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Design and synthesis of novel 5-aminosalicylate (5-ASA)–4-thiazolinone hybrid derivatives with promising antiproliferative activity

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ABSTRACT

Two privileged pharmacophores were assembled in one molecular frame involving 5-aminosalicylate and 4-thiazolinones that can be found in different stereochemical features. The compounds were fully characterized and evaluated for antiproliferative activity against four human cancer cell lines and some are equipotent to doxorubicin with lower cytotoxicity to normal cells. The most interesting finding relates to compound **10**, which shows an IC_{50} value of 70 nM against MCF-7 cells, while the IC_{50} against human fibroblasts is 10 μM. The results of this study indicate that the new compounds are optimal anti-cancer leading compounds and merit further studies to optimize their structure, detect their biotargets and in vivo activity.

Although a range of treatment options are available for cancer, chemotherapy is fraught with significant levels of toxicity to healthy cells, and drug resistance develops in some treatment regimes. To decrease the current cancer burden, drug discovery is directed at the development of highly effective and potent medications with reduced side effects. 5-Aminosalicylic acid (5-ASA), as well as its prodrugs that are currently used in the management of inflammatory bowel diseases have been shown recently to possess cancer chemopreventive and chemotherapeutic properties.[1] The mechanism of this activity is attributed to improved maintenance of genomic stability that counteracts carcinogenesis,[2] thus contributing to its chemopreventive and chemotherapeutic properties, in particular, against colorectal cancer.[3,4] 1,2,3-Triazolyl-salicylamides exhibited antiproliferative activity as lavendustin mimetics and potent aurora kinase inhibitors.[5] Recently, it was suggested that the antioxidant properties of salicylate derivatives is a possible mechanism of anti-inflammatory activity.[6]

On the other hand, 2-arylaminothiazolidin-4-ones having 5-un/substituted benzylidenes were the most promising in inhibiting growth of several human cancer cell lines but not normal fibroblasts in a dose dependent manner.[7–13] Several mechanisms were suggested for these effects involving reversible blockage of cell cycle progression at the G2/M phase border, induction of apoptosis,[14] antagonizing stimulatory effect of free fatty acids at cell proliferation,[15] inhibition of translation initiation,[16] interaction with Sphingosine Kinase and non-membrane protein tyrosine phosphatase (SHP-2).[17]

For complex diseases like cancer; a balanced modulation of several targets can provide advanced therapeutic effects and a favorable side effects profile compared to the action of selective ligand. Accordingly, it is thought of interest to accommodate thiazolin-4-one and 5-ASA moieties in a single molecular framework as the key pharmacophore and screen the antiproliferative activity. Interestingly, the target compounds can be considered as lavendustin analogs.[18] Our design is based, also, on an interesting SAR study in 2-amino-4-thiazolinones which allowed to identify optimal H-bond donating OH substituent at *para* position of arylamino fragment, as well as small lipophilic substituents of the benzylidene moiety at positions 3 and 4 are favored.[19] Several studies revealed that the presence and the nature of the moiety at position 5 of thiazolidinone play the key role in realization of the pharmacological effects.[19–22] Accordingly, the structural variations were selected by introducing different benzylidenes at that position.

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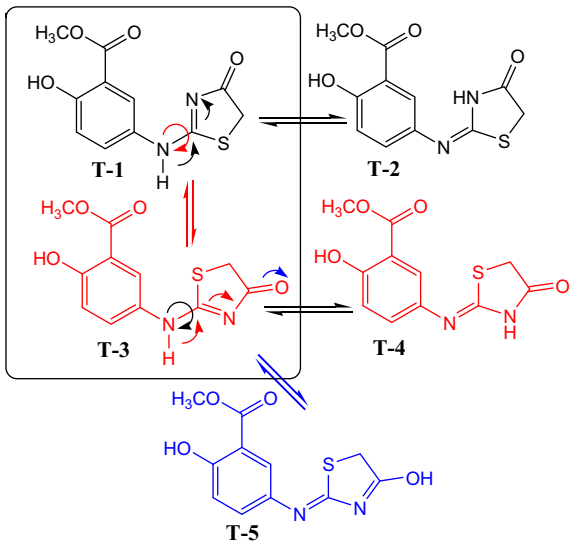
The target compounds were synthesized as shown in Scheme 1 (see ESI for Experimental details). 5-ASA was chloroacetylated by heating with chloroacetyl chloride in dry benzene.[23] Heterocyclization of the product in the presence of ammonium thiocyanate in refluxing ethanol efficiently produced methyl 2-hydroxy-5-[(4-oxo-4,5-dihydro-1,3-thiazol-2-yl)-amino]benzoate (**1**).[24] The two active methylene protons appear as separate doublets at $\delta = 4.00$ and 3.96 ppm. The target compounds (**2**–**19**) were obtained by refluxing **1** with commercially available aromatic aldehydes using a Knoevenagel condensation procedure in the presence of sodium acetate, in glacial acetic acid. The purity of the synthesized compounds has been checked by TLC. The structures of the synthesized compounds were confirmed by analytical and spectral data (IR, ^{1}H, ^{13}C NMR and ESI-HRMS).[25] In ^{1}H NMR spectra of compounds **1**–**19** characteristic doubling of the signals is seen that corresponds to the presence of tautomerism or *syn*/*anti* arrangement rotamers. On the basis of spectra the correlation of the two isomers in the solution make approximately 1:1 mixture. For example, NH proton appears as two singlets at about ~11.60 ppm and ~12.45 ppm. The 5-ASA moiety forms subspectrum of multiplets at ~7.05–7.55 ppm. Only *Z*-isomers were obtained as in the Knoevenagel reaction, because it is thermodynamically stable.[26]

