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Original article

Stereoselective synthesis and QSAR study of cytotoxic 2-(4-oxo-thiazolidin-2-ylidene)-2-cyano-*N*-arylacetamides

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ABSTRACT

(2*Z*,5*Z*) 2-[(5-Arylidene-4-oxo-3-phenyl)-thiazolidin-2-ylidine]-2-cyano-*N*-arylacetamides **4a**–**l** were stereoselectively prepared via condensation of aromatic aldehydes with 4-thiazolidinones **3a**–**c**. The latters were obtained via electrophilic attack of phenylisothiocyanate on 2-cyano-*N*-arylacetamides **1a**–**c** followed by reaction with chloroacetyl chloride under basic condition. Single crystal X-ray study of **3a** allows good confirmation for the assigned structure. Additionally, 5-arylhydrazono analogs **5a**–**e** were prepared via condensation of the appropriate diazonium salts with 4-thiazolidinones **3a**,**b**. Many of the synthesized compounds exhibited promising antitumor properties against colon HCT116, breast MCF7 and liver HEPG2 cell lines. 3D-Pharmacophore modeling and QSAR analysis were combined to explain the observed antitumor properties.

1. Introduction

Cancer is a major health problem worldwide; it is considered the second leading cause of death after the heart diseases [1]. Deaths from cancer worldwide are projected to continue rising over 11 million deaths in the year 2030 [2]. Among the various types of malignant tumors, breast cancer causes the second leading deaths in women [3]. Colorectal cancer is the third leading cause of death and in United States about 50% patients were dead in 2010 [1]. Hepatocellular carcinoma is also one of the most common causes of cancer death and its incidence is increasing worldwide [4]. Although major advances have been made in the chemotherapeutic management of some patients, continued research is still needed toward development of new antitumor active hits with fewer side effects and higher potency.

4-Thiazolidinone ring system is a core structure in many synthetic compounds exhibiting broad pharmacological spectrum and affinity for various biotargets [5,6]. Some derivatives are peroxisome proliferator activated receptor (PPAR-γ) agonists showing hypoglycemic activity [7] or aldose reductase inhibitors guarding against diabetic complications [8]. Others are dual cyclo-oxygenase-2/5-lipoxygenase (COX-2/5-LOX) inhibitors [9], selective COX-2 inhibitors [10], phospholipase A2 (PLA2) inhibitors [11,12] and PPAR-γ agonists [13] possessing anti-inflammatory action. Thymidylate synthase X inhibitors with antimicrobial effects were also described [14]. Additionally, recent publications reported the anticancer properties of many 4-thiazolidinone analogs possessing different arylidene residues [15–28]. This is exemplified by compounds **I**–**IV** (Fig. 1), that exerted promising anticancer activity against colon HCT116 and/or breast MCF7 cancer cell lines [24–26]. Furthermore, the hydrazono moiety was proved to exert anticancer activities [29–32]. Compound **V** (Fig. 1) is an example of thiazolidine nucleus substituted with hydrazono group that exhibited promising anticancer activity in Ehrlich ascites assay [32].

The present work is directed toward not only synthesis of 5-arylidene-4-thiazolidinones and 5-arylhydrazono analogs utilizing easily accessible chemicals and facile synthetic pathways, but also investigation of the antitumor properties of the constructed compounds against a variety of human tumor cell lines (HCT116 “colon”, MCF7 “breast” and HEPG2 “liver” cancers). QSAR study is also initiated for understanding and validating the pharmacological observations.

2. Results and discussion

2.1. Chemistry

Scheme 1 outlines the synthetic pathway for preparation of 4-thiazolidinone derivatives **3a**–**c**. The starting compounds, 2-cyano-*N*-arylacetamides **1a**–**c**, were prepared according to the

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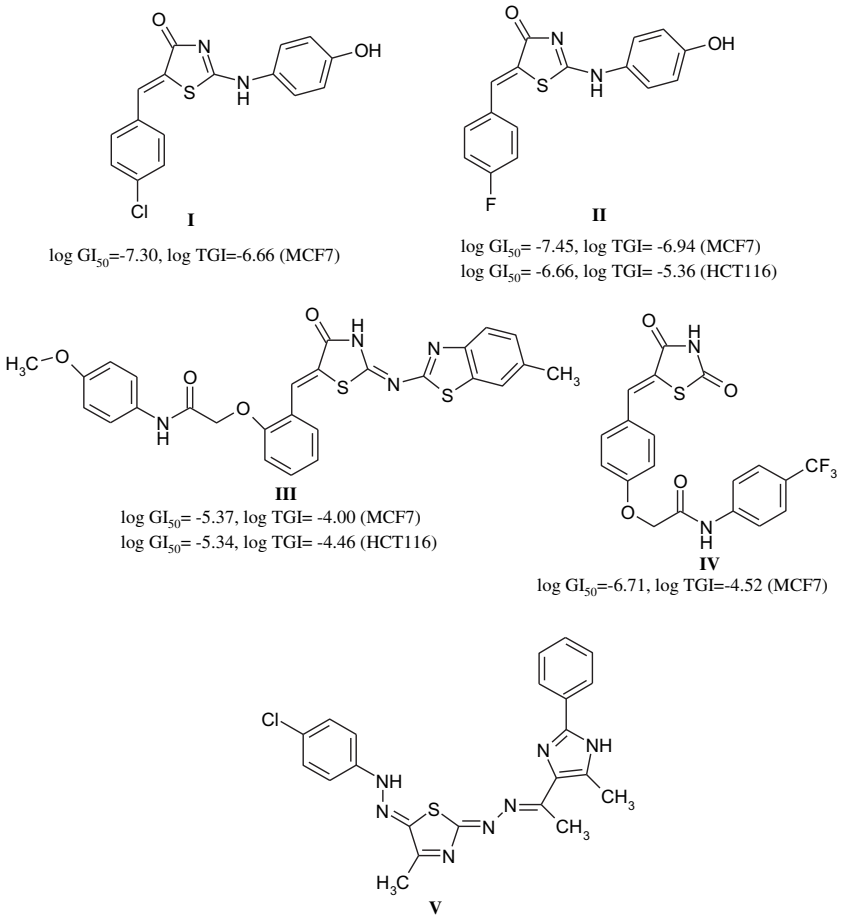


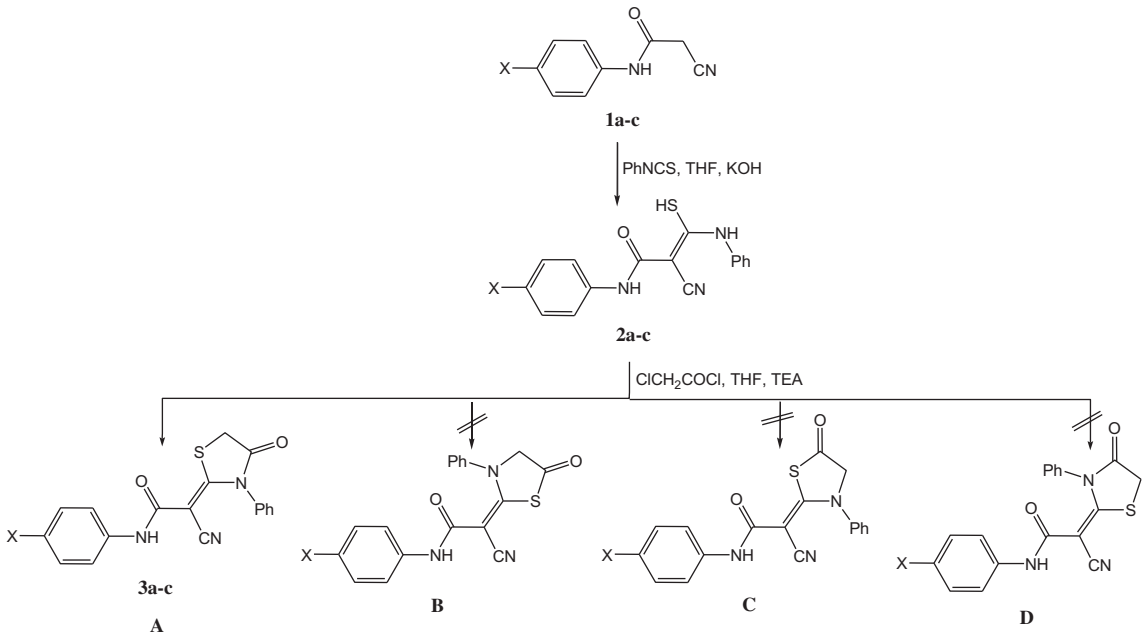
Fig. 1. Structures of some thiazolidine-based anticancer agents.

previously reported procedures [33,34]. 2-Cyano-3-mercapto-3-phenylamino-*N*-arylacrylamides **2a**–**c** were synthesized via electrophilic attack of phenylisothiocyanate on the active methylene of compounds **1a**–**c**. The reaction was conducted in dry tetrahydrofuran (THF) in the presence of potassium hydroxide. The structures of the isolated compounds **2a**–**c** were confirmed by ^{1}H NMR spectral data that lacked any signal assignable for the active methylene function and exhibited signals due to the aromatic ring protons at δ = 7.14–7.55, and three exchangeable signals corresponding to two NH and one SH protons at δ = 4.52–4.53, 12.05–12.09 and 12.70–12.80, respectively.

