97; N, 11.93.

4.2. In vitro anticancer screening

The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. For present screening experiment, cells were inoculated into 96 well microtiter plates in 90 μL at plating densities ranging of 5000 cells/well. 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, one plate of each cell line was 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 (Tz). Test compounds were solubilized in DMSO solvent at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of compound addition, an aliquot of frozen concentrate was thawed and diluted to 10 times the desired final maximum test concentration with complete medium containing 1% gentamicin. Additional three, 10-fold serial dilutions were made to provide a total of four concentrations plus control. Aliquots of 10 μl of these different dilutions were added to the appropriate microtiter wells already containing 90 μl of medium, resulting in the required final concentrations.

After compound addition, plates were incubated at standard conditions for 48 h at 37 °C, 5% CO_2, 95% air and 100% relative humidity and assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 μl of cold 30% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 μl) at 0.4% (w/v) in 1% acetic acid was added to each of the wells, and plates were incubated for 20 min at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1% acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on an Elisa plate reader at a wavelength of 540 nm with 690 nm reference wavelength.

Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells * 100.

Using the six absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as:

$[(Ti - Tz)/(C - Tz)] \times 100$ for concentrations for which $Ti > / = Tz$ $(Ti - Tz)$ positive or zero.

$[(Ti - Tz)/Tz] \times 100$ for concentrations for which $Ti < Tz$. $(Ti - Tz)$ negative.

The dose response parameters were calculated for each test article. Growth inhibition of 50% (GI50) was calculated from $[(Ti - Tz)/(C - Tz)] \times 100 = 50$, 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) was calculated from $Ti = Tz$.

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 values for that parameter were expressed as greater or less than the maximum or minimum concentration tested.

Appendix. Supplementary material

Supplementary material associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2010.07.014.

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