Full Paper

Synthesis and Anticancer Activity of Indolin-2-one Derivatives Bearing the 4-Thiazolidinone Moiety

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A novel series of indolin-2-one derivatives containing the 4-thiazolidinone moiety (**5a–5p**) was synthesized and the cytotoxicity of these derivatives was evaluated *in vitro* against three human cancer cell lines (HT-29, H460 and MDA-MB-231) by standard MTT assay. Some prepared compounds exhibited significant cytotoxicity against different human cancer cell lines. Several potent compounds were further evaluated against one normal cell line (WI-38). In particular, the promising compound **5h** showed remarkable cytotoxicity and selectivity against HT-29 and H460 cancer cell lines (IC_{50} = 0.016 μmol/L, 0.0037 μmol/L, respectively).

Keywords: Anticancer activity / Indolin-2-one / 4-Thiazolidinone

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Introduction

Cancer is the worldwide health problem and the most frightening disease of human. Recently, considerable attention has been devoted to the construction of new derivatives of indolin-2-one or 4-thiazolidinone moieties on the account of their reported anticancer activities.

A variety of 3-substituted indolin-2-ones have been utilized as anticancer drugs or drug candidates [1–5]. A representative member of this class is sunitinib (SU11248, Sutent™; Pfizer, Inc.) which is currently used in the clinics as a multi-targeting tyrosine kinase inhibitor with antiangiogenic activity (Fig. 1) [6, 7]. 6-Methoxycarbonyl group substituted indolin-2-ones (BIBF1000, BIBF1120) are potent inhibitors of VEGFR-1/2/3, PDGFRα, and FGFR-1, with low cross-reactivity against a panel of other kinases (Fig. 1) [8]. Notably, BIBF1120 is currently being evaluated in phase III clinical trials in the treatment of non small cell lung cancer and is in clinical development for other tumor types. Indirubin was identified as the active ingredient of a traditional Chinese recipe (Danggui Longhui Wan) that was used for the treatment of chronic myelogenous leukemia (CML) (Fig. 1) [9].

Thiazolidinone derivatives are known for their broad spectrum of biological activities [10], including anticancer effect [11–16]. Among them, 4-thiazolidinone-3-carboxylic acid derivatives are promising anticancer agents. Recently, a series of 5-benzylidene-2-thioxo-4-thiazolidinone-3-carboxylic acids (Fig. 1) have been reported as inhibitors for anti-apoptotic Bcl-2 proteins [17, 18]. Their analogues are highly active inhibitors of JNK-stimulating phosphatase-1 (JSP-1) [19]. Moreover, novel 4-thiazolidinone-3-carboxylic acid amides having furan moiety exhibited significant cytotoxicity and induction of apoptosis in human leukemia cell (Fig. 1) [20].

Based on aforementioned compounds, indolin-2-one and 5-benzylidene-4-thiazolidinone moieties are promising scaffolds for design of anticancer drugs. In addition, different anticancer biotargets and mechanism of indolin-2-one or 4-thiazolidinone derivatives encourage us to design hybrids containing these two moieties within their structures. They may display improved anticancer activity and be less susceptible to the development of multi-drug resistance (MDR). To our knowledge, there were hardly any studies about combination of indolin-2-one and 4-thiazolidinone moieties at the 2 position of the 4-thiazolidinone ring so far. Thus, a series of novel indolin-2-one derivatives with 5-benzylidene-4-thiazolidinone moieties (**5a–5p**) were designed (Fig. 1). The acidic group at the 3 position of the 4-thiazolidinone ring may prevent entry of the compounds into the

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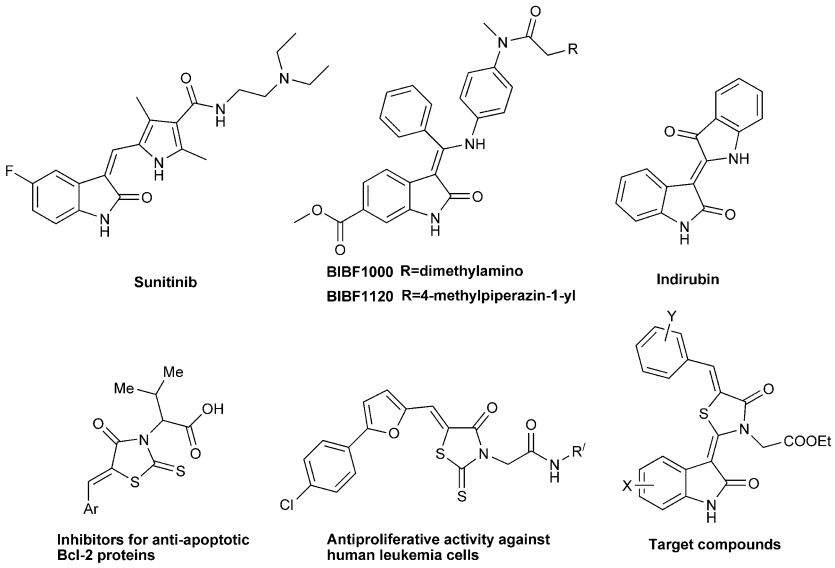