Reaction of **2a**–**c** with chloroacetyl chloride in the presence of triethylamine in dry THF afforded the 4-thiazolidinone derivatives **3a**–**c**. IR spectra of **3a**–**c** revealed only one NH band at ν = 3348–3332 cm^{-1} in addition to two C=O bands at ν = 1747–1736 and 1651–1647 cm^{-1} regions. ^{1}H NMR spectra of **3a**–**c** exhibited the methylene function as a sharp singlet signal at δ = 3.87–3.89. ^{13}C NMR (APT) spectrum of **3a**, as a representative example of the synthesized analogs, showed the methylene carbon of the thiazolidinone ring at δ = 31.8 in addition to the carbonyl carbons of the amide and 4-thiazolidinone ring functions at δ = 162.8 and 169.5 respectively. Single crystal X-ray study of **3a** (Fig. 2) allowed good confirmation for the assigned structure confirming that the isolated product is a *Z*-isomer (structure A) excluding any other hypothetical isomeric forms (*Z*-isomer "structure C" or *E*-isomers "structures B, D") indicating that the conducted reaction proceeded in a stereoselective manner under the described applied reaction conditions (Scheme 1).

Geometric parameters obtained experimentally through single crystal X-ray study and theoretically calculated ones with both AM1 and PM3 methods are presented in Table 1 (see Supplementary data). The geometries of **3a** were optimized by the molecular mechanics force field (MM+) followed by either semi-empirical AM1 [35] or PM3 [36,37] methods implemented in the HyperChem 8.0 package. The structures were fully optimized without fixing any parameters, thus bringing all geometric variables to their equilibrium values. The energy minimization protocol employed the Polak–Ribiere conjugated gradient algorithm. Convergence to a local minimum was achieved when the energy gradient was $\leq$0.01 kcal mol^{-1}. The RHF method was used in the spin pairing for the two semi-empirical tools [38–40].

2-(5-Arylidene-4-oxo-3-phenyl-thiazolidin-2-ylidene)-2-cyano-*N*-arylacetamides **4a**–**l** were obtained by condensation of the appropriate aromatic aldehydes with **3a**–**c** in dimethylformamide (DMF) in the presence of triethylamine (Scheme 2). The structures of **4a**–**l** were established through different spectroscopic techniques (IR, ^{1}H NMR, MS) and elemental analyses data. The disappearance of the singlet signal due the methylene protons and the presence of the sharp singlet signal due to the ylidene proton in ^{1}H NMR spectra added a good confirmation for the assigned structures **4a**–**l**. It was noticed that the ylidene proton of compounds **4a**–**c**, **4e**–**g** and **4i**–**k** were exhibited at δ = 7.83–7.91 confirming the formation of *Z*-isomers [25,41–46]. Meanwhile, the ylidene proton of compounds **4d**, **4h** and **4l** were revealed at δ = 8.03–8.04, relatively downfielded than the other synthesized analogs, which could be attributed to the anisotropic effect of the hydroxyl



1a, 2a, 3a; X = H
1b, 2b, 3b; X = CH_3
1c, 2c, 3c; X = Cl

Scheme 1. Preparation of compounds **3a**–**c**.

group oriented at the *o*-position of the arylidene function. Reaction of the appropriate diazonium salt derived from different anilines (aniline, *p*-anisidine and *p*-chloroaniline) with 4-thiazolidinones **3a**,**b** in DMF in the presence of sodium acetate afforded 2-(5-arylhydrazono-4-oxo-3-phenyl-thiazolidin-2-ylidene)-2-cyano-*N*-arylacetamides **5a**–**e** whose structures were established via different spectroscopic and elemental analyses data (c.f. Experimental section).

2.2. Antitumor activity

In-vitro antitumor activity of the tested compounds was performed utilizing in-vitro sulfo-rodamine B (SRB) standard method [47] in the National Cancer Institute, Cairo University, Egypt. Fifteen synthesized compounds (**4a**,**b**, **4d**–**i**, **4k**, **4l** and **5a**–**e**) were tested for their antitumor properties against HCT116 "colon", MCF7 "breast" and HEPG2 "liver" cancer cell lines. From the observed

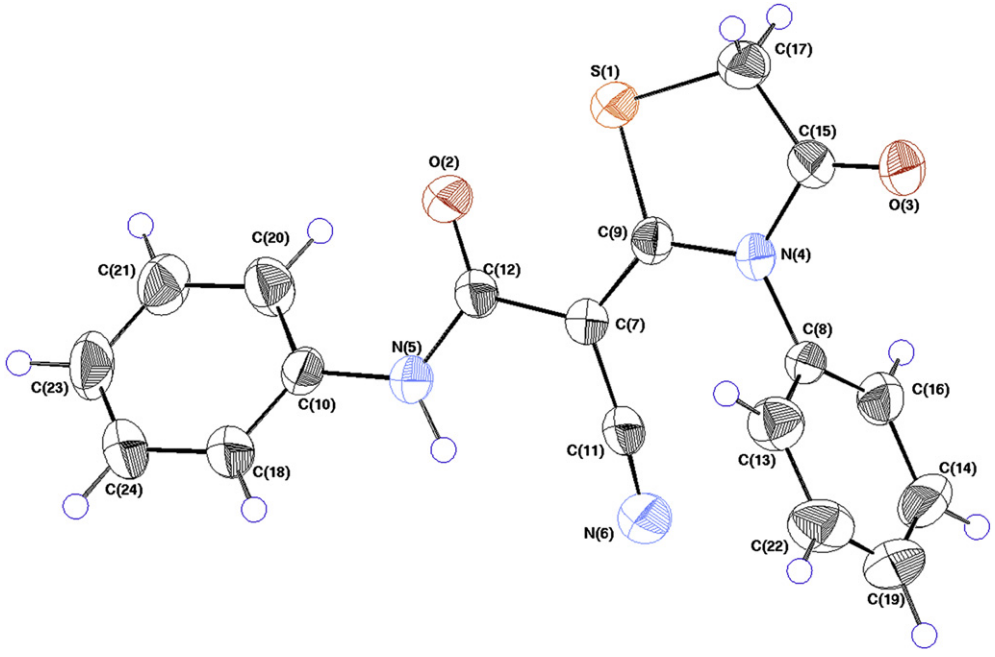


Fig. 2. ORTEP projection of single crystal X-ray diffraction of **3a**.

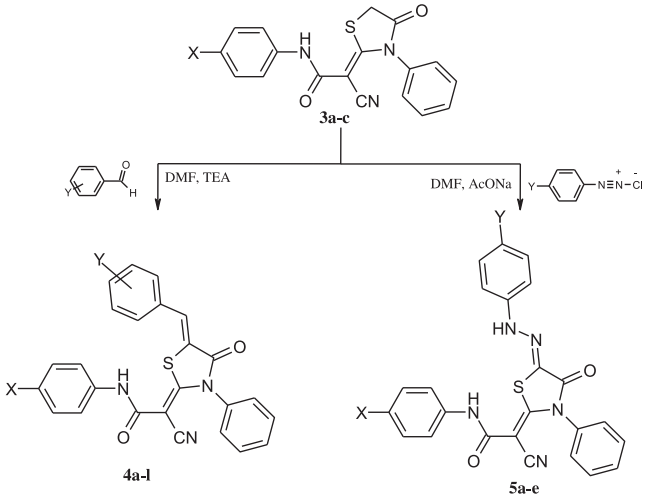
Table 1
Log *P* and IC_{50} of the tested compounds against human tumor cell lines.

