Facile Synthesis and Quantitative Structure–Activity Relationship Study of Antitumor Active 2-(4-Oxo-thiazolidin-2-ylidene)-3-oxo-propionitriles

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2-(5-Arylidene-4-oxo-3-phenyl-thiazolidin-2-ylidene)-3-oxo-propionitriles 4a–j were prepared via condensation of aromatic aldehydes with 4-thiazolidinones 3a, b. The latter was obtained via electrophilic attack of phenylisothiocyanate on 3-oxo-propionitriles 1a, b followed by reaction with chloroacetyl chloride under basic condition. Additionally, 2-(5-heteroalicyclic methylene) analogues 5a–h were prepared via Mannich reaction of the appropriate secondary amines and formaldehyde with 4-thiazolidinones 3a, b. Many of the synthesized compounds exhibited promising antitumor properties against colon HCT116 and breast T47D cell lines. 3D-Pharmacophore modeling and quantitative structure–activity relationship (QSAR) analysis were combined to explain the observed antitumor properties.

Key words thiazolidinone; quantitative structure–activity relationship; antitumor activity; 3-oxo-propionitrile; HCT116; T47D

Cancer ranks second in diseases leading to mortality, following only cardiovascular diseases. One-quarter of all deaths in the United States are caused by cancer.1) Out of the many cancer diseases, breast cancer is the most prevalent cancer in women and represents the second highest leading cause of cancer death in this population after lung cancer.2) Colorectal cancer is the third most common cancer in both men and women, 91% of cases are diagnosed in individuals 50 years of age and older.3) Chemotherapy is widely used to treat and control cell growth and limit the spread of cancer cells to other sites. Although, there is a success with certain forms of cancer, drug therapy has only limited impact against the three major killers: carcinoma of lung, breast and colorectal system. Therefore, there is a need to develop novel antitumor agents to treat and combat this disease.

Several promising antitumor agents containing thiazolidine and thiazolidinone scaffolds have been identified to have a broad range of anticancer activities.3–14) Previously, we reported promising antitumor properties of a variety of 5-arylidene thiazolidinone derivatives 1a–c (Fig. 1) exhibiting considerable cytotoxic activity against colon HCT116 cancer cell lines compared with Doxorubicin (reference standard, IC50=0.00686 mM).15) In continuation of these previous findings and in order to optimize novel antitumor active agents possessing the same thiazolidinone core, it is intended in the present work to perform some modifications in the adopted structures via inserting heteroalicyclic amines (morpholinyl or piperidinyl functions, 4a–j) instead of the aromatic amines 1a–c due to their hydrophilic properties. Moreover, a series of heteroalicyclic methylene containing compounds 5a–h will be also prepared replacing the arylidene moiety of 1a–c (Fig. 1). Additionally, many morpholine and piperidine containing compounds were known as anticancer active agents that exerted their actions via inhibition of different targets such as phosphatidylinositol 3-kinases (PI3Ks) and histone deacetylase (HDAC).16–20) Moreover, the piperidine derivatives IIa–e (Fig. 2) were reported through antitumor activity data obtained by US-NCI to be active against different cell lines exemplified by T47D (breast cancer, GI50=0.00089 mM), HL-60 (TB) (leukemia, GI50=0.00085 mM), SNB-75 (CNS cancer, GI50=0.00185 mM), OVCAR-5 (ovarian cancer, GI50=0.00055 mM), EKVX (non-small lung cell cancer, GI50=0.00202 mM) and PC-3 (prostate cancer, GI50=0.00087 mM).21)

Quantitative structure–activity relationship (QSAR) will be also taken into consideration during the present work in order to study the pharmacophoric features of the antitumor active compounds and to determine the parameters controlling the pharmacological properties.

The authors declare no conflict of interest.

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Fig. 1. Reported (1a–c) and Proposed (4a–j, 5a–h) Thiazolidinone Derivatives
Results and Discussion

Chemistry The target 2-(5-arylidene-4-oxo-3-phenyl-thiazolidin-2-ylidene)-3-oxo-propionitriles 4a–j were prepared as depicted in Charts 1 and 2. The starting 3-(morpholin-4-yl)-1a and 3-(piperidin-1-yl)-3-oxo-propionitriles 1b were synthesized according to the previously reported procedures.22 Electrophilic attack of phenylisothiocyanate on the active methylene group of the expected arylidene derivatives 2a,b via condensation reaction of different aromatic aldehydes (benzaldehyde, salicylaldehyde or vanillin) with the appropriate aromatic aldehydes (benzaldehyde, salicylaldehyde or vanillin) afforded 3-oxo-2-(4-oxo-3-phenyl-[1,3]-thiazinan-2-ylidene)-3-oxo-propionitriles 6a,b (Chart 3). Single X-ray crystallography of 6a (Fig. 3) allowed good confirmation for the assigned structure confirming that the isolated product is E-isomer.

The target arylidenes 4a–j were obtained by condensation of the appropriate aromatic aldehydes with 3a,b in dimethylformamide (DMF) in the presence of TEA. The structures of 4a–j were established through different spectroscopic techniques (IR, 1H-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 1H-NMR spectra added a good confirmation for the assigned structures 4a–j. The appearance of the ylidene proton of compounds 4a–c, 4f–h at δ=7.78–7.86 confirmed the formation of Z-isomers.9,15,23–28 On the other hand, the ylidene proton of compounds 4d and 4i were revealed at δ=8.00–8.16, slightly downfield shifted than the other synthesized analogues, which could be attributed to the anisotropic effect of the hydroxyl group oriented at the $\alpha$-position of the arylidine function. Furthermore, reaction of 4-thiazolidinones 3a,b with formaldehyde and the appropriate heteroalicyclic amines (pyrrolidine, piperidine, morpholine and N-methylpiperazine) through Mannich reaction yielded 5a–h. The structures of the obtained products 5a–h were confirmed by 1H-NMR that revealed the expected heteroalicyclic protons signals in addition to the other skeleton protons (cf. Experimental).