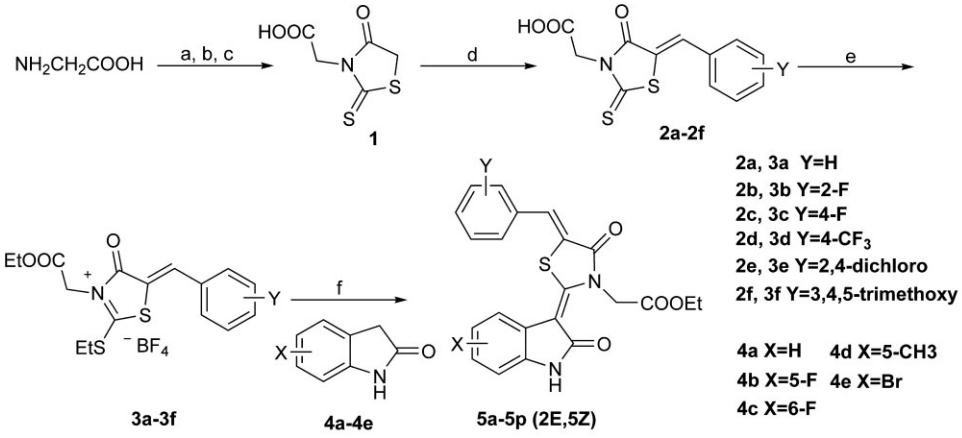
Figure 1. Structures of indolin-2-ones, 4-thiazolidinone and target compounds.

cancer cell. In order to increase cancer cell membrane permeability of these structures, the ester group was retained at the 3 position of the 4-thiazolidinone ring.

Results and discussion

Chemistry

The synthetic route of the target compounds **5a–5p** is illustrated in Scheme 1. The indolin-2-ones **4a–4e** were synthesized from corresponding anilines according to the reported procedures [1, 2]. Rhodanine-3-acetic acid **1** was synthesized *via* reaction of the glycine and carbon disulfide under basic conditions followed by ring closure with chloroacetic acid. Knoevenagel condensation of **1** with appropriate benzaldehydes in the refluxing acetic acid afforded the 2-(5-benzylidene-2-thioxo-4-thiazolidin-3-yl)acetic acids **2a–2f**. The presence of only one signal for the methyne proton at more downfield 7.83–7.95 ppm in ^{1}H-NMR spectra of **2a–2f** suggested that a single *Z*-configuration isomer was present. The exclusive formation of the thermodynamically stable *Z*-isomers of **2a–2f** is in agreement with the literature reports for similar compounds [21, 22]. *S*-Ethylation of **2a–2f** with



Reagents and conditions: (a) 25% NH_4OH, CS_2, 23 °C, 1 h; (b) $ClCH_2CO_2Na$, 23 °C, 3 h; (c) HCl, reflux, 1 h; (d) benzaldehydes, AcONa, AcOH, reflux, 3-4 h; (e) $HC(OEt)_3$, $BF_3 \cdot Et_2O$, 1,4-dioxane, 80 °C, 4 h; (f) Et_3N, CH_3CN, 60 °C, 3 h.

Scheme 1. Synthesis of target compounds.

boron trifluoride diethyl etherate and triethyl orthoformate produced thiazolinium salts **3a–3f**, which reacted with indolin-2-ones **4a–4e** in the presence of triethylamine to yield compounds **5a–5p**.

Because of the exocyclic double bond at 2-position of 4-thiazolidinone ring, compounds **5a–5p** existed as either exclusively (2*E*, 5*Z*) isomer or a mixture of the (2*E*, 5*Z*) and (2*Z*, 5*Z*) isomers with the (2*E*, 5*Z*) isomer being the predominant one (>90%) and the single (2*E*, 5*Z*) isomer was obtained by recrystallization. The two isomers were assigned on the basis of different chemical shift of α-methylene proton. In ^{1}H-NMR spectra the α-methylene proton of the (2*E*, 5*Z*) isomer was more downfield (5.25–5.36 ppm) than that of the (2*Z*, 5*Z*) isomer (4.95–5.01 ppm) due to the deshielding effect of the carbonyl group at the 2-position of the indolin-2-one ring (Fig. 2). In ^{1}H-NMR spectra of compounds **5a–5p** the NH proton of indolin-2-one appeared as one singlet at 10.56–10.84 ppm. The IR spectra of compounds **5a–5p** showed three strong absorption bands (1761.1–1736.3 cm^{-1}, 1719.0–1708.3 cm^{-1}, 1683.0–1656.8 cm^{-1}) corresponding to two lactam and one ester carbonyl groups.

Biological results and discussion

The cytotoxicity of compounds **5a–5p** and precursors **4a–4e** were evaluated against three cancer cell lines, i.e. human colon cancer cell line (HT-29), human lung cancer cell line (H460) and human breast cancer cell line (MDA-MB-231). In order to investigate the cytotoxicity of these compounds against a normal cell line, several potent compounds (**5a**, **5d**, **5f**, **5h**, **5l**) were further evaluated against human fetal lung fibroblasts (WI-38). For comparison purposes, the cytotoxicity of sunitinib, a standard anticancer drug, was evaluated under the same conditions. The cytotoxicity was determined by standard MTT assay and the results expressed as IC_{50} were summarized in Table 1. The IC_{50} values are the average of two independent experiments.

According to the cytotoxicity data in Table 1, some of the new compounds exhibited potential anticancer activities. Compound **5h** showed the highest potency against HT-29 and H460 cancer cell lines while compounds **5e–5g** were nearly as active as sunitinib against H460 and MDA-MB-231 cancer cell lines. All tested precursors **4a–4e** were inactive. As shown in Table 1, compounds **5a–5p** were more potent against H460 cancer cell line than against HT-29 and MDA-MB-231 cancer cell lines. These results suggested this series of compounds possessed selectivity for H460 cancer cell line.