Compound	Substitution		Log *P*	Tested human tumor cell lines, IC_{50} μg/ml (μM)		
	X	Y		Colon HCT116	Breast MCF7	Liver HEPG2
Doxorubicin	–	–		3.73 (6.86)	2.97 (5.46)	4.00 (7.36)
4a	H	H	4.02	14.10 (33.29)	31.90 (75.33)	4.65 (10.98)
4b	H	4-OCH_3	3.77	3.00 (6.61)	3.58 (7.89)	13.80 (30.43)
4d	H	2-OH	3.73	3.43 (7.80)	3.89 (8.85)	36.50 (83.05)
4e	CH_3	H	4.49	4.80 (10.97)	24.30 (55.54)	16.70 (38.17)
4f	CH_3	4-OCH_3	4.23	4.65 (9.95)	>50.00 (>106.94)	17.50 (37.43)
4g	CH_3	4-Cl	5.00	13.40 (28.39)	13.50 (28.60)	22.80 (48.31)
4h	CH_3	2-OH	4.20	3.14 (6.92)	>50.00 (>110.25)	3.74 (8.25)
4i	Cl	H	4.54	17.80 (38.87)	35.50 (77.52)	22.50 (49.13)
4k	Cl	4-Cl	5.06	4.50 (9.15)	>50.00 (>101.63)	>50.00 (>101.63)
4l	Cl	2-OH	4.25	3.28 (6.92)	3.74 (7.89)	5.87 (12.39)
5a	H	H	4.72	>50.00 (>113.77)	NT[a]	8.24 (18.75)
5b	H	OCH_3	4.47	21.76 (46.35)	8.25 (17.57)	NT[a]
5c	CH_3	H	5.19	>50.00 (>110.25)	NT[a]	11.00 (24.25)
5d	CH_3	OCH_3	4.94	>50.00 (>103.40)	NT[a]	16.47 (34.06)
5e	CH_3	Cl	5.71	7.25 (14.86)	>50.00 (>102.47)	NT[a]

[a] NT = not tested.

antitumor activity data (Table 1), see also Figs. 1–3 of the Supplementary data, it has been noticed that most of the tested compounds exerted significant activity against HCT116 "colon" cell line compared with the other assayed cell lines: MCF7 "breast" and HEPG2 "liver" cancers.

Considering the observed antitumor screening data against MCF7 "breast" cancer cell line, only compounds **4b**, **4d** and **4l** revealed promising pharmacological activity (IC_{50} = 7.89, 8.85, 7.89 μM, respectively) compared with Doxorubicin which was used as a reference standard during this study (IC_{50} = 5.46 μM). Otherwise, compounds **4a**, **4h** and **4l** exhibited moderate activity against HEPG2 "liver" cell line (IC_{50} = 10.98, 8.25, 12.39 μM, respectively) compared with Doxorubicin (IC_{50} = 7.36 μM). However, compound **4b** exhibited the most promising antitumor properties among all the tested analogs against HCT116 "colon" cancer cell line (IC_{50} = 6.61 μM) compared with Doxorubicin (IC_{50} = 6.86 μM). Additionally, compounds **4d**, **4h** and **4l** revealed also promising antitumor properties against the same cell line (IC_{50} = 7.80, 6.92 and 6.92 μM respectively).



4a X=H, Y=H
4b X=H, Y=4-OCH_3
4c X=H, Y=4-Cl
4d X=H, Y=2-OH
4e X=CH_3, Y=H
4f X=CH_3, Y=4-OCH_3
4g X=CH_3, Y=4-Cl
4h X=CH_3, Y=2-OH
4i X=Cl, Y=H
4j X=Cl, Y=4-OCH_3
4k X=Cl, Y=4-Cl
4l X=Cl, Y=2-OH
5a X=H, Y=H
5b X=H, Y=OCH_3
5c X= CH_3, Y=H
5d X= CH_3, Y=OCH_3
5e X= CH_3, Y=Cl

Scheme 2. Preparation of compounds **4a**–**l** and **5a**–**e**.

Structure activity relationship based on the observed antitumor properties of the synthesized compounds against HCT116 "colon" cancer cell line, indicated that attachment of arylidene function at the 5-position of 4-oxo-thiazolidin-2-ylidine ring system **4a**–**l**, seemed more favorable for exhibiting antitumor properties compared with the case when arylhydrazono moiety was incorporated at the same position **5a**–**e**. This may be attributed to the presence of an additional NH spacer which can confer some hindrance to the proper binding of these compounds to their biotarget. Moreover, it could be noted that the impact of the electronic nature of the substituents X and Y viz. H, CH_3, Cl, OCH_3 and OH functions upon activity is not absolute.

An attempt was made to correlate between the activity of the compounds and their partition coefficient (log *P*) (Table 1). It was observed that (except in case of compounds **4k** and **5e**) there is an inverse relationship between log *P* value and antitumor activity i.e. the lower the log *P* values, the higher was the antitumor properties as exhibited in compounds **4a**, **4b**, **4d** (IC_{50} = 33.29, 6.61, and 7.80 μM correlated with log *P* = 4.02, 3.77, and 3.73, respectively), compounds **4e**, **4f**, **4g** and **4h** (IC_{50} = 10.97, 9.95, 28.39 and 6.92 μM correlated with log *P* = 4.49, 4.23, 5.00 and 4.20, respectively), compounds **4i** and **4l** (IC_{50} = 38.87 and 6.92 μM correlated with log *P* = 4.54, 4.25, respectively), and compounds **5a** and **5b** (IC_{50} = >113.77 and 46.35 μM correlated with log *P* = 4.72 and 4.47 respectively).

2.3. Log P value calculations

Log *P* values of the tested compounds were determined for the optimized structures of the tested compounds developed by HyperChem software. Whereby, the structures were pre-optimized by the molecular mechanics force field (MM+) followed by fine adjustment with semi-empirical AM1. The structures were fully optimized without fixing any parameters, thus bringing all geometric variables to their equilibrium values. The energy minimization protocol employed the Polak–Ribiere conjugated gradient algorithm. Convergence to a local minimum was achieved when the energy gradient was ≤0.05 kcal mol^{-1} [38–40].

2.4. QSAR study

2.4.1. 3D-QSAR pharmacophore modeling

This study was performed using Discovery Studio 2.5 software (Accelrys Inc., San Diego, CA, USA), which is accessible for pharmacophore generation, structural alignment, activity prediction

and 3D database creation [48]. 3D-QSAR based on pharmacophore was constructed using collections of molecules (training set of 13 compounds, **4a**, **4d**–**i**, **4k**, **4l**, **5b**–**e**) with activities ranging over a number of orders of magnitude. The observed HYPOGEN identifies a 3D array of a maximum of four chemical features common to the adopted training set. The chemical features considered are, two hydrogen bond acceptors (HBA-1), (HBA-2) and two hydrophobics (H-1), (H-2) (Fig. 3). Table 2 exhibits constraint distances and angles between features of the generated pharmacophores. *Fit* values and estimated activities of the training set due to the generated 3D-pharmacophore model was presented in Table 3 (Fig. 4 of the Supplementary data exhibited mapping of the training set analogs into the pharmacophoric hypothesis).

2.4.2. QSAR modeling

Despite the significance of pharmacophoric hypotheses in understanding ligand molecule affinity and 3D search queries, their predictive value as 3D-QSAR models is generally limited by steric shielding and bioactivity-modulating auxiliary groups (electron-donating or withdrawing functionalities) [49]. Thus, classical QSAR analysis was employed searching for the best combination of orthogonal pharmacophores using *Fit* value and other structural descriptors (connectivity, topological, …etc.) capable of explaining bio-activity variation across a collected list of descriptors allowing different pharmacophoric models competing within 3D-QSAR context.

A set of 13 compounds (**4a**, **4d**–**i**, **4k**, **4l**, **5b**–**e**) was used as a training set for QSAR modeling. The remaining 2 compounds (**4b** and **5a**) were adopted as an external test subset for validating the QSAR model. Many molecular descriptors were calculated for each compound employing calculate molecular properties module. The

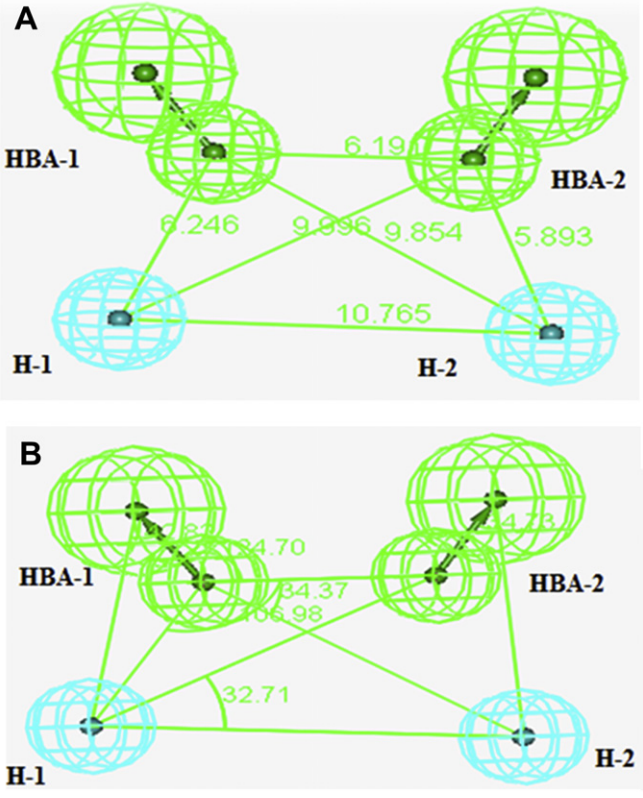


Fig. 3. (A) Constraint distances (Å) and (B) constraint angles (°) between features of the generated pharmacophores.