Furthermore, reaction of the 3-mercapto-3-phenylamino-acrylonitriles 2a,b with 3-chloropropionyl chloride in THF in the presence of TEA afforded 3-oxo-2-(4-oxo-3-phenyl-[1,3]-thiazinan-2-ylidene)propionitriles 6a,b (Chart 3). Single X-ray crystallography of 6a (Fig. 3) allowed good confirmation for the assigned structure confirming that the isolated product is E-isomer.

Many trials had been performed toward preparation of 2-(5-arylidene-4-oxo-3-phenyl-[1,3]thiazinan-2-ylidene)-3-oxo-propionitriles 7 via condensation reaction of different aromatic aldehydes (benzaldehyde, p-anisaldehyde, p-chlorobenzaldehyde, salicylaldehyde or vanillin) with 6a,b under different reaction conditions. When this reaction was conducted in DMF/TEA, absolute ethanol/piperidine or absolute ethanol/potassium hydroxide at room temperature, or either warming the reaction in DMF/TEA at 60°C, no reaction was occurred. However, upon conducting the reaction at reflux temperature in absolute ethanol/piperidine or absolute ethanol/potassium hydroxide, the hydrolysed products 2a,b were isolated instead of the expected arylidine derivatives 7 (where the structures

![Chart 1. Synthetic Pathway for Preparation of Compounds 4a–j and 5a–h](image1.png)

![Chart 2. Synthetic Pathway for Preparation of Compounds 4a–j](image2.png)
were established by comparative IR and melting point data. These results could be attributed to the lower activity of the methylene group neighbouring to the cyclic ketonic function of the six membered ring system than the corresponding five membered thiazolidinone system.

**Antitumor Activity** *In-vitro* antitumor activity of the tested compounds was screened utilizing Sulfo-Rodamine B (SRB) standard method\(^{15,29-35}\) in the National Cancer Institute, Cairo University, Egypt. All the prepared target compounds (4a–j, 5a–h) were tested for their antitumor properties against HCT116 “colon” and T47D “breast” cancer cell lines. From the observed antitumor activity data (Table 1), (see also Figs. 1, 2 of the Supplementary data), it has been noticed that the tested compounds showed moderate to potent antitumor activity having IC\(_{50}\) values ranging from 0.00586 to 0.04848mM against HCT116 cancer cell lines and from 0.00646 to 0.05059mM against T47D cell lines. In most cases, the 5-arylidene derivatives 4a–j exerted promising antitumor activity against both cell lines than the 5-heteroalicyclic methyl analogues 5a–h.

Considering the observed antitumor screening data of the synthesized compounds against HCT116 “colon” cancer cell line, the aryldiene piperidine derivatives 4f–i exerted higher potency than the morpholine analogues 4a–d. Compound 4g was the most effective one with IC\(_{50}\) = 0.00586mM compared with Doxorubicin (IC\(_{50}\) = 0.00686mM). This derivative was selected by US-NCI for more antitumor activity screening.