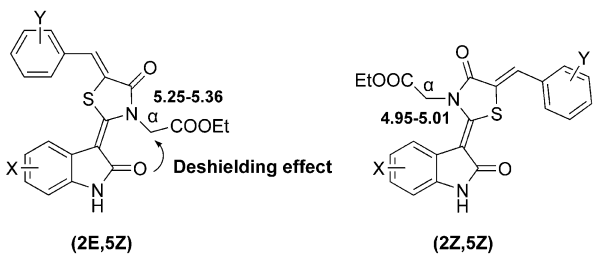
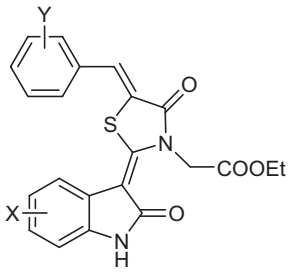


Figure 2. Deshielding effect of carbonyl group in (2*E*, 5*Z*) isomers.

As shown in Table 1, the indolin-2-one and 5-benzylidene-4-thiazolidinone hybrid derivatives **5a–5p** were more active than the corresponding precursors **4a–4e** in most cases. It suggested that the substituted 5-benzylidene-4-thiazolidinone ring is a necessary moiety for these compounds to possess potent cytotoxicity. Compounds **5a–5d** showed moderate cytotoxicity against one or more cancer cell lines. Introduction of smaller electron-withdrawing fluoro-atom at 5-position of the indolin-2-one ring was more favorable for increasing cytotoxicity against all three cancer cell lines (**5c** vs. **5f**, **5d** vs. **5h**). In contrast, introduction of a methyl group (electron-donating group) reduced their cytotoxicity against both HT-29 and H460 cancer cell lines (**5a** vs. **5i**, **5b** vs. **5k**, **5d** vs. **5l**). However, compound **5i** exhibited better activity and selectivity against MDA-MB-231 cancer cell line than compound **5a**. Compound **5p** possessing bulky electron-withdrawing bromo-atom only showed marginal activity against MDA-MB-231 cancer cell line. These results suggested that small electron-withdrawing fluoro-substitution at the 5-position of indolin-2-one ring is more favorable than electron-donating methyl group and bulky bromo-atom. In addition, the position of substituents appears to play an important role in activity, since the change of fluoro from 5- to 6-position of indolin-2-one ring led to a clear loss of activity against HT-29 and MDA-MB-231 cancer cell lines (**5e** vs. **5n**). These results are not surprising, as substitution at the 5 position of indolin-2-one ring has previously been associated with increased biological activity for a range of indole-based compounds.

Compounds (**5d**, **5h**, **5l**) with three electron-donating groups (such as methoxy in this study) in the phenyl ring, exhibited moderate to excellent cytotoxicity against both HT-29 and H460 cancer cell lines. Replacement of the electron-donating group with electron-withdrawing group (such as fluoro, trifluoromethyl, chloro) or hydrogen atom resulted in compounds (**5a–5c**, **5e–5g**, **5i–5k**) possessing less cytotoxicity against HT-29 and H460 cancer cell lines. However, in the case of MDA-MB-231 cancer cell line, compounds (except **5b**) without electron-donating group in the phenyl ring were more active against MDA-MB-231 cancer cell line (**5e–5g** vs. **5h**; **5i–5k** vs. **5l**; **5a**, **5c vs. 5d**). Interestingly, compound **5i**, possessing 5-methylindolin-2-one ring and phenyl ring without substitutions, exhibited the most potent cytotoxicity against MDA-MB-231 cancer cell line. Therefore, we tentatively concluded that the electronic influences of the substituent in the phenyl ring appear to play an important role in activity, and this behavior depends on the cancer cell lines to which they are

Table 1. The cytotoxicity of compounds **5a-5p** and precursors **4a-4e** against HT-29, H460, MDA-MB-231 and WI-38 cell lines *in vitro* [a]



5a-5p (2E,5Z)

Compd.	X	Y	IC_{50} (μmol/L)[b] ± SD			
			HT-29[c]	H460[c]	MDA-MB-231[c]	WI-38[c]
5a	H	H	3.45 ± 0.14	1.9 ± 0.07	7.25 ± 0.21	13.5 ± 0.30
5b	H	4-F	NA[d]	5.2 ± 0.14	NA[d]	ND[e]
5c	H	4-CF_3	NA[d]	12.8 ± 1.13	18.6 ± 0.85	ND[e]
5d	H	3,4,5-trimethoxy	1.78 ± 0.10	0.8 ± 0.07	27.1 ± 1.84	1.23 ± 0.35
5e	5-F	2-F	19.1 ± 1.56	2.28 ± 0.11	3.47 ± 0.18	ND[e]
5f	5-F	4-CF_3	46.3 ± 1.84	2.74 ± 0.19	3.79 ± 0.12	13.4 ± 0.41
5g	5-F	2,4-dichloro	NA[d]	2.13 ± 0.10	3.49 ± 0.12	ND[e]
5h	5-F	3,4,5-trimethoxy	0.016 ± 0.006	0.0037 ± 0.0004	10.5 ± 0.42	7.60 ± 0.22
5i	5-CH_3	H	NA[d]	56.5 ± 3.53	2.3 ± 0.28	ND[e]
5j	5-CH_3	2-F	NA[d]	NA[d]	20 ± 2.83	ND[e]
5k	5-CH_3	4-F	NA[d]	NA[d]	37.8 ± 1.13	ND[e]
5l	5-CH_3	3,4,5-trimethoxy	7.8 ± 0.85	2.1 ± 0.28	52 ± 2.83	3.6 ± 0.28
5m	6-F	H	NA[d]	NA[d]	NA[d]	ND[e]
5n	6-F	2-F	NA[d]	3.51 ± 0.15	NA[d]	ND[e]
5o	6-F	4-F	NA[d]	NA[d]	NA[d]	ND[e]
5p	5-Br	4-F	NA[d]	NA[d]	56 ± 4.24	ND[e]
4a	H	–	NA[d]	NA[d]	NA[d]	ND[e]
4b	5-F	–	NA[d]	NA[d]	NA[d]	ND[e]
4c	6-F	–	NA[d]	NA[d]	NA[d]	ND[e]
4d	5-CH_3	–	NA[d]	NA[d]	NA[d]	ND[e]
4e	5-Br	–	NA[d]	NA[d]	NA[d]	ND[e]
Sunitinib[f]	–	–	1.83 ± 0.16	2.59 ± 0.13	3.46 ± 0.06	6.20 ± 0.25