Table 2
Constraint distances (Å) and angles (°) between features of the generated pharmacophores.

Constraint distances (Å)	Constraint angles (°)
(HBA-1)–(HBA-2), 6.19; (HBA-2)–(H-2), 5.893; (H-1)–(HBA-2), 9.996; (HBA-1)–(H-1), 6.246; (HBA-1)–(H-2), 9.854; (H-1)–(H-2), 10.765.	(H-1)–(HBA-1)–(HBA-2), 106.98; (H-2)–(HBA-1)–(HBA-2), 34.37; (H-2)–(H-1)–(HBA-2), 32.71; (H-2) (HBA-2) Vector, 34.73; (H-1) (HBA-1) Vector, 42.82; (HBA-2) (HBA-1) Vector, 124.70

calculated descriptors including various simple and valence connectivity indices, electro-topological state indices, single point quantum-mechanical descriptors (via the AM1 model) and other molecular descriptors were considered. Furthermore, the training set compounds were fitted against the corresponding pharmacophore hypotheses generated by the HYPOGEN automatic runs and their *Fit* values (produced by the best-fit command) were added as additional molecular descriptors. Genetic function approximation (GFA) was employed to search for the best possible QSAR regression equation capable of correlating the variations in biological activities of the training set compounds with variations in the generated descriptors, i.e. multiple linear regression modeling (MLR) [50]. Equation (1), shows our best-performing QSAR model (Fig. 4 exhibits the corresponding scatter plots of observed versus estimated activity values for the training set compounds against HCT116 colon cancer cell line). The goodness of the model was validated by squared correlation coefficient (R^2 = 0.823) and residuals between the estimated and experimental activity of the training set (Table 4).

Equation (1): Potency (IC_{50}) against HCT116 (colon cancer) cell line (N "number of molecules in the training set" = 13, R^2 "squared correlation coefficient value" = 0.823)

$$IC_{50} = 1994.08 - 1091.92\,[\text{Average bond length}] - 71.42\,[\text{Fit value}]$$

Searching for set descriptors (D), containing D descriptors of optimal subset (d), where $d << D$ ones with minimum standard deviation (S), by means of multivariable linear regression (MLR) technique.

$$S = \frac{1}{(N-d-1)}\sum_{i=1}^{N}\text{resi}$$

Where; N, is the number of molecules of the training set; resi, is the

Table 3
Best fit values and estimated activities for compounds of the training set (**4a**, **4d**–**4i**, **4k**, **4l**, **5b**–**e**) mapped with the generated 3D-pharmacophore model due to HCT116 colon cancer cell line.

Entry	Compd. no.	Estimated activity	Observed activity	*Fit* value
1	**4a**	16.8137	33.29	7.63604
2	**4d**	7.28358	7.8	7.99935
3	**4e**	9.777	10.97	7.87149
4	**4f**	8.60996	9.95	7.9267
5	**4g**	19.4939	28.39	7.5718
6	**4h**	7.57676	6.92	7.98222
7	**4i**	68.1687	38.87	7.02812
8	**4k**	8.89853	9.15	7.91238
9	**4l**	19.1485	6.92	7.57956
10	**5b**	33.494	46.35	7.33673
11	**5c**	77.5942	>110	6.97187
12	**5d**	62.7711	>103	7.06394
13	**5e**	18.7868	14.86	7.58785

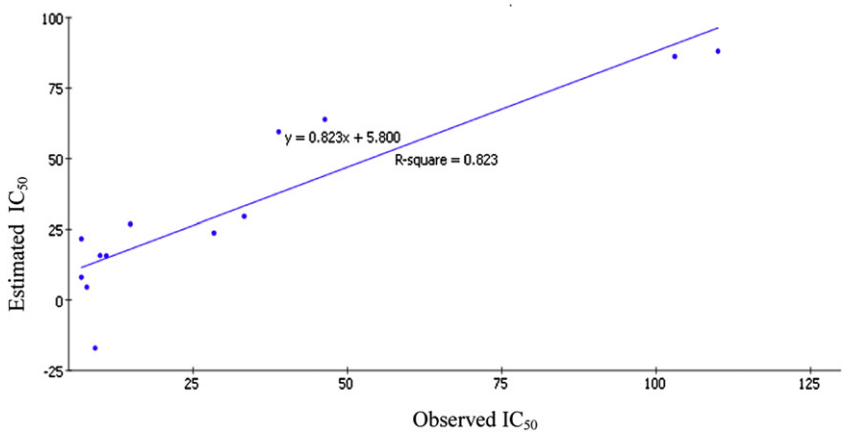


Fig. 4. Estimated activity versus observed activity (IC_{50}) of the tested compounds against HCT116 (colon) human tumor cell line.

Table 4
Estimated activity data of the training set against HCT116 (colon cancer) cell line and calculated descriptors governing activity according to equation (1).

Entry	Compd.	Estimated activity	Observed activity	Residuals	Average bond length	*Fit* value
1	**4a**	29.5247	33.29	3.76532	1.2997	7.63604
2	**4d**	4.55884	7.8	3.24116	1.2988	7.99935
3	**4e**	15.6564	10.97	−4.6864	1.297	7.87149
4	**4f**	15.7533	9.95	−5.80327	1.2933	7.9267
5	**4g**	23.7396	28.39	4.65038	1.3092	7.5718
6	**4h**	8.07535	6.92	−1.15535	1.2967	7.98222
7	**4i**	59.5133	38.87	−20.6433	1.312	7.02812
8	**4k**	−17.0735	9.15	26.2235	1.3243	7.91238
9	**4l**	21.6567	6.92	−14.7367	1.3106	7.57956
10	**5b**	63.8961	46.35	−17.5461	1.2878	7.33673
11	**5c**	88.0991	>110	21.9009	1.2895	6.97187
12	**5d**	86.2184	>103	16.7816	1.2852	7.06394
13	**5e**	26.8518	14.86	−11.9918	1.3053	7.58785

residual for molecule; *i*, is the difference between the experimental property (p) and predicted property (ppred).

More precisely, The Kubinyi function (*Fit*) [51,52] is a statistical parameter which is closely related to the Fisher ratio (F), but avoids the main disadvantage of the latter that is, too sensitive to changes in small d values and poorly sensitive to changes in large d values. The *Fit* (d) criterion has a low sensitivity to changes in small d values and a substantially increasing sensitivity for large d values. The greater the *Fit* value the better the linear equation [53]. It is given by the following equation, "where R (d) is the correlation coefficient for a model with (d) descriptors". The observed *Fit* values are 2.45 corresponding to models due to HCT116 cancer cell lines.

$$Fit = \frac{R(d)^2(N-d-1)}{(N+d^2)(1-R^2)}$$

Where $N = 13$, $R = 0.907$, $S = 0.46$, $Fit = 2.45$.

Table 5
External validation for the established QSAR models utilizing promising **4b** and mild **5a** antitumor active agents.

Compd. no.	3D-QSAR pharmacophore		Classical QSAR			
	Observed activity (IC_{50})	Estimated activity (IC_{50})	Observed activity (IC_{50})	Estimated activity (IC_{50})	*Fit* value	Average bond length
4b	6.61	10.63	6.61	8.53	7.834	1.306
5a	>113.77	116.22	>113.77	114.92	6.803	1.276

2.4.3. External validation of QSAR

External validation of the determined QSAR equation was performed utilizing two of our synthesized analogs exhibiting promising **4b** and mild **5a** antitumor properties. The observed activity supporting the established QSAR study is presented in Table 5.