### Table 1. IC\(_{50}\) of the Tested Compounds against Human Tumor Cell Lines

<table>
<thead>
<tr>
<th>Compound</th>
<th>X</th>
<th>R</th>
<th>Tested human tumor cell lines, IC(_{50}) µg/mL (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Colon (HCT 116)</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>—</td>
<td>—</td>
<td>3.73 (0.00686)</td>
</tr>
<tr>
<td>4a</td>
<td>O</td>
<td>H</td>
<td>3.64 (0.00872)</td>
</tr>
<tr>
<td>4b</td>
<td>O</td>
<td>4-OCH(_3)</td>
<td>3.64 (0.00813)</td>
</tr>
<tr>
<td>4c</td>
<td>O</td>
<td>4-Cl</td>
<td>3.48 (0.00777)</td>
</tr>
<tr>
<td>4d</td>
<td>O</td>
<td>2-OH</td>
<td>10.91 (0.02517)</td>
</tr>
<tr>
<td>4e</td>
<td>O</td>
<td>3-OCH(_2), 4-OH</td>
<td>12.17 (0.02626)</td>
</tr>
<tr>
<td>4f</td>
<td>CH(_2)</td>
<td>H</td>
<td>2.92 (0.00703)</td>
</tr>
<tr>
<td>4g</td>
<td>CH(_2)</td>
<td>4-OCH(_3)</td>
<td>2.61 (0.00586)</td>
</tr>
<tr>
<td>4h</td>
<td>CH(_2)</td>
<td>4-Cl</td>
<td>2.92 (0.00649)</td>
</tr>
<tr>
<td>4i</td>
<td>CH(_2)</td>
<td>2-OH</td>
<td>3.64 (0.00844)</td>
</tr>
<tr>
<td>4j</td>
<td>CH(_2)</td>
<td>3-OCH(_2), 4-OH</td>
<td>15.00 (0.0325)</td>
</tr>
<tr>
<td>5a</td>
<td>O</td>
<td>—</td>
<td>20.00 (0.04848)</td>
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<tr>
<td>5b</td>
<td>O</td>
<td>—</td>
<td>13.64 (0.03198)</td>
</tr>
<tr>
<td>5c</td>
<td>O</td>
<td>—</td>
<td>6.67 (0.01557)</td>
</tr>
<tr>
<td>5d</td>
<td>O</td>
<td>—</td>
<td>6.96 (0.01576)</td>
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<tr>
<td>5e</td>
<td>CH(_2)</td>
<td>—</td>
<td>11.67 (0.02843)</td>
</tr>
<tr>
<td>5f</td>
<td>CH(_2)</td>
<td>—</td>
<td>15.91 (0.03747)</td>
</tr>
<tr>
<td>5g</td>
<td>CH(_2)</td>
<td>—</td>
<td>14.09 (0.03303)</td>
</tr>
<tr>
<td>5h</td>
<td>CH(_2)</td>
<td>—</td>
<td>13.18 (0.02998)</td>
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utilizing 56 cell lines belonging to nine types of cancers (leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer and breast cancer). The observed data expressed as GI_{50} (the concentration resulting in a 50% growth inhibition of the tumor compared with the control experiments), TGI (the concentration resulting in a 100% growth inhibition of the tumor compared with the control experiments) and LC_{50} were presented in Table 2. It showed promising activity against 38 cell lines especially, HOP-92, NCI-H226 (non-small cell lung cancer), GI_{50} = 0.000865, 0.00127 mm, respectively, SF-539, SNB-75 (CNS cancer), GI_{50} = 0.00144, 0.000811 mm, respectively, MALME-3M, SK-MEL-5 (melanoma), GI_{50} = 0.0006, 0.0015 mm, respectively, OVCAR-4, SK-OV-3 (ovarian cancer), GI_{50} = 0.00102, 0.00115 mm, respectively, 786-0, A498, RXF 393, UO-31 (renal cancer), GI_{50} = 0.00112, 0.00111, 0.00118, 0.00148 mm, respectively and MDA-MB-231/ATCC, HS 578T (breast cancer), GI_{50} = 0.00133, 0.00113 mm, respectively.

On the other hand, the antitumor activity of the tested compounds against T47D breast cell lines exhibiting that compound 4c was the most active with IC_{50} value = 0.00646 mm compared with Doxorubicin (IC_{50} = 0.01407 mm), the 5-aryl-morpholine derivatives 4c and 4d (IC_{50} = 0.00646, 0.00879 mm, respectively) were more potent than their piperidine analogues 4h and 4i (IC_{50} = 0.0074, 0.01877 mm, respectively) (Table 1). However, the piperidine containing compounds 4f and 4g exhibited higher antitumor activity (IC_{50} = 0.00688, 0.00714 mm, respectively) than the morpholine derivatives 4a and 4b (IC_{50} = 0.03375, 0.03631 mm, respectively). In addition, compound 5d showed good activity with IC_{50} value = 0.0132 mm.

**QSAR Study.** 3D-QSAR Pharmacophore Modeling This study was performed using Discovery Studio 2.5 software (Accelrys Inc., San Diego, CA, U.S.A.). A given

<table>
<thead>
<tr>
<th>Panel/cell lines</th>
<th>GI_{50}</th>
<th>TGI</th>
<th>LC_{50}</th>
</tr>
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<td>Leukemia</td>
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<td>HL-60(TB)</td>
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<td>&gt;0.05</td>
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<td>K-562</td>
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<td>&gt;0.05</td>
</tr>
<tr>
<td>RPMI-8226</td>
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<td>&gt;0.05</td>
<td>&gt;0.05</td>
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<tr>
<td>SR</td>
<td>0.0113</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Non-small cell lung cancer</td>
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<td></td>
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</tr>
<tr>
<td>A549/ATCC</td>
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<td>0.00938</td>
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<td>0.00286</td>
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<td>Colon cancer</td>
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<td>SW-620</td>
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<td>CNS cancer</td>
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<td>SF-268</td>
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<td>SF-295</td>
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</tr>
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<td>UACC-62</td>
<td>0.00211</td>
<td>&gt;0.05</td>
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</tr>
</tbody>
</table>

\textsuperscript{a} ND = not determined.
hypothesis could be combined with a known activity data to create a 3D-QSAR model that identifies overall aspects of molecular structure governing activity. 3D-QSAR based on pharmacophore was constructed using collections of molecules with activities ranging over a number of orders of magnitude. Pharmacophores explain the variability of bioactivity with respect to the geometric localization of the chemical features present in the molecules.15,35

Twenty nine compounds (4a–f, 4h–j, 5a, b, 5d–h and the previously prepared 1a–j and IIa–e15) were taken as a training set and the remaining two compounds, 4g (with potent antitumor activity) and 5e (with mild activity) were used as a test set. The observed HYPOGEN identifies a 3D array of a maximum of three chemical features common to the training set that provide relative alignment for each input molecule, consistent with its binding mode to a proposed common receptor site. The chemical features considered were: two hydrophobic sites (H1, H2) and one hydrogen bond acceptors (HBA)–(H1)–(H2)–(HBA vector) = 70.48° (Fig. 4A, B, Table 3 exhibit constraint distances and angles between features of the generated pharmacophore). Through the pharmacophore mapping study, it was found that the major structural factors affecting the potency of these compounds were related to their basic skeleton. The controlling features were two hydrophobic sites represented by the arylidene and morpholine or piperidine moieties together with a hydrogen bond acceptor of the amide carbonyl group for 4a–j. While compounds 5a–h had not been fitted well to the generated pharmacophore as they lacked the arylidene moiety, therefore, their fit values were smaller than those of 4a–j that explained their mild antitumor activity (Table 4).