[a] Several potent compounds (**5a**, **5d**, **5f**, **5h**, **5l**) were evaluated against a normal cell line (WI-38). [b] IC_{50}: Concentration of the compound (μmol/L) producing 50% cell growth inhibition after 72 h of drug exposure, as determined by the MTT assay. Each experiment was run at least twice, and the results are presented as average values ± standard deviation. [c] HT-29, human colon cancer cell line; H460, human lung cancer cell line; MDA-MB-231, human breast cancer cell line; WI-38, human fetal lung fibroblasts. [d] NA: Compound showing IC_{50} value >200 μmol/L. [e] ND: Not determined. [f] Used as a positive control.

faced to. Compounds **5h** exerted markedly weaker cytotoxicity against WI-38 normal cell line than against HT-29 and H460 cancer cell lines. The selective index (IC_{50} normal cell/IC_{50} cancer cell) for HT-29 and H460 cancer cell lines was 475 and 2.05×10^3, respectively.

In the present study, the coupling between indolin-2-one and 5-benzylidene-4-thiazolidinone systems led to several compounds with better anticancer activity than the previously reported analogues [15, 23, 24]. Two neutral conjugate systems integrated at the 4-thiazolidinone moiety may contribute to the anticancer activity of these compounds, which has already been partially confirmed by anticancer activity of the rhodacyanine dyes [25].

Experimental

Chemistry

All melting points were obtained on a Büchi Melting Point B-540 apparatus (Büchi Labortechnik, Flawil, Switzerland) and were uncorrected. The IR spectra were recorded by means of the KBr pellet technique on a Bruker FTS 135 spectrometer. ^{1}H-NMR spectra were performed using Bruker 300-MHz spectrometers (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal

standard. Mass spectra (MS) were taken in ESI mode on Agilent 1100 LC–MS (Agilent, Palo Alto, CA, USA). Elemental analysis was determined on a Carlo-Erba 1106 Elemental analysis instrument (Carlo Erba, Milan, Italy). All chemicals were obtained from commercial suppliers and used without purification. TLC analysis was carried out on silica gel plates GF254 (Qindao Haiyang Chemical, China). The indolin-2-ones **4a–4e** were synthesized from corresponding anilines according to the reported procedures [1, 2].

*2-(4-Oxo-2-thioxothiazolidin-3-yl)acetic acid **1***

Glycine (24.0 g, 320 mmol) was dissolved with 25% NH_4OH (70 mL) in water (10 mL). Then, carbon disulfide (24.3 g, 320 mmol) was added to the reaction mixture, which was stirred vigorously for 1 h. An aqueous solution of sodium chloroacetate (37.1 g, 320 mmol) was added and stirring was continued at 23°C for 3 h. Then the reaction mixture was acidified with dilute HCl until pH 1.0 and refluxed for 1 h. The reaction mixture was neutralized with saturated $NaHCO_3$ solution. The resultant solution was acidified again with dilute HCl. The cyclized product was extracted in ethyl acetate, dried over anhydrous sodium sulfate and evaporated under vacuum and the residue was purified by recrystallization with water to obtain intermediate **1**. Yield: 86.0%. M.p.: 145–148°C; ^{1}H-NMR (DMSO-d_6, ppm): δ 4.41 (s, 2H), 4.56 (s, 2H); MS (ESI): m/z 190.2 $(M-H)^-$.

*General procedure for the synthesis of (Z)-2-(5-benzylidene-4-oxo-2-thioxothiazolidin-3-yl)acetic acid derivatives **2a–2f***

A mixture of rhodanine-3-acetic acid **1** (30 mmol), appropriate benzaldehyde (33 mmol) and anhydrous sodium acetate (30 mmol) was refluxed for 3–4 h in glacial acetic acid (60 mL) and the reaction was monitored by TLC. After cooling, the precipitated product was filtered off, washed with water and recrystallized with ethanol.

*(Z)-2-(5-Benzylidene-4-oxo-2-thioxothiazolidin-3-yl)acetic acid **2a***

Yield: 66.0%. M.p.: 249–250°C; ^{1}H-NMR (DMSO-d_6, ppm): δ 4.75 (s, 2H), 7.21–7.52 (m, 3H), 7.67–7.70 (m, 2H), 7.91 (s, 1H), 13.45 (br s, 1H); MS (ESI): m/z 278.1 $(M-H)^-$.

*(Z)-2-(5-(2-Fluorobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid **2b***

Yield: 62.0%. M.p.: 201–204°C; ^{1}H-NMR (DMSO-d_6, ppm): δ 4.76 (s, 2H), 7.40–7.46 (m, 2H), 7.59–7.66 (m, 2H), 7.85 (s, 1H); MS (ESI): m/z 296.3 $(M-H)^-$.