3. Conclusion

From all the above it could be concluded that, compounds 2-cyano-2-(4-oxo-3-phenyl-thiazoldin-2-ylidene)-*N*-arylacetamides **3a**–**c** were stereoselectively synthesized as *Z*-isomers via reaction of *N*-aryl-2-cyano-3-mercapto-3-phenylaminoacrylamides **2a**–**c** with chloroacetyl chloride in the presence of triethylamine. Reaction of **3a**–**c** with either aromatic aldehydes or aryl diazonium compounds afforded (2*Z*,5*Z*) 2-(5-arylidene-4-oxo-3-phenyl-thiazoldin-2-ylidene)-2-cyano-*N*-arylacetamides **4a**–**l** and 2-(5-arylhydrazono) analogs **5a**–**e**, respectively.

Compound **4b** revealed higher antitumor property against HCT116 "colon" cancer cell line with potency ($IC_{50} = 6.61$ μM) than Doxorubicin ($IC_{50} = 6.86$ μM). Additionally, compounds **4h** and **4l** exhibited promising antitumor property against the same cell line ($IC_{50} = 6.92$ μM). Meanwhile, compounds **4b** and **4l** revealed moderate antitumor activity against MCF7 "breast" cancer cell line ($IC_{50} = 7.89$ μM) compared with Doxorubicin ($IC_{50} = 5.46$ μM). Additionally, compounds **4h** exhibited good activity against HEPG2 "liver" cancer cell line ($IC_{50} = 8.25$ μM) compared with Doxorubicin ($IC_{50} = 7.36$ μM).

It has been noticed that the most controlling factor affecting the observed antitumor activity against HCT116 "colon" cancer cell line was the log *P* value. i.e. the lower the log *P* value, the higher the observed antitumor properties.

QSAR study revealed that the average bond length and the *Fit* value descriptors affected the activity as concluded from equation (1).

4. Experimental

4.1. Chemistry

Melting points were measured with an Electrothermal Stuart SMP_3 digital melting point apparatus. IR spectra (KBr disc) were recorded on Shimadzu FT-IR 8400S infrared spectrophotometer. NMR spectra were recorded on a Varian Mercury VX 300 spectrometer (^1H: 300, ^{13}C APT: 75 MHz) using TMS as an internal standard, ^{13}C NMR of compounds **4b**, **5b** and **5d** were recorded on

JOEL (Eclipse) 400. Mass spectra were measured on a Shimadzu GCMS-QP1000 EX spectrometer (EI, 70 eV). Elemental analyses were carried out at the Microanalytical center, Faculty of Science, Cairo University, Egypt. Reagents and solvents used in synthesis were purchased from Sigma–Aldrich. Compounds **1a**–**c** were prepared according to the reported procedures [33,34].

*4.1.1. General procedure for the preparation of (**2a**–**c**)*

To an ice cold mixture of **1a**–**c** (5 mmol) and finely powdered potassium hydroxide (0.28 g, 5 mmol) in dry THF (25 ml) was added dropwise a solution of phenylisothiocyanate (0.68 g, 0.61 ml, 5 mmol) in dry THF (10 ml). The mixture was stirred at room temperature for 48 h, and it was poured on water (200 ml) with stirring. The obtained solution was neutralized with dilute HCl and the obtained precipitate was filtered, washed with water and crystallized from ethanol to obtain **2a**–**c** in a pure form.

*4.1.1.1. 2-Cyano-3-mercapto-N-phenyl-3-phenylaminoacrylamide (**2a**) [54].* Yellow crystals, mp 117–119 °C, 61% yield; IR (cm^{-1}): ν 3363, 3336 (2NH), 3047 (aromatic CH), 2195 (C≡N), 1632 (C=O), 1581 (bending NH), 1528 (C=C); ^{1}H NMR (*C*DCl$_3$): δ 4.52, 12.09, 12.80 (3s, 3H, 2NH, SH exchanged with D_2O), 7.16–7.54 (m, 10H, aromatic H); Anal. Calcd. for $C_{16}H_{13}N_3OS$ (295.37): C, 65.06; H, 4.44; N, 14.23. Found: C, 65.08; H, 4.14; N, 14.16.

*4.1.1.2. 2-Cyano-3-mercapto-N-(4-methylphenyl) 3-phenylaminoacrylamide **(2b)**.* Yellow crystals, mp 127–129 °C, 42% yield; IR (cm^{-1}): ν 3360, 3341 (2NH), 3050 (aromatic CH), 2930 (aliphatic CH), 2195 (C≡N), 1636 (C=O), 1574 (bending NH), 1520 (C=C); ^{1}H NMR (*C*DCl$_3$): δ 2.34 (s, 3H, CH_3), 4.52, 12.09, 12.79 (3s, 3H, 2NH, SH exchanged with D_2O), 7.14–7.49 (m, 9H, aromatic H); MS (*m*/*z*, %): 309 (M^+, 15), 310 [(M^+ + 1), 4], 107 (100); Anal. Calcd. for $C_{17}H_{15}N_3OS$ (309.39): C, 66.00; H, 4.89; N, 13.58. Found: C, 66.29; H, 4.91; N, 13.49.

*4.1.1.3. N-(4-Chlorophenyl)-2-cyano-3-mercapto-3-phenylaminoacrylamide (**2c**).* Orange crystals, mp 165–167 °C, 35% yield; IR (cm^{-1}): ν 3340 (2NH), 3097 (aromatic CH), 2187 (C≡N), 1632 (C=O), 1558 (bending NH), 1528 (C=C); ^{1}H NMR (*C*DCl$_3$): δ 4.53, 12.05, 12.70 (3s, 3H, 2NH, SH exchanged with D_2O), 7.26–7.55 (m, 9 H, aromatic H); Anal. Calcd. for $C_{16}H_{12}ClN_3OS$ (329.81): C, 58.27; H, 3.67; N, 12.74. Found: C, 58.19; H, 3.60; N, 12.77.

*4.1.2. General procedure for the preparation of (**3a**–**c**)*

A solution of **2a**–**c** (3 mmol) and triethylamine (0.61 g, 0.84 ml, 6 mmol) in dry THF (15 ml) was cooled to −5 °C in an ice/salt bath and a solution of chloroacetyl chloride (0.34 g, 0.24 ml, 3 mmol) in dry THF (5 ml) was added dropwise. The mixture was stirred overnight at room temperature, and the formed precipitate was filtered and dried. The residue was suspended in water, stirred for 5 min, and filtered. The crude product was crystallized from ethanol.

*4.1.2.1. (2Z) 2-Cyano-2-(4-oxo-3-phenyl-thiazolidin-2-ylidene)-N-phenylacetamide (**3a**).* Buff crystals, mp 260–262 °C (dec.), 73% yield; IR (cm^{-1}): ν 3341 (NH), 3059–3039 (aromatic CH), 2935 (aliphatic CH), 2199 (C≡N), 1747 (thiazolidinone C=O), 1647 (amide C=O), 1597 (bending NH), 1535 (C=C); ^{1}H NMR (*C*DCl$_3$): δ 3.89 (s, 2H, CH_2), 7.12–7.82 (m, 11H, 10 aromatic H + NH exchanged with D_2O); ^{13}C NMR (APT) (DMSO-d_6): δ 31.8 (CH_2), 78.7 (C≡N–*C*=C), 113.2 (*C*≡N), 120.9, 123.9, 128.4, 129.2, 129.3, 130.3 (aromatic C), 135.1 (aromatic C attached to N of the 4-thiazolidinone ring), 138.0 (aromatic C attached to amide NH), 162.8 (amide *C*=O), 169.5 (thiazolidinone *C*=O), 173.2 (C≡N–C=*C*); Anal. Calcd. for $C_{18}H_{13}N_3O_2S$ (335.39): C, 64.46; H, 3.91; N, 12.53. Found: C, 64.30; H, 3.90; N, 12.76.

*4.1.2.2. 2-Cyano-N-(4-methylphenyl)-2-(4-oxo-3-phenyl-thiazolidin-2-ylidene)acetamide **(3b)**.* Buff crystals, mp 236–237 °C (dec.), 67% yield; IR (cm^{-1}): ν 3348 (NH), 3066–3020 (aromatic CH), 2924 (aliphatic CH), 2203 (C≡N), 1747 (thiazolidinone C=O), 1647 (amide C=O), 1597 (bending NH), 1535 (C=C); ^{1}H NMR (*C*DCl$_3$): δ 2.32 (s, 3H, CH_3), 3.87 (s, 2H, CH_2), 7.12–7.77 (m, 10H, 9 aromatic H + NH exchanged with D_2O); Anal. Calcd. for $C_{19}H_{15}N_3O_2S$ (349.41): C, 65.31; H, 4.33; N, 12.03. Found: C, 65.48; H, 4.36; N, 12.07.