**QSAR Modeling** Despite of the significance of pharmacophoric hypotheses for 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).15,35–38 Thus, a classical QSAR analysis was employed to search for the best combination of orthogonal pharmacophores using a fit value and other structural descriptors (connectivity, topological, etc.) capable of explaining bioactivity variation across a collected list of the descriptors, allowing different pharmacophoric models competing within the 3D-QSAR framework.

A set of 29 compounds (4a–f, 4h–j, 5a, b, 5d–h and the previously reported 1a–j and IIa–e15) was used as a training set for a QSAR modeling. The remaining 2 compounds (4g and 5e) were adopted as an external test subset for validating the QSAR model. Many molecular descriptors were calculated for each compound employing a calculated molecular properties module. The 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).15,35,39 Equation 1 shows our best-performing QSAR model (Fig. 5 exhibits the corresponding scatter plots of experimental versus estimated bioactivity values for the

![Figure 4](image-url)
of the model was validated by squared correlation coefficient ($R^2$) and residuals between the predicted and experimental activity of the training set (Table 5).

Potency (IC$_{50}$) against HCT116 (colon cancer) cell line ($N$ “number of molecules in the training set”=$29$, $R^2$ “squared correlation coefficient value”=$0.726$)

$$\text{IC}_{50} = 604.41 - 0.298 \times [\text{Molecular SurfaceArea}] - 398.59 \times [\text{Jurs_RNCG}] - 67.65 \times [\text{FitValue}]$$

(1)

Where the Jurs_RNCG is a relative negative charge descriptor that described the charge of most negative atom divided by total negative charge.

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

Table 4. Best Fit Values and Estimated Activities for 29 Compounds of the Training Set Mapped with the Generated 3D-Pharmacophore Model

<table>
<thead>
<tr>
<th>Compound</th>
<th>X</th>
<th>R</th>
<th>Observed activity</th>
<th>Estimated activity</th>
<th>Fit value</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>O</td>
<td>H</td>
<td>$8.72 \times 10^{-3}$</td>
<td>$18.04 \times 10^{-3}$</td>
<td>5.86</td>
</tr>
<tr>
<td>4b</td>
<td>O</td>
<td>4-OCH$_3$</td>
<td>$8.13 \times 10^{-3}$</td>
<td>$10.91 \times 10^{-3}$</td>
<td>5.92</td>
</tr>
<tr>
<td>4c</td>
<td>O</td>
<td>4-Cl</td>
<td>$7.7 \times 10^{-3}$</td>
<td>$10.80 \times 10^{-3}$</td>
<td>6.05</td>
</tr>
<tr>
<td>4d</td>
<td>O</td>
<td>2-OH</td>
<td>$25.17 \times 10^{-3}$</td>
<td>$11.65 \times 10^{-3}$</td>
<td>5.88</td>
</tr>
<tr>
<td>4e</td>
<td>O</td>
<td>3-OCH$_3$, 4-OH</td>
<td>$26.26 \times 10^{-3}$</td>
<td>$11.64 \times 10^{-3}$</td>
<td>5.93</td>
</tr>
<tr>
<td>4f</td>
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Fig. 5. Estimated Activity versus Observed Activity (IC$_{50}$) of the Tested Compounds against HCT116 (Colon) Human Tumor Cell Line
More precisely, the Kubinyi function (FIT)\(^{15,35}\) is a statistically sensitive to changes in small value the better the linear equation.\(^{15,35,40}\) It is given by the sensitivity to changes in small

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

\[ \text{FIT} = \frac{R(d)^2(N-d-1)}{(N+d^2)(1-R^2)} \]

Where, \(N\) is the number of molecules of the training set; resi, is the residual for molecule; \(i\), is the difference between the experimental property (\(p\)) and predicted property (\(p_{\text{pred}}\)). More precisely, the Kubinyi function (FIT)\(^{15,35}\) is a statistical parameter which is closely related to the Fisher ratio (\(F\)), but avoids the main disadvantage of the latter of being 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.\(^{15,35,40}\) It is given by the following equation, “where \(R(d)\) is the correlation coefficient for a model with (\(d\)) descriptors.” The observed FIT value is 2.91 corresponding to model due to HCT116 cancer cell lines.

**Conclusion**

3-Oxo-2-(4-oxo-3-phenyl-thiazolidin-2-ylidene)propionitriles \(3a, b\) were synthesized via reaction of 3-mercapto-3-oxo-3-phenylamino-acrylonitriles \(2a, b\) with chloroacetyl chloride in the presence of TEA. Reaction of \(3a, b\) with either aromatic aldehydes or heterocyclic secondary amines and formaldehyde afforded 2-(5-aryliden-4-oxo-3-phenyl-thiazolidin-2-ylidene)-2-cyano-3-oxo-propionitriles \(4a-j\) and 2-(5-heterocyclic methyl) analogues \(5a-h\), respectively.
Furthermore, (2E) 3-oxo-2-(4-oxo-3-phenyl-[1,3]thiazinan-2-ylidene)-propionitriles 6a, b were prepared stereoselectively via reaction of 2a, b with 3-chloropropionyl chloride in the presence of TEA. Many trials to obtain the arylidene derivatives 7 via condensation of different aromatic aldehydes with 6a, b were unsuccessful.