*(Z)-2-(5-(4-Fluorobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid **2c***

Yield: 70.3%. M.p.: 256–259°C; ^{1}H-NMR (DMSO-d_6, ppm): δ 4.75 (s, 2H), 7.40–7.45 (m, 2H), 7.74–7.79 (m, 2H), 7.93 (s, 1H), 13.47 (br s, 1H); MS (ESI): m/z 296.3 $(M-H)^-$.

*(Z)-2-(5-(4-Trifluoromethylbenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid **2d***

Yield: 45.2%. M.p.: 240–243°C; ^{1}H-NMR (DMSO-d_6, ppm): δ 4.75 (s, 2H), 7.41 (d, 2H, J = 8.8 Hz), 7.75 (d, 2H, J = 8.8 Hz), 7.93 (s, 1H), 13.50 (br s, 1H); MS (ESI): m/z 345.9 $(M-H)^-$.

*(Z)-2-(5-(2,4-Dichlorobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid **2e***

Yield: 56.5%. M.p.: 263–266°C; ^{1}H-NMR (DMSO-d_6, ppm): δ 4.76 (s, 2H), 7.67 (dd, 1H, J = 8.7, 2.1 Hz), 7.88 (d, 1H, J = 2.1 Hz), 7.92 (d, 1H, J = 8.7 Hz), 7.95 (s, 1H); MS (ESI): m/z 346.0 $(M-H)^-$.

*(Z)-2-(5-(3,4,5-Trimethoxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid **2f***

Yield: 32.5%. M.p.: 217–219°C; ^{1}H-NMR (DMSO-d_6, ppm): δ 3.76 (s, 3H), 3.86 (s, 6H), 4.71 (s, 2H), 6.98 (s, 2H), 7.83 (s, 1H); MS (ESI): m/z 368.0 $(M-H)^-$.

*General procedure for the synthesis of ethyl 2-(5-benzylidene-2-(2-oxo-indolin-3-ylidene)-4-oxothiazolidin-3-yl)acetate derivatives **5a–5p***

To a solution of (Z)-2-(5-benzylidene-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (**2a–2f**) (3 mmol) in 1,4-dioxane (15 mL) was added $HC(OEt)_3$ (2 mL) and $BF_3 \cdot Et_2O$ (2 mL). The reaction mixture was heated to 80°C and stirring was continued at the same temperature for 4 h. The resulting thiazolium fluoroborate (**3a–3f**) was precipitated, filtered off, dried, without any additional purification, as starting material for the following reactions. To a mixture of thiazolium fluoroborate (**3a–3f**) (3 mmol) and indolin-2-one (**4a–4e**) (3 mmol) in acetonitrile (15 mL) was added triethylamine (0.91 g, 9 mmol) dropwise at 25°C, and the mixture was stirred for 3 h at 60°C. The orange precipitate was collected and washed with ethyl acetate (8 mL). The crude product thus obtained was recrystallized from methanol or acetone to give compound (**5a–5p**).

*(2E,5Z) Ethyl 2-(5-benzylidene-2-(2-oxo-indolin-3-ylidene)-4-oxothiazolidin-3-yl)acetate **5a***

Yield: 42.5%. M.p.: 168–171°C; IR (KBr, cm^{-1}): 3428.9, 3191.7, 1761.1, 1710.1, 1657.6, 1563.9, 1203.7, 1154.7; MS (ESI) m/z: 407.1 $(M+H)^+$; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.20 (t, 3H, J = 7.2 Hz), 4.12 (q, 2H, J = 7.2 Hz), 5.33 (s, 2H), 6.87 (d, 1H, J = 7.5 Hz), 7.06–7.22 (m, 2H), 7.52–7.64 (m, 3H), 7.80–7.83 (m, 3H), 7.89 (s, 1H), 10.69 (s, 1H); anal. calcd. for $C_{22}H_{18}N_2O_4S$ (%): C, 65.01; H, 4.46; N, 6.89. Found (%): C, 65.07; H, 4.51; N, 6.93.

*(2E,5Z) Ethyl 2-(5-(4-fluorobenzylidene)-2-(2-oxo-indolin-3-ylidene)-4-oxothiazolidin-3-yl)acetate **5b***

Yield: 43.0%. M.p.: 156–158°C; IR (KBr, cm^{-1}): 3448.2, 3119.9, 1739.5, 1719.0, 1674.5, 1524.4, 1509.3, 1214.5, 1198.7, 1160.3, 1144.2; MS (ESI) m/z: 425.1 $(M+H)^+$; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.20 (t, 3H, J = 7.2 Hz), 4.11 (q, 2H, J = 7.2 Hz), 5.33 (s, 2H), 6.87–7.91 (m, 9H), 10.69 (s, 1H); anal. calcd. for $C_{22}H_{17}FN_2O_4S$ (%): C, 62.25; H, 4.04; N, 6.60. Found (%): C, 62.30; H, 4.08; N, 6.67.

*(2E,5Z) Ethyl 2-(5-(4-trifluoromethylbenzylidene)-2-(2-oxo-indolin-3-ylidene)-4-oxothiazolidin-3-yl)acetate **5c***

Yield: 42.6%. M.p.: 185–187°C; IR (KBr, cm^{-1}): 3428.1, 3161.2, 1741.4, 1711.6, 1676.3, 1532.5, 1326.1, 1216.0, 1165.2; MS (ESI) m/z: 475.3 $(M+H)^+$; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.21 (t, 3H, J = 7.2 Hz), 4.12 (q, 2H, J = 7.2 Hz), 5.32 (s, 2H), 6.87–8.03 (m, 9H), 10.72 (s, 1H); anal. calcd. for $C_{23}H_{17}F_3N_2O_4S$ (%): C, 58.22; H, 3.61; N, 5.90; Found (%): C, 58.12; H, 3.60; N, 5.85.