*4.1.2.3. (2Z) N-(4-Chlorophenyl)-2-cyano-2-(4-oxo-3-phenyl-thiazolidin-2-ylidene)acetamide (**3c**).* White crystals, mp 266–267 °C (dec.), 65% yield; IR (cm^{-1}): ν 3332 (NH), 3060 (aromatic CH), 2950 (aliphatic CH), 2203 (C≡N), 1736 (thiazolidinone C=O), 1651 (amide C=O), 1593 (bending NH), 1535 (C=C); ^{1}H NMR (*C*DCl$_3$): δ 3.89 (s, 2H, CH_2), 7.12–7.80 (m, 10H, 9 aromatic H + NH exchanged with D_2O); MS (*m*/*z*, %):369 (M^+, 45), 370 [(M^+ + 1), 30], 371 [(M^+ + 2), 25], 215 (100); Anal. Calcd. for $C_{18}H_{12}N_3O_2S$ (369.83): C, 58.46; H, 3.27; N, 11.36. Found: C, 58.29; H, 3.50; N, 11.11.

*4.1.3. General procedure for the preparation of (**4a**–**l**)*

A solution of **3a**–**c** (1 mmol), the appropriate aldehyde (1 mmol) and triethylamine (0.10 g, 0.14 ml, 1 mmol) in DMF (5 ml) was stirred at room temperature for 6 h. The obtained precipitate was filtered, washed and crystallized from ethanol.

*4.1.3.1. (2Z,5Z) 2-(5-Benzylidene-4-oxo-3-phenyl-thiazoldin-2-ylidene)-2-cyano-N-phenylacetamide (**4a**).* Yellow crystals, mp 347–348 °C (dec.), 38% yield; IR (cm^{-1}): ν 3416 (NH), 3070–3017 (aromatic CH), 2200 (C≡N), 1712 (thiazolidinone C=O), 1655 (amide C=O), 1597 (bending NH), 1512 (C=C); ^{1}H NMR (*C*DCl$_3$): δ 7.18–7.89 (m, 16H, 15 aromatic H + NH exchanged with D_2O), 7.91 (s, 1H, =CH); Anal. Calcd. for $C_{25}H_{17}N_3O_2S$ (423.50): C, 70.90; H, 4.05; N, 9.92. Found: C, 70.97; H, 4.38; N, 10.25.

*4.1.3.2. (2Z,5Z) 2-Cyano-2-[5-(4-methoxybenzylidene)-4-oxo-3-phenyl-thiazoldin-2-ylidene]-N-phenylacetamide (**4b**).* Canary yellow crystals, mp 314–315 °C, 31% yield; IR (cm^{-1}): ν 3390 (NH), 3055–3017 (aromatic CH), 2950 (aliphatic CH), 2195 (C≡N), 1712 (thiazolidinone C=O), 1651 (amide C=O), 1589 (bending NH), 1512 (C=C); ^{1}H NMR (*C*DCl$_3$): δ 3.91 (s, 3H, OCH_3), 7.02–7.85 (m, 15H, 14 aromatic H + NH exchanged with D_2O), 7.90 (s, 1H, =CH); ^{13}C NMR (DMSO-d_6): δ 55.6 (OCH_3), 78.6 (C≡N–*C*=C), 115.2 (*C*≡N), 118.1, 121.2, 126.0, 128.4, 129.3 (aromatic C), 129.6 (*C* of thiazolidinone attached to olefinic CH), 132.7 (aromatic *C* attached to olefinic CH), 133.6 (aromatic *C* attached to N of thiazolidinone), 135.0 (C=*C*H), 137.9 (aromatic *C* attached to NH), 161.2 (aromatic *C* attached to OCH_3), 162.1 (thiazolidinone *C*=O), 162.7 (amide *C*=O), 166.3 (C≡N–C=*C*); Anal. Calcd. for $C_{26}H_{19}N_3O_3S$ (453.52): C, 68.86; H, 4.22; N, 9.27. Found: C, 68.62; H, 4.48; N, 9.72.

*4.1.3.3. (2Z,5Z) 2-[5-(4-Chlorobenzylidene)-4-oxo-3-phenyl-thiazoldin-2-ylidene]-2-cyano-N-phenylacetamide **(4c)**.* Orange crystals, mp > 350 °C (dec.), 32% yield; IR (cm^{-1}): ν 3395 (NH), 3050–3020 (aromatic CH), 2200 (C≡N), 1712 (thiazolidinone C=O), 1655 (amide C=O), 1582 (bending NH), 1519 (C=C); ^{1}H NMR (*C*DCl$_3$): δ 7.18–7.91 (m, 15H, 14 aromatic H + NH exchanged with D_2O), 7.91 (s, 1H, =CH); MS (*m*/*z*, %):457 (M^+, 23), 458 [(M^+ + 1), 23], 459 [(M^+ + 2), 12], 336 (54), 77 (100); Anal. Calcd. for $C_{25}H_{16}ClN_3O_2S$ (457.94): C, 65.57; H, 3.52; N, 9.18. Found: C, 65.39; H, 3.76; N, 9.55.

*4.1.3.4. (2Z,5Z) 2-Cyano-2-[5-(2-hydroxybenzylidene)-4-oxo-3-phenyl-thiazoldin-2-ylidene]-N-phenylacetamide **(4d)**.* Orange crystals, mp 281–283 °C (dec.), 30% yield; IR (cm^{-1}): ν 3402 (NH), 3225 (OH), 3059–3043 (aromatic CH), 2195 (C≡N), 1713 (thiazolidinone C=O), 1655 (amide C=O), 1597 (bending NH), 1520 (C=C); ^{1}H NMR (DMSO-

d_6): δ 6.99–7.58 (m, 14H, 14 aromatic H), 8.04 (s, 1H, =CH), 9.56, 10.61 (s, 2H, NH + OH exchanged with D_2O); MS (*m*/*z*, %):439 (M^+, 5), 438 [(M^+ − 1), 21], 347 (18), 77 (100); Anal. Calcd. for $C_{25}H_{17}N_3O_3S$. H_2O (457.51): C, 65.64; H, 4.19; N, 9.18. Found: C, 65.31; H, 4.64; N, 9.52.

4.1.3.5. (2Z,5Z) 2-(5-Benzylidene-4-oxo-3-phenyl-thiazolidin-2-ylidene)-2-cyano-N-(4-methylphenyl)acetamide ***(4e)****.* Canary yellow crystals, mp 342–343 °C (dec.), 33% yield; IR (cm^{-1}): ν 3410 (NH), 3050–3020 (aromatic CH), 2924 (aliphatic CH), 2191 (C≡N), 1709 (thiazolidinone C=O), 1655 (amide C=O), 1597 (bending NH), 1508 (C=C); ^1H NMR ($CDCl_3$): δ 2.34 (s, 3H, CH_3), 7.15–7.72 (m, 14H, 14 aromatic H), 7.81 (s, 1H, NH exchanged with D_2O), 7.88 (s, 1H, =CH); ^{13}C NMR (APT) (DMSO-d_6): δ 20.2 (CH_3), 78.8 (C≡N–*C*=C), 112.7 (*C*≡N), 121.3, 128.7, 129.1, 129.2, 129.3, 130.2, 130.3 (aromatic C), 121.2 (*C* of thiazolidinone attached to olefinic CH), 133.0 (C=*C*H), 133.2 (aromatic *C* attached to N of thiazolidinone), 133.3 (aromatic *C* attached to NH), 134.8 (aromatic *C* attached to CH_3),135.1 (aromatic *C* attached to olefinic CH), 161.4 (thiazolidinone *C*=O), 162.1 (amide *C*=O), 166.3 (C≡N–C=*C*); MS (*m*/*z*, %):437 (M^+, 11), 438 [(M^+ + 1), 8], 331 (39), 77 (100); Anal. Calcd. for $C_{26}H_{19}N_3O_2S$ (437.52): C, 71.38; H, 4.38; N, 9.60. Found: C, 71.26; H, 4.19; N, 10.00.

4.1.3.6. (2Z,5Z) 2-Cyano-2-[5-(4-methoxybenzylidene)-4-oxo-3-phenyl-thiazolidin-2-ylidene]-N-(4-methylphenyl)acetamide ***(4f)****.* Canary yellow crystals, mp 325–326 °C (dec.), 53% yield; IR (cm^{-1}): ν 3400 (NH), 3050–3020 (aromatic CH), 2924 (aliphatic CH), 2200 (C≡N), 1713 (thiazolidinone C=O), 1643 (amide C=O), 1585 (bending NH), 1504 (C=C); ^1H NMR ($CDCl_3$): δ 2.34 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 7.02–7.69 (m, 14H, 13 aromatic H + NH exchanged with D_2O), 7.84 (s, 1H, =CH); MS (*m*/*z*, %):467 (M^+, 44), 468 [(M^+ + 1), 14], 361 (93), 77 (100); Anal. Calcd. for $C_{27}H_{21}N_3O_3S$ (467.55): C, 69.36; H, 4.53; N, 8.99. Found: C, 69.08; H, 4.38; N, 8.86.