The synthesized 4a–j and 5a–h were tested for their anticancer activity against colon (HCT116) and breast (T47D) cancer cell lines. It was concluded that the 5-arylidene moiety is essential for the antitumor activity, and in most cases, the piperidine derivatives exhibited better activity than morpholine analogues. Compound 4g was the most active compound against colon HCT116 with IC₅₀ = 0.00586 mm compared with Doxorubicin (IC₅₀ = 0.00686 mm) and 4h had the highest activity against breast T47D with IC₅₀ = 0.00646 mm compared with Doxorubicin (IC₅₀ = 0.01407 mm). QSAR study confirmed the obtained antitumor results, where the three pharmacophoric features for HCT116 activity were two hydrophobic sites and a hydrogen bond acceptor. Classical QSAR studies of HCT116 (colun) cancer cell lines delivered Eq. 1 of three descriptors with R² = 0.726. The most important descriptor in the equation was the fit value derived from mapping of the synthesized analogues into the generated pharmacophore. The other controlling descriptors were the molecular surface area and JursRNCG.

External validation of the established QSAR models utilizing two of our synthesized analogues exhibiting promising (4g) and mild (5c) antitumor properties, revealed good agreement between the experimental and the calculated data. It can be concluded that combination of 3D-pharmacophore modeling and QSAR provides an effective technique for understanding the observed pharmacological properties and thus could be adopted for developing effective lead structures.

Experimental
Chemistry Melting points were measured with an Electrothermal Stuart SMP, 1 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, 13C: 75 MHz) using TMS as an internal standard and on JOEL (Eclipse) 400 (1H: 400, 13C: 100 MHz). Mass spectra were measured on a Shimadzu GCMS-QP2010 Plus spectrometer (EI, 70 eV). Elemental analyses were carried out at the Microanalytical center, Faculty of Science, Cairo University, Egypt, and at the Regional center for mycology and controlling descriptors were the molecular surface area and JursRNCG.

General Procedure for the Preparation of (2a,b) To an ice cold mixture of 1a,b (5 mmol) and fine 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,b in a pure form.

3-Mercapto-2-(morpholine-4-carbonyl)-3-phenylamino-acrylonitrile (2a): Yellow crystals, 61% yield, mp 122–124°C. 1H-NMR (CDCl₃, 300 MHz) δ: 3.45–3.82 (8H, m, morpholine protons), 5.37 (1H, s, NH exch. D₂O), 7.11–7.77 (5H, m, aromatic H), 11.08 (1H, s, SH exch. D₂O). IR (KBr) cm⁻¹: 3271 (NH), 3050–3010 (aromatic CH), 2967–2859 (aliphatic CH), 1727 (C≡N), 1650 (C=O), 1632 (bending NH), 1593–1535 (C= C). Anal. Calcd for C₁₁H₁₁N₂O₃S (238.36): C, 58.11; H, 5.23; N, 13.14. Found: C, 58.13; H, 4.69; N, 12.93.

3-Oxo-2-(4-oxo-3-phenyl-thiazolidin-2-ylidene)propionitrile (3a): White crystals, 80% yield, mp 188–190°C. 1H-NMR (CDCl₃, 300 MHz) δ: 3.56 (4H, t, J = 4.2 Hz, (CH₂)₂N morpholine protons), 3.67 (4H, t, J = 4.2 Hz, (CH₂)₂O morphine protons), 3.90 (2H, s, CH₂), 7.26–7.59 (5H, m, aromatic H). IR (KBr) cm⁻¹: 3059 (aromatic CH), 2974–2855 (aliphatic CH), 2199 (C=O), 1736 (C=O thiazolidinone), 1636 (C=O), 1515–1512 (C=C). Anal. Calcd for C₁₀H₁₀N₂O₃S (292.38): C, 58.35; H, 4.59; N, 12.76. Found: C, 58.13; H, 4.69; N, 12.93.

General Procedure for the Preparation of (4a–j) A solution of 3a,b (3 mmol) and TEA (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.

3-Mercapto-2-(4-oxo-3-phenyl-thiazolidin-2-ylidene)propionitrile (2b): White crystals, 80% yield, mp 188–190°C. 1H-NMR (CDCl₃, 300 MHz) δ: 3.32 (3H, s, NH exch. D₂O), 7.32–7.78 (5H, m, aromatic H). IR (KBr) cm⁻¹: 3059 (aromatic CH), 2974–2855 (aliphatic CH), 2199 (C=O), 1736 (C=O thiazolidinone), 1636 (C=O), 1515–1512 (C=C). Anal. Calcd for C₁₀H₁₀N₂O₃S (292.38): C, 58.35; H, 4.59; N, 12.76. Found: C, 58.13; H, 4.69; N, 12.93.
attached to olefinic CH), 161.7 (C=O of thiazolidinone), 162.2 (C=O), 166.3 (C=C–CN). IR (KBr) cm⁻¹: 3050–3028 (aromatic CH), 2967–2859 (aliphatic CH), 2191 (C≡N), 1717 (C=O thiazolidinone), 1647 (C=O), 1547–1493 (C=C). Anal. Calcd for C₂₃H₂₆N₆O₄S: (471.49): C, 66.17; H, 4.59; N, 10.06. Found: C, 66.33; H, 4.62; N, 10.32.