(2E,5Z) Ethyl 2-(5-(3,4,5-trimethoxybenzylidene)-2-(2-oxo-indolin-3-ylidene)-4-oxothiazolidin-3-yl)acetate ***5d***

Yield: 43.8%. M.p.: 140–142°C; IR (KBr, cm^{-1}): 3435.8, 3095.5, 1744.5, 1709.2, 1674.0, 1529.0, 1504.4, 1322.2, 1221.0, 1140.8; MS (ESI) *m/z*: 497.5 $(M+H)^+$; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.20 (t, 3H, J = 7.2 Hz), 3.77 (s, 3H), 3.90 (s, 6H), 4.11 (q, 2H, J = 7.2 Hz), 5.33 (s, 2H), 6.86–7.73 (m, 6H), 7.83 (s, 1H), 10.68 (s, 1H); anal. calcd. for $C_{25}H_{24}N_2O_7S$ (%): C, 60.47; H, 4.87; N, 5.64. Found (%): C, 60.52; H, 4.92; N, 5.73.

(2E,5Z) Ethyl 2-(5-(2-fluorobenzylidene)-2-(2-oxo-5-fluoroindolin-3-ylidene)-4-oxothiazolidin-3-yl)acetate ***5e***

Yield: 46.5%. M.p.: 172–174°C; IR (KBr, cm^{-1}): 3432.7, 3160.1, 1749.2, 1710.7, 1668.8, 1540.0, 1507.9, 1211.8, 1162.0, 1141.9; MS (ESI) *m/z*: 443.2 $(M+H)^+$; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.20 (t, 3H, J = 7.2 Hz), 4.11 (q, 2H, J = 7.2 Hz), 5.30 (s, 2H), 6.83 (dd, 1H, J = 8.4, 4.8 Hz), 6.99–7.06 (m, 1H), 7.42–7.48 (m, 2H), 7.60 (dd, 1H, J = 9.9, 2.4 Hz), 7.89–7.94 (m, 3H), 10.69 (s, 1H); anal. calcd. for $C_{22}H_{16}F_2N_2O_4S$ (%): C, 59.72; H, 3.65; N, 6.33. Found (%): C, 59.76; H, 3.77; N, 6.40.

(2E,5Z) Ethyl 2-(5-(4-trifluoromethylbenzylidene)-2-(2-oxo-5-fluoroindolin-3-ylidene)-4-oxothiazolidin-3-yl)-acetate ***5f***

Yield: 52.5%. M.p.: 162–164°C; IR (KBr, cm^{-1}): 3428.7, 3173.0, 1757.8, 1709.5, 1656.8, 1541.2, 1325.3, 1214.3, 1170.4; MS (ESI) *m/z*: 493.1 $(M+H)^+$; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.21 (t, 3H, J = 7.2 Hz), 4.12 (q, 2H, J = 7.2 Hz), 5.29 (s, 2H), 6.83 (d, 1H, J = 8.4, 4.8 Hz), 7.00–7.07 (m, 1H), 7.59 (dd, 1H, J = 9.6, 2.1 Hz), 7.93 (d, 2H, J = 8.4 Hz), 7.98 (s, 1H), 8.02 (d, 2H, J = 8.4 Hz), 10.72 (s, 1H); anal. calcd. for $C_{23}H_{16}F_4N_2O_4S$ (%): C, 56.10; H, 3.27; N, 5.69. Found (%): C, 56.12; H, 3.30; N, 5.72.

(2E,5Z) Ethyl 2-(5-(2,4-dichlorobenzylidene)-2-(2-oxo-5-fluoroindolin-3-ylidene)-4-oxothiazolidin-3-yl)acetate ***5g***

Yield: 45.7%. M.p.: 153–155°C; IR (KBr, cm^{-1}): 3425.9, 3160.5, 1744.2, 1715.9, 1669.2, 1555.1, 1478.8, 1372.6, 1218.9, 1171.7, 1137.6; MS (ESI) *m/z*: 493.0 $(M+H)^+$; ^{1}H-NMR (300 MHz, DMSO-d_6) δ:1.21 (t, 3H, J = 6.9 Hz), 4.12 (q, 2H, J = 6.9 Hz), 5.28 (s, 2H), 6.83 (dd, 1H, J = 8.7, 4.8 Hz), 6.99–7.06 (m, 1H), 7.54 (dd, 1H, J = 9.9, 2.4 Hz), 7.67 (dd, 1H, J = 8.7, 2.1 Hz), 7.88 (d, 1H, J = 2.1 Hz), 7.92 (d, 1H, J = 8.7 Hz), 7.95 (s, 1H), 10.72 (s, 1H); anal. calcd. for $C_{22}H_{15}Cl_2FN_2O_4S$ (%): C, 53.56; H, 3.06; N, 5.68. Found (%): C, 53.62; H, 3.17; N, 5.80.

(2E,5Z) Ethyl 2-(5-(3,4,5-trimethoxybenzylidene)-2-(2-oxo-5-fluoroindolin-3-ylidene)-4-oxothiazolidin-3-yl)-acetate ***5h***

Yield: 52.5%. M.p.: 176–178°C; IR (KBr, cm^{-1}): 3311.2, 1746.4, 1708.3, 1677.8, 1524.4, 1504.9, 1332.1, 1295.1, 1132.9; MS (ESI) *m/z*: 515.5 $(M+H)^+$; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.20 (t, 3H, J = 7.2 Hz), 3.77 (s, 3H), 3.91 (s, 6H), 4.12 (q, 2H, J = 7.2 Hz), 5.34 (s, 2H), 6.82–7.04 (m, 2H), 7.11 (s, 2H), 7.50 (dd, 1H, J = 10.2, 2.4 Hz), 7.87 (s, 1H), 10.69 (s, 1H); anal. calcd. for $C_{25}H_{23}FN_2O_7S$ (%): C, 58.36; H, 4.51; N, 5.44. Found (%): C, 58.43; H, 4.56; N, 5.50.