4.1.3.7. (2Z,5Z) 2-[5-(4-Chlorobenzylidene)-4-oxo-3-phenyl-thiazolidin-2-ylidene]-2-cyano-N-(4-methylphenyl)acetamide ***(4g)****.* Orange crystals, mp 330–331 °C (dec.), 37% yield; IR (cm^{-1}): ν 3395 (NH), 3059–3028 (aromatic CH), 2920 (aliphatic CH), 2195 (C≡N), 1713 (thiazolidinone C=O), 1659 (amide C=O), 1585 (bending NH), 1508 (C=C); ^1H NMR ($CDCl_3$): δ 2.34 (s, 3H, CH_3), 7.15–7.81 (m, 14H, 13 aromatic H + NH exchanged with D_2O), 7.86 (s, 1H, =CH); Anal. Calcd. for $C_{26}H_{18}ClN_3O_2S$ (471.97): C, 66.17; H, 3.84; N, 8.90. Found: C, 66.28; H, 3.94; N, 8.65.

4.1.3.8. (2Z,5Z) 2-Cyano-2-[5-(4-hydroxybenzylidene)-4-oxo-3-phenyl-thiazolidin-2-ylidene]-N-(4-methylphenyl)acetamide ***(4h)****.* Orange crystals, mp 276–278 °C (dec.), 28% yield; IR (cm^{-1}): ν 3400 (NH), 3230 (OH), 3059–3030 (aromatic CH), 2920 (aliphatic CH), 2200 (C≡N), 1690 (thiazolidinone C=O), 1655 (amide C=O), 1589 (bending NH), 1512 (C=C); ^1H NMR (DMSO-d_6): δ 2.26 (s, 3H, CH_3), 6.99–7.56 (m, 13H, 13 aromatic H), 8.03 (s, 1H, =CH), 9.46, 10.61 (s, 2H, OH + NH exchanged with D_2O); ^{13}C NMR (APT) (DMSO-d_6): δ 20.3 (CH_3), 78.7 (C≡N–*C*=C), 113.0 (*C*≡N), 116.2, 119.7, 120.5, 120.6, 121.2, 128.7, 128.8, 129.2, 129.4, 130.4 (aromatic C), 119.5 (aromatic *C* attached to olefinic CH), 120.3 (*C* of thiazolidinone attached to olefinic CH), 132.4 (C=*C*H), 133.2 (aromatic *C* attached to N of thiazolidinone), 135.0 (aromatic *C* attached to NH), 135.3 (aromatic *C* attached to CH_3), 157.3 (aromatic *C* attached to OH), 161.9 (thiazolidinone *C*=O), 162.3 (amide *C*=O), 166.1 (C≡N–C=*C*); Anal. Calcd. for $C_{26}H_{19}N_3O_3S$ (453.52): C, 68.86; H, 4.22; N, 9.27. Found: C, 68.56; H, 4.33; N, 9.37.

4.1.3.9. (2Z,5Z) 2-(5-Benzylidene-4-oxo-3-phenyl-thiazolidin-2-ylidene)-N-(4-chlorophenyl)-2-cyanoacetamide ***(4i)****.* Yellow crystals, mp 347–348 °C (dec.), 27% yield; IR (cm^{-1}): ν 3391 (NH), 3059–3030 (aromatic CH), 2195 (C≡N), 1713 (thiazolidinone C=O), 1659 (amide C=O), 1597 (bending NH), 1512 (C=C); ^1H NMR ($CDCl_3$): δ 7.27–7.72 (m, 15H, 14 aromatic H + NH exchanged with D_2O), 7.90 (s, 1H, =CH); MS (*m*/*z*, %): 457 (M^+,15), 458 [(M^+ + 1), 6], 459 [(M^+ + 2), 6], 460 [(M^+ + 3), 2], 331 (72), 77 (100); Anal. Calcd. for $C_{25}H_{16}ClN_3O_2S$ (457.94): C, 65.57; H, 3.52; N, 9.18. Found: C, 65.47; H, 3.82; N, 9.50.

4.1.3.10. (2Z,5Z) N-(4-Chlorophenyl)-2-cyano-2-[5-(4-methoxybenzylidene)-4-oxo-3-phenyl-thiazolidin-2-ylidene]acetamide ***(4j)****.* Canary yellow crystals, mp 310–311 °C (dec.), 29% yield; IR (cm^{-1}): ν 3398 (NH), 3059–3030 (aromatic CH), 2938 (aliphatic CH), 2194 (C≡N), 1713 (thiazolidinone C=O), 1659 (amide C=O), 1589 (bending NH), 1512 (C=C); ^1H NMR ($CDCl_3$): δ 3.91 (s, 3H, OCH_3), 7.02–7.85 (m, 14H, 13 aromatic H + NH exchanged with D_2O), 7.88 (s, 1H, =CH); Anal. Calcd. for $C_{26}H_{18}ClN_3O_3S$ (487.97): C, 64.00; H, 3.72; N, 8.61. Found: C, 63.87; H, 4.01; N, 8.95.

4.1.3.11. (2Z,5Z) 2-[5-(4-Chlorobenzylidene)-4-oxo-3-phenyl-thiazolidin-2-ylidene]-N-(4-chlorophenyl)-2-cyanoacetamide ***(4k)****.* Yellow crystals, mp 339–341 °C (dec.), 24% yield; IR (cm^{-1}): ν 3418 (NH), 3050–3024 (aromatic CH), 2191 (C≡N), 1713 (thiazolidinone C=O), 1670 (amide C=O), 1597 (bending NH), 1520 (C=C); ^1H NMR ($CDCl_3$): δ 7.27–7.68 (m, 13H, 13 aromatic H), 7.83 (s, 1H, =CH), 7.90 (s, 1H, NH exchanged with D_2O); MS (*m*/*z*, %): 492 (M^+, 18), 493 [(M^+ + 1), 4], 494 [(M^+ + 2), 4], 365 (50), 367 (13), 77 (100); Anal. Calcd. for $C_{25}H_{15}Cl_2N_3O_2S$ (492.39): C, 60.98; H, 3.07; N, 8.53. Found: C, 60.87; H, 3.37; N, 8.88.

4.1.3.12. (2Z,5Z) N-(4-Chlorophenyl)-2-cyano-2-[5-(2-hydroxybenzylidene)-4-oxo-3-phenyl-thiazolidin-2-ylidene]acetamide ***(4l)****.* Orange crystals, mp 269–271 °C (dec.), 30% yield; IR (cm^{-1}): ν 3390 (NH), 3232 (OH), 3059 (aromatic CH), 2199 (C≡N), 1690 (thiazolidinone C=O), 1659 (amide C=O), 1589 (bending NH), 1508 (C=C); ^1H NMR (DMSO-d_6): δ 6.98–7.59 (m, 13H, 13 aromatic H), 8.04 (s, 1H, =CH), 9.70, 10.63 (s, 2H, NH + OH exchanged with D_2O); Anal. Calcd. for $C_{25}H_{16}ClN_3O_3S$ (473.94): C, 63.36; H, 3.40; N, 8.87. Found: C, 63.32; H, 3.61; N, 9.07.

4.1.4. General procedure for the preparation of compounds ***5a–e***

To a cold solution of appropriate aniline (1 mmol) in dilute hydrochloric acid (1 ml) was added dropwise a solution of sodium nitrite (0.07 g in 2 ml water, 1 mmol). This mixture was added to a suspension of the 4-thiazolidinone derivatives **3a**,**b** (1 mmol) in DMF (5 ml) and was rendered alkaline with a solution of sodium acetate (0.24 g, 3 mmol) in water (5 ml). The reaction was stirred at room temperature overnight and the separated residue was filtered, washed with water and crystallized from ethanol to obtain the hydrazono derivatives in pure form.

4.1.4.1. (2Z,5Z) 2-Cyano-2-[4-oxo-3-phenyl-5-(phenylhydrazono)-thiazolidin-2-ylidene]-N-phenylacetamide ***(5a)****.* Orange crystals, mp 181–183 °C (dec.), 30% yield; IR (cm^{-1}): ν 3402 (NH), 3055–3012 (aromatic CH), 2191 (C≡N), 1701 (thiazolidinone C=O), 1651 (amide C=O), 1601 (bending NH), 1532 (C=N), 1508 (C=C); ^1H NMR (DMSO-d_6): δ 7.10–7.59 (m, 15H, 15 aromatic H), 9.65 (s, 1H, NH exchanged with D_2O), 11.22 (s, 1H, NH exchanged with D_2O); Anal. Calcd. for $C_{24}H_{17}N_5O_2S$ (439.49): C, 65.59; H, 3.90; N, 15.94. Found: C, 65.29; H, 4.11; N, 15.81.