2-[5-(4-Methoxybenzylidene)-4-oxo-3-phenyl-thiazolidin-2-ylidene]-3-morpholin-4-yl-3-oxo-propionitrile (4b): Yellow crystals, 35% yield, mp 295–297°C (dec.). ¹³NMR (CDCl₃, 300 MHz): δ: 3.59–3.61 (4H, m, (CH₂)₂N morpholine protons), 3.69–3.71 (4H, m, (CH₂)₂O morpholine protons), 3.90 (3H, s, OCH₃), 7.02 (2H, d, J=8.7 Hz, protons o-OCH₂), 7.35–7.63 (7H, m, aromatic H), 7.81 (1H, s, =CH). ¹³C-NMR (DMSO-d₆, 100 MHz): δ: 45.3 [(CH₂)₂N of morpholine], 55.5 (OCH₃), 66.0 [(CH₂)₂O of morpholine], 69.7 (C=C–CN), 115.1 (C=N), 119.7, 120.0, 121.9, 122.5, 123.6, 124.7 (aromatic carbons), 126.9 (aromatic C attached to olefinic CH), 129.4 (C of thiazolidinone attached to olefinic CH), 132.4 (aromatic C attached to N of the 4-thiazolidinone ring), 134.9 (C=CH), 153.1 (aromatic C attached to OCH₃ group), 162.2 (C=O of thiazolidinone), 163.0 (C=O), 166.1 (C=C–CN). IR (KBr) cm⁻¹: 3065–3020 (aromatic CH), 2965–2865 (aliphatic CH), 2191 (C≡N), 1713 (C=O thiazolidinone), 1655 (C=O), 1589–1508 (C=C–CN). MS m/z: 448.35 (M⁺), 449.30 (M⁺+1), 450.30 (M⁺+2), 361, 334.20, 164.10, 77.10. Anal. Calcd for C₂₃H₂₆N₆O₄S (472.52): C, 64.41; H, 4.73; N, 9.39. Found: C, 64.59; H, 4.80; N, 9.56.

2-[5-(4-Chlorobenzylidene)-4-oxo-3-phenyl-thiazolidin-2-ylidene]-3-morpholin-4-yl-3-oxo-propionitrile (4e): Yellow crystals, 32% yield, mp 334–336°C (dec.). ¹³NMR (CDCl₃, 300 MHz): δ: 3.61 (4H, t, J=4.2 Hz, (CH₂)₂N morpholine protons), 3.71 (4H, t, J=4.2 Hz, (CH₂)₂O morpholine protons), 7.35–7.63 (9H, m, aromatic H), 7.79 (1H, s, =CH). IR (KBr) cm⁻¹: 3051 (aromatic CH), 2982–2855 (aliphatic CH), 2191 (C≡N), 1701 (C=O thiazolidinone), 1647 (C=O), 1593–1543 (C=C). MS m/z: 451.30 (M⁺), 452.30 (M⁺+1), 453.30 (M⁺+2), 365.20 (42.93), 367.20 (13.38), 168.05 (41.75), 170.05 (18.77), 77.10 (100). Anal. Calcd for C₂₃H₂₆ClN₆O₄S (451.94): C, 61.13; H, 4.01; N, 9.30. Found: C, 61.41; H, 4.13; N, 9.68.

2-[5-(2-Hydroxybenzylidene)-4-oxo-3-phenyl-thiazolidin-2-ylidene]-3-morpholin-4-yl-3-oxo-propionitrile (4d): Orange crystals, 30% yield, mp 248–250°C (dec.). ¹³NMR (DMSO-d₆, 300 MHz): δ: 3.48–3.57 (8H, m, morpholine protons), 6.99–7.55 (9H, m, aromatic H), 8.00 (1H, s, =CH), 10.62 (1H, s, OH exch. D₂O). ¹³C-NMR (DMSO-d₆, 75 MHz): δ: 46.8 [(CH₂)₂N of morpholine], 66.0 [(CH₂)₂O of morpholine], 78.0 (C=C–CN), 112.8 (CN), 116.2 (aromatic C attached to olefinic CH), 118.7, 119.8, 120.2, 120.9, 127.8, 128.9, 129.0 (aromatic carbons), 130.4 (C of thiazolidinone attached to olefinic CH), 132.4 (aromatic C attached to N of the 4-thiazolidinone ring), 134.8 (C=CH), 157.2 (aromatic C attached to OH), 159.8 (C=O of thiazolidinone), 162.2 (C=O), 166.1 (C=C–CN). IR (KBr) cm⁻¹: 3445 (OH), 3028 (aromatic CH), 2965–2865 (aliphatic CH), 2195 (C≡N), 1713 (C=O thiazolidinone), 1636 (C=O), 1585–1489 (C=C). MS m/z: 449.30 (M⁺), 450.30 (M⁺+1), 451.30 (M⁺+2), 338.20, 168.05, 77.10. Anal. Calcd for C₂₃H₂₆N₆O₄S (449.54): C, 67.40; H, 5.20; N, 9.43. Found: C, 67.30; H, 5.47; N, 9.81.