(2E,5Z) Ethyl 2-(5-benzylidene-2-(2-oxo-5-methylindolin-3-ylidene)-4-oxothiazolidin-3-yl)acetate ***5i***

Yield: 54.3%. M.p.: 145–147°C; IR (KBr, cm^{-1}): 3430.7, 3156.7, 1742.5, 1714.2, 1674.2, 1531.0, 1212.9, 1162.4; MS (ESI) *m/z*: 421.5 $(M+H)^+$; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.20 (t, 3H, J = 7.2 Hz), 2.39 (s, 3H), 4.11 (q, 2H, J = 7.2 Hz), 5.31 (s, 2H, CH_2), 6.76 (d, 1H, J = 10.8 Hz), 6.99 (d, 1H, J = 10.8 Hz), 7.52–7.65 (m, 4H), 7.79–7.82 (m, 2H), 7.88 (s, 1H), 10.57 (s, 1H); anal. calcd. for $C_{23}H_{20}N_2O_4S$ (%): C, 65.70; H, 4.79; N, 6.66. Found (%): C, 65.76; H, 4.87; N, 6.70.

(2E,5Z) Ethyl 2-(5-(2-fluorobenzylidene)-2-(2-oxo-5-methylindolin-3-ylidene)-4-oxothiazolidin-3-yl)acetate ***5j***

Yield: 38.2%. M.p.: 135–137°C; IR (KBr, cm^{-1}): 3429.4, 3149.1, 1751.9, 1710.2, 1673.2, 1531.8, 1506.2, 1198.7, 1159.3, 1142.7; MS (ESI) *m/z*: 439.1 $(M+H)^+$; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.20 (t, 3H, J = 6.9 Hz), 2.39 (s, 3H), 4.14 (q, 2H, J = 6.9 Hz), 5.31 (s, 2H), 6.76 (d, 1H, J = 7.8 Hz), 6.99 (d, 1H, J = 7.8 Hz), 7.43–7.49 (m, 2H), 7.57 (s, 1H), 7.86–7.90 (m, 3H), 10.57 (s, 1H); anal. calcd. for $C_{23}H_{19}FN_2O_4S$ (%): C, 63.00; H, 4.37; N, 6.39. Found (%): C, 63.08; H, 4.40; N, 6.42.

(2E,5Z) Ethyl 2-(5-(4-fluorobenzylidene)-2-(2-oxo-5-methylindolin-3-ylidene)-4-oxothiazolidin-3-yl)acetate ***5k***

Yield: 42.2%. M.p.: 147–149°C; IR (KBr, cm^{-1}): 3430.7, 3156.7, 1751.9, 1710.0, 1673.5, 1532.5, 1506.4, 1198.8, 1159.2; MS (ESI) *m/z*: 439.5 $(M+H)^+$; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.20 (t, 3H, J = 7.2 Hz), 2.39 (s, 3H), 4.11 (q, 2H, J = 7.2 Hz), 5.31 (s, 2H), 6.76–7.90 (m, 8H), 10.57 (s, 1H); anal. calcd. for $C_{23}H_{19}FN_2O_4S$ (%): C, 63.00; H, 4.37; N, 6.39. Found (%): C, 63.10; H, 4.45; N, 6.45.

(2E,5Z) Ethyl 2-(5-(3,4,5-trimethoxybenzylidene)-2-(2-oxo-5-methylindolin-3-ylidene)-4-oxothiazolidin-3-yl)-acetate ***5l***

Yield: 56.5%. M.p.: 157–159°C; IR (KBr, cm^{-1}): 3432.9, 2939.7, 1736.3, 1713.0, 1671.9, 1531.3, 1502.8, 1325.9, 1213.9, 1156.4, 1136.3; MS (ESI) *m/z*: 511.2 $(M+H)^+$; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.20 (t, 3H, J = 7.2 Hz), 2.30 (s, 3H), 3.76 (s, 3H), 3.92 (s, 6H), 4.18 (q, 2H, J = 7.2 Hz), 5.36 (s, 2H), 6.58–7.56 (m, 5H), 7.81 (s, 1H), 10.56 (s, 1H); anal. calcd. for $C_{26}H_{26}N_2O_7S$ (%): C, 61.16; H, 5.13; N, 5.49. Found (%): C, 61.22; H, 5.20; N, 5.56.

(2E,5Z) Ethyl 2-(5-benzylidene-2-(2-oxo-6-fluoroindolin-3-ylidene)-4-oxothiazolidin-3-yl)acetate ***5m***

Yield: 46.2%. M.p.: 183–185°C; IR (KBr, cm^{-1}): 3427.8, 3165.5, 1757.9, 1709.6, 1661.9, 1566.6, 1205.5, 1142.9; MS (ESI) *m/z*: 425.1 $(M+H)^+$; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.20 (t, 3H, J = 7.2 Hz), 4.12 (q, 2H, J = 7.2 Hz), 5.28 (s, 2H), 6.68 (dd, 1H, J = 9.0, 2.4 Hz), 6.84–6.91 (m, 1H), 7.51–7.63 (m, 3H), 7.79–7.83 (m, 3H), 7.88 (s, 1H), 10.83 (s, 1H); anal. calcd. for $C_{22}H_{17}FN_2O_4S$ (%): C, 62.25; H, 4.04; N, 6.60. Found (%): C, 62.14; H, 4.11; N, 6.69.