4.1.4.2. (2Z,5Z) 2-Cyano-2-[5-(4-methoxyphenylhydrazono)-4-oxo-3-phenyl-thiazolidin-2-ylidene]-N-phenylacetamide ***(5b)****.* Red crystals, mp 167–170 °C (dec.), 39% yield; IR (cm^{-1}): ν 3406 (NH), 3050–3010 (aromatic CH), 2935 (aliphatic CH), 2199 (C≡N), 1736 (thiazolidinone C=O), 1655 (amide C=O), 1601 (bending NH), 1543 (C=N), 1501 (C=C); ^1H NMR ($CDCl_3$): δ 3.74 (s, 3H, OCH_3),

6.82–7.80 (m, 15H, 14 aromatic H + NH), 8.05 (s, 1H, NH exchanged with D_2O); ^{13}C NMR (DMSO-d_6): δ 55.9 (OCH_3), 88.2 (C≡N–*C*═C), 115.4 (*C*≡N), 115.9, 121.3, 124.0, 126.0, 129.3, 130.0, 131.3 (aromatic C), 131.7 (aromatic *C* attached to N of thiazolidinone), 133.7 (aromatic *C* attached to hydrazono NH), 135.5 (aromatic *C* attached to NH), 149.3 (aromatic *C* attached to OCH_3), 154.3 (*C* of thiazolidinone attached to hydazono N), 155.7 (thiazolidinone *C*═O), 162.3 (C≡N–C═*C*), 163.6 (amide *C*═O); Anal. Calcd. for $C_{25}H_{19}N_5O_3S$ (469.52): C, 63.95; H, 4.08; N, 14.92. Found: C, 63.65; H, 4.38; N, 14.61.

4.1.4.3. (2Z,5Z) 2-Cyano-N-(4-methylphenyl)-2-[4-oxo-3-phenyl-5-(phenylhydrazono)-thiazolidin-2-ylidene]acetamide ***(5c).*** Orange crystals, mp 177–179 °C (dec.), 40% yield; IR (cm^{-1}): ν 3406 (NH), 3055–3013 (aromatic CH), 2970 (aliphatic CH), 2187 (C≡N), 1705 (thiazolidinone C═O), 1655 (amide C═O), 1601 (bending NH), 1545 (C═N), 1501 (C═C); ^1H NMR ($CDCl_3$): δ 2.31 (s, 3H, CH_3), 7.08–8.20 (m, 16H, 14 aromatic H + 2NH exchanged with D_2O); Anal. Calcd. for $C_{25}H_{19}N_5O_2S$ (453.52): C, 66.21; H, 4.22; N, 15.44. Found: C, 65.98; H, 4.32; N, 15.18.

*4.1.4.4. (2Z,5Z) 2-Cyano-2-[5-(4-methoxyphenylhydrazono)-4-oxo-3-phenyl-thiazolidin-2-ylidene]-N-(4-methylphenyl)acetamide (****5d****).* Red crystals, mp 162–164 °C (dec.), 47% yield; IR (cm^{-1}): ν 3406 (NH), 3055–3009 (aromatic CH), 2924 (aliphatic CH), 2199 (C≡N), 1721 (thiazolidinone C═O), 1651 (amide C═O), 1601 (bending NH), 1545 (C═N), 1504 (C═C); ^1H NMR (DMSO-d_6): δ 2.18 (s, 3H, CH_3), 3.94 (s, 3H, OCH_3), 6.88–7.55 (m, 15H, 13 aromatic H + 2NH exchanged with D_2O); ^{13}C NMR (DMSO-d_6): δ 18.5 (CH_3), 55.8 (OCH_3), 88.5 (C≡N–C═C), 114.2 (*C*≡N), 120.7, 122.4, 124.2, 128.4, 129.2 (aromatic C), 129.6 (aromatic *C* attached to N of thiazolidinone), 130.6 (aromatic *C* attached to NH), 135.3 (aromatic *C* attached to CH_3), 138.1 (aromatic *C* attached to hydrazono NH), 149.1 (aromatic *C* attached to OCH_3), 149.4 (*C* of thiazolidinone attached to hydazono N), 159.9 (thiazolidinone *C*═O), 161.8 (C≡N–C═*C*), 163.4 (amide *C*═O); Anal. Calcd. for $C_{26}H_{21}N_5O_3S$ (483.54): C, 64.58; H, 4.38; N, 14.48. Found: C, 64.39; H, 4.83; N, 14.30.

*4.1.4.5. (2Z,5Z) 2-[5-(4-Chlorophenylhydrazono)-4-oxo-3-phenyl-thiazolidin-2-ylidene]-2-cyano-N-(4-methylphenyl)acetamide (****5e****).* Orange crystals, mp 167–169 °C (dec.), 40% yield; IR (cm^{-1}): ν 3402 (NH), 3059–3028 (aromatic CH), 2920 (aliphatic CH), 2199 (C≡N), 1736 (thiazolidinone C═O), 1655 (amide C═O), 1601 (bending NH), 1550 (C═N), 1508 (C═C); ^1H NMR (DMSO-d_6): δ 2.25 (s, 3H, CH_3), 7.10–7.56 (m, 13H, 13 aromatic H), 9.59 (s, 1H, NH exchanged with D_2O), 11.27 (s, 1H, NH exchanged with D_2O); Anal. Calcd. for $C_{25}H_{18}ClN_5O_2S$ (487.96): C, 61.54; H, 3.72; N, 14.35. Found: C, 61.31; H, 4.02; N, 14.34.

4.2. Single crystal X-ray crystallographic data of **3a**

For X-ray crystallographic studies, compound **3a** was recrystallized as prismatic yellow crystals from ethanol. The crystallographic data were collected at $T = 298$ K on a Kappa CCD Enraf Nonius FR 590 diffractometer using a graphite monochromator with Mo-K_α radiation ($\lambda = 0.71073$ Å). The crystal structures were determined by SIR92 [55] and refined by maXus [56] (Bruker Nonius, Delft and MacScience, Japan). Chemical formula $C_{18}H_{13}N_3O_2S$, $M_r = 335.385$, monoclinic, crystallizes in space group $P2_1/c$, Cell lengths "$a = 10.4778$ (3), $b = 16.1711$ (5), $c = 10.9619$ (7) Å", Cell angles "$\alpha = 90.00$, $\beta = 12.$ (18) $\times 10^{1^\circ}$, $\gamma = 90.00^\circ$", $V = 1617.86$ (13) Å^3, $Z = 4$, $D_c = 1.377$ mg/m^3, θ values 2.910–25.028°, absorption coefficient μ (Mo-K_α) = 0.22 mm^{-1}, $F(000) = 696$. The unique reflections measured 3142 of which 1685 reflections with threshold expression $I > 3\sigma(I)$ were used in the structural analysis. Convergence for 217 variable parameters by least-squares refinement on F^2 with $w = 1/[\sigma^2(F_0^2) + 0.10000\,F_0^2]$. The final agreement factors were $R = 0.038$ and $wR = 0.065$ with a goodness-of-fit of 1.436.

4.3. Antitumor activity

The potential cytotoxicity of the tested compounds was evaluated using the method of Skehan et al. [47]. Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the prepared compounds to allow the attachment of cells to the wall of the plate. The tested compounds were dissolved in dimethylsulfoxide (DMSO) and diluted 1000-fold in the assay. Concentrations 0, 5, 12.5, 25, and 50 μg/ml of the tested compounds were added to the cell monolayer. The monolayer cells were incubated with the compounds for 48 h at 37 °C, in atmosphere of 5% CO_2. After 48 h, the cells were fixed, washed and stained with Sulfo-Rhodamine-B stain (SRB). Excess stain was washed with acetic acid. The attached stain was recovered with Tris EDTA buffer. Cell survival and drug activity were determined by measuring color intensity using an ELISA reader. Data are representative of the individual experiment, performed in three replicates for each individual dose and measured by SRB assay. Control values did not exhibit significant changes compared to the DMSO vehicle. The IC_{50} was determined by using a program Graph-Pad PRISM version 5. Mean and standard error were determined by SPSS 11 software.

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Appendix. Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.ejmech.2011.11.006.

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