2-[5-(4-Hydroxy-3-methoxybenzylidene)-4-oxo-3-phenyl-thiazolidin-2-ylidene]-3-morpholin-4-yl-3-oxo-propionitrile (4e): Yellow crystals, 30% yield, mp 319–321°C (dec.). ¹³NMR (DMSO-d₆, 300 MHz): δ: 1.49–1.57 (6H, m, piperidine protons), 3.43 (4H, br s, (CH₂)₂N piperidine protons), 7.56–7.72 (9H, m, aromatic H), 7.79 (1H, s, =CH). ¹³C-NMR (DMSO-d₆, 75 MHz): δ: 24.0 (C₃C₅ of piperidine), 25.4 (C₄ of piperidine), 44.1 [C₂C₆ of piperidine], 69.7 (C=C–CN), 113.1 (CN), 118.7, 120.0, 121.9, 123.5, 125.1, 126.0, 127.2 (aromatic carbons), 129.5 (C of thiazolidinone attached to olefinic CH), 131.7 (aromatic C attached to N of the 4-thiazolidinone ring), 133.7 (C=CH), 134.4 (aromatic C attached to olefinic CH), 134.7 (aromatic C attached to C–H), 153.6 (C=O of thiazolidinone), 155.0 (C=O), 166.4 (C=C–CN). IR (KBr) cm⁻¹: 3059–3028 (aromatic CH), 2928–2855 (aliphatic CH), 2195 (C≡N), 1713 (C=O thiazolidinone), 1636 (C=O), 1585–1489 (C=C). MS m/z: 449.30 (M⁺), 450.30 (M⁺+1), 451.30 (M⁺+2), 338.20, 168.05, 77.10. Anal. Calcd for C₂₃H₂₆N₆O₄S (449.96): C, 64.06; H, 4.48; N, 9.34. Found: C, 64.19; H, 4.71; N, 9.62.
exch. D$_2$O). IR (KBr) cm$^{-1}$: 3350 (OH), 3051 (amorphous CH), 2932–2851 (aliphatic CH), 2191 (C=N), 1701 (C=O thiazo- lolidine), 1636 (C=O), 1597–1493 (C=C). Anal. Caled for C$_{23}$H$_{28}$N$_{4}$O$_2$S: 25.3 (C$_4$ of piperidine), 25.8 (C$_3$,C$_4$,C$_5$ of piperidine), 40.5 (3-Oxo-2-(4-oxo-3-phenyl-5-piperidin-1-ylmethyl-thiazolidine-2-yldiene)-3-piperidin-1-yl-propionitrile (5f)). Yellow crystals, 46% yield, mp 183–186°C (dec.). IR (KBr) cm$^{-1}$: 3067 (aromatic CH), 2967–2859 (aliphatic CH), 2203 (C≡C of piperazine), 2.70 (4H, br s, (CH$_2$)$_2$N piperazine), 3.00 (2H, br s, CH$_3$, 3.50–3.61 (9H, m, 4H(C$_2$H$_2$)N morpholine + 4H (CH$_3$)$_2$O morpholine+CH of thiazolidine), 7.38–7.72 (5H, aromatic protons). IR (KBr) cm$^{-1}$: 3060 (aromatic CH), 2963–2801 (aliphatic CH), 2199 (C=N), 1732 (C=O thiazolidine), 1628 (C=O), 1558–1493 (C=C). Anal. Caled for C$_{25}$H$_{28}$N$_4$O$_4$: 24.6 (C$_3$,C$_5$ of piperidine), 134.2 (aromatic C attached to N of thiazolidine), 135.1 (C=CH), 148.1 (aromatic C attached to OH), 150.2 (aromatic C attached to OCH$_3$), 158.8 (C=O of thiazolidine), 162.8 (C=O), 166.2 (C=C–CN). IR (KBr) cm$^{-1}$: 3556 (OH), 3062–3032 (aromatic CH), 2940–2851 (aliphatic CH), 2915 (C=N), 1717 (C=O thiazolidine), 1663 (C=O), 1616–1516 (C=C). Anal. Caled for C$_{23}$H$_{28}$N$_4$O$_2$: 25.3 (C$_4$ of piperidine), 25.8 (C$_3$,C$_4$,C$_5$ of piperidine), 40.5 (3-Oxo-2-(4-oxo-3-phenyl-5-piperidin-1-ylmethyl-thiazolidine-2-yldiene)-3-piperidin-1-yl-propionitrile (5f)). Yellow crystals, 46% yield, mp 214–216°C (dec.). IR (KBr) cm$^{-1}$: 3067 (aromatic CH), 2967–2859 (aliphatic CH), 2203 (C=N), 1732 (C=O thiazolidine), 1628 (C=O), 1558–1493 (C=C). Anal. Caled for C$_{23}$H$_{28}$N$_4$O$_2$: 25.3 (C$_4$ of piperidine), 25.8 (C$_3$,C$_4$,C$_5$ of piperidine), 40.5 (3-Oxo-2-(4-oxo-3-phenyl-5-piperidin-1-ylmethyl-thiazolidine-2-yldiene)-3-piperidin-1-yl-propionitrile (5f)). Yellow crystals, 46% yield, mp 183–186°C (dec.). IR (KBr) cm$^{-1}$: 3067 (aromatic CH), 2967–2859 (aliphatic CH), 2203 (C=N), 1732 (C=O thiazolidine), 1628 (C=O), 1558–1493 (C=C). Anal. Caled for C$_{23}$H$_{28}$N$_4$O$_2$: 25.3 (C$_4$ of piperidine), 25.8 (C$_3$,C$_4$,C$_5$ of piperidine), 40.5 (3-Oxo-2-(4-oxo-3-phenyl-5-piperidin-1-ylmethyl-thiazolidine-2-yldiene)-3-piperidin-1-yl-propionitrile (5f)). Yellow crystals, 46% yield, mp 222–227°C (dec.). IR (KBr) cm$^{-1}$: 3060 (aromatic CH), 2936–2808 (aliphatic CH), 2199 (C=N), 1732 (C=O thiazolidine), 1628 (C=O), 1558–1493 (C=C). Anal. Caled for C$_{25}$H$_{28}$N$_4$O$_4$: 24.6 (C$_3$,C$_5$ of piperidine), 134.2 (aromatic C attached to N of thiazolidine), 135.1 (C=CH), 148.1 (aromatic C attached to OH), 150.2 (aromatic C attached to OCH$_3$), 158.8 (C=O of thiazolidine), 162.8 (C=O), 166.2 (C=C–CN). IR (KBr) cm$^{-1}$: 3526 (OH), 3062–3032 (aromatic CH), 2940–2851 (aliphatic CH), 2915 (C=N), 1717 (C=O thiazolidine), 1663 (C=O), 1616–1516 (C=C). Anal. Caled for C$_{23}$H$_{28}$N$_4$O$_2$: 25.3 (C$_4$ of piperidine), 25.8 (C$_3$,C$_4$,C$_5$ of piperidine), 40.5 (3-Oxo-2-(4-oxo-3-phenyl-5-piperidin-1-ylmethyl-thiazolidine-2-yldiene)-3-piperidin-1-yl-propionitrile (5f)). Yellow crystals, 46% yield, mp 222–227°C (dec.). IR (KBr) cm$^{-1}$: 3060 (aromatic CH), 2936–2808 (aliphatic CH), 2199 (C=N), 1732 (C=O thiazolidine), 1628 (C=O), 1558–1493 (C=C). Anal. Caled for C$_{23}$H$_{28}$N$_4$O$_2$: 25.3 (C$_4$ of piperidine), 25.8 (C$_3$,C$_4$,C$_5$ of piperidine), 40.5 (3-Oxo-2-(4-oxo-3-phenyl-5-piperidin-1-ylmethyl-thia-
2-yliden)3-oxo-3-piperidin-1-yl-proponitrile (5g): Orange crystals, 50% yield, mp 224–226°C (dec.). 1H-NMR (CDCl3, 400 MHz) δ: 1.28–1.49 (6H, m, piperidin protons), 2.57–2.74 (4H, m, (CH2)3N of morpholine), 2.85–2.95 (2H, m, CH2), 3.38–3.40 (4H, m, (CH2)3N of piperidine), 3.65–3.68 (5H, m, (CH2)O of morpholine+CH of thiazolidinone), 7.35–7.75 (5H, m, aromatic protons). IR (KBr) cm−1: 3050 (aromatic CH), 2940–2855 (aliphatic CH), 2199 (C=O thiazolidinone), 1624 (C=O, 157.1 (C(aromatic carbons), 135.0 (aromatic C attached to N of thiazolidinone), 1628 (C=O thiazinanone), 1643 (C=O, 1570–1493 (C=O thiazolidinone), 1620 (C=O), 1524–1493 (C=C). Anal. Calcd for C17H17N3O3S, Mf = 343.405, monoclinic, crystalsizes in space group P21/c, cell lengths “a=0.2306 (2), b=17.5581 (4), c=12.8758 (4) Å,” cell angles “α=90.00°, β=12 (18)×10°, γ=90.00°,” V = 1617.86 (13) Å3, Z=4, Dc = 1.392 mg/mm3, mθ values 2.910–2.7485°, absorption coefficient μ (Mo-Kα) = 0.22 mm−1, F(000)=720. The unique reflections measured 4148 of which 2406 reflections with threshold expression I>3σ(I) were used in the structural analysis. Convergence for 217 variable parameters by least-squares refinement on F2 with w=1/[σ2(Fo)2+0.10000F2]. The final agreement factors were R=0.044 and wR=0.175 with a goodness-of-fit of 1.119.