(2E,5Z) Ethyl 2-(5-(2-fluorobenzylidene)-2-(2-oxo-6-fluoroindolin-3-ylidene)-4-oxothiazolidin-3-yl)acetate ***5n***

Yield: 43.0%. M.p.: 188–190°C; IR (KBr, cm^{-1}): 3434.7, 3131.0, 1740.6, 1712.3, 1683.0, 1540.7, 1507.7, 1236.2, 1146.5; MS (ESI) *m/z*: 443.2 $(M+H)^+$; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.20 (t, 3H, J = 7.2 Hz), 4.12 (q, 2H, J = 7.2 Hz), 5.28 (s, 2H), 6.67 (dd, 1H, J = 9.0, 2.4 Hz), 6.83-6.90 (m, 1H), 7.41–7.46 (m, 2H), 7.78–7.89 (m, 4H), 10.83 (s, 1H); anal. calcd. for $C_{22}H_{16}F_2N_2O_4S$ (%): C, 59.72; H, 3.65; N, 6.33. Found (%): C, 59.79; H, 3.70; N, 6.39.

(2E,5Z) Ethyl 2-(5-(4-fluorobenzylidene)-2-(2-oxo-6-fluoroindolin-3-ylidene)-4-oxothiazolidin-3-yl)acetate ***5o***

Yield: 46.3%. M.p.: 134–136°C; IR (KBr, cm^{-1}): 3438.5, 3135.7, 1738.9, 1711.1, 1680.7, 1540.0, 1506.9, 1232.8, 1191.3, 1144.9; MS (ESI) *m/z*: 443.1 $(M+H)^+$; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.20 (t, 3H, $J = 7.2$ Hz), 4.11 (q, 2H, $J = 7.2$ Hz), 5.28 (s, 2H), 6.68–7.90 (m, 8H), 10.84 (s, 1H); anal. calcd. for $C_{22}H_{16}F_2N_2O_4S$ (%): C, 59.72; H, 3.65; N, 6.33. Found (%): C, 59.80; H, 3.70; N, 6.42.

(2E,5Z) Ethyl 2-(5-(4-fluorobenzylidene)-2-(2-oxo-5-bromoindolin-3-ylidene)-4-oxothiazolidin-3-yl)acetate ***5p***

Yield: 56.3%. M.p.: 162–164°C; IR (KBr, cm^{-1}): 3430.7, 3154.5, 1759.0, 1708.3, 1659.8, 1560.1, 1508.5, 1207.3, 1156.4; MS (ESI) *m/z*: 503.3 $(M+H)^+$; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.20 (t, 3H, $J = 7.2$ Hz), 4.11 (q, 2H, $J = 7.2$ Hz), 5.25 (s, 2H), 6.83–7.89 (m, 7H), 7.96 (m, 1H), 10.82 (s, 1H); anal. calcd. for $C_{22}H_{16}BrFN_2O_4S$ (%): C, 52.50; H, 3.20; N, 5.57. Found (%): C, 52.53; H, 3.26; N, 5.65.

Pharmacology

The cytotoxicities of compounds **5a–5p** and precursors **4a–4e** were evaluated with HT-29, H460 and MDA-MB-231 cell lines by the standard MTT assay *in vitro*, with sunitinib as the positive control. Compounds (**5a**, **5d**, **5f**, **5h**, **5l**) were further evaluated against WI-38 normal cell line. The cancer or normal cell lines were cultured in minimum essential medium (MEM) supplement with 10% fetal bovine serum (FBS). Approximately 4×10^3 cells, suspended in MEM medium, were plated onto each well of a 96-well plate and incubated in 5% CO_2 at 37°C for 24 h. The test compounds at indicated final concentrations were added to the culture medium and the cell cultures were continued for 72 h. Fresh MTT was added to each well at a terminal concentration of 5 μg/mL and incubated with cells at 37°C for 4 h. The formazan crystals were dissolved in 100 μL DMSO each well, and the absorbency at 492 nm (for absorbance of MTT formazan) and 630 nm (for the reference wavelength) was measured with the ELISA reader. All of the compounds were tested twice in each of the cell lines. The results expressed as IC_{50} (inhibitory concentration 50%) were the averages of two determinations and calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software.

Conclusion

In summary, a series of indolin-2-ones with 4-thiazolidinone moiety were designed and synthesized. The cytotoxicity of all synthesized compounds was evaluated against three human cancer cell lines (HT-29, H460 and MDA-MB-231). Several potent compounds were further evaluated against one normal cell line (WI-38). Some of the prepared compounds displayed moderate to excellent cytotoxicity. In particular, compound **5h** showed potent cytotoxicity against HT-29 and H460 cancer cell lines. Moreover, compound **5h** exhibited markedly weaker toxicity for normal cell line WI-38, when compared to HT-29 and H460 cancer cell lines. The preliminary structure–activity relationship (SAR) studies revealed that combination of indolin-2-one core structure and 5-benzylidene-4-thiazolidinone moiety at the 2-position of the 4-thiazolidinone ring could enhance anticancer activities, and 5-fluoroindolin-2-one core was more favorable. As for 5-benzylidene moiety, introduction of electron-donating group at the phenyl ring is beneficial for the cytotoxicity and selectivity against HT-29 and H460 cancer cell lines. Further studies are in progress in our laboratories and will be reported upon in the future.

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