Antitumor Activity The potential cytotoxicity of the tested compounds was evaluated using the method of Skelan et al.15,29–35 Cells were plated in 96-multwell plate (104 cells/well) for 24h 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 48h at 37°C in atmosphere of 5% CO2. After 48h, 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 enzyme-linked immuno-sorbsent assay (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 IC50 was determined by using a program Graph-Pad PRISM version 5. Mean and standard error were determined by SPSS 11 software.

Acknowledgement The authors are thankful to Dr. Nasser S. M. Ismail, Pharmaceutical Chemistry Department, Faculty of Pharmacy, Ain Shams University, for his assistance in performing the QSAR study.

References

Single Crystal X-Ray Crystallographic Data of (6a)22

For X-ray crystallographic studies, compound 6a was recrystallized as prismatic colourless 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-Kα radiation (λ=0.71073 Å). The crystal structures were determined by SIR9243 and refined by maXus46 (Bruker Nonius, Delft and MacScience, Japan). Chemical formula C17H17N3O3S, Mf = 343.405, monoclinic, crystallizes in space group P21/c, cell lengths “a=0.2306 (2), b=17.5581 (4), c=12.8758 (4) Å,” cell angles “α=90.00°, β=12 (18)×10°, γ=90.00°,” V = 1617.86 (13) Å3, Z=4, Dc = 1.392 mg/mm3, mθ values 2.910–2.7485°, absorption coefficient μ (Mo-Kα) = 0.22 mm−1, F(000)=720. The unique reflections measured 4148 of which 2406 reflections with threshold expression I>3σ(I) were used in the structural analysis. Convergence for 217 variable parameters by least-squares refinement on F2 with w=1/[σ2(Fo)2+0.10000F2]. The final agreement factors were R=0.044 and wR=0.175 with a goodness-of-fit of 1.119.

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References