2-Arylamino-2-thiazolin-4-ones and their 5-substituted derivatives can primarily display amino/imino due to prototropic tautomerism. Additionally, both tautomeric forms may exist as a mixture of two conformers (T-1, T-2 and T-3, T-4; Scheme 2), which are particularly stabilized by the formation of intramolecular hydrogen bonds. It is worth noting that amino/imino tautomerism in 2-amino(imino)-1,3-thiazolidin-4-one derivatives have been extensively investigated. Results from these studies showed that the tautomer with the carbonyl–imine group in the five-membered heterocyclic ring and an exocyclic amine N atom is predominant.[27]

Similarly, X-ray single crystal analysis of **1** revealed that the amino form is predominant and the compound is crystallized with two independent molecules in the asymmetric unit that differ primarily in the rotational orientation of the five-membered heterocyclic ring (Fig. 1).[24] This finding was also studied by computational chemistry.

The possible conformational (*S-trans* and *S-cis*, such as T-1 and T-3, respectively) and the configurational (*E* and *Z* such as T2 and T4, respectively) isomers are shown in Scheme 2. The structures and the vibrational frequencies of all stable rotamers and tautomers have been calculated. Final energies have been obtained and recorded in Table 1. The results show that the rotamers T1 and T3 are almost of equal heat of formation and both are much more stable than their corresponding tautomers T-2 and T-4, respectively. This finding explains why this precursor **1** is found as inseparable 1:1 mixture of conformers (T-1 and T-3). To our knowledge this is the 1st crystal structure shows conformers (not tautomers) and in equal ratio. Systematic investigation of the reported X-ray structure shows that the two rotamers are stacked together oppositely and face to face via the amino-thiazoline entities.



Scheme 2. Tautomers and conformational isomers of compound **1**.

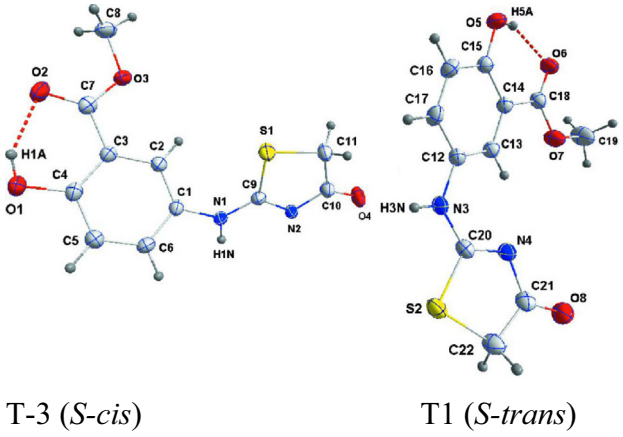
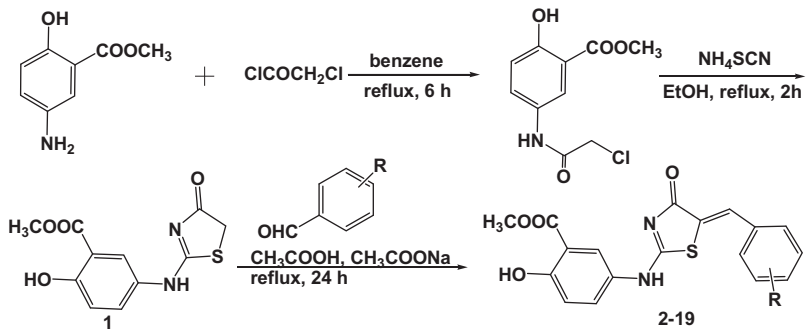


Figure 1. The structures of the rotamers of compound **1**. The asymmetric unit with labeling scheme and 50% probability ellipsoids.[24]



2: R = H; **3**: R = 4-Me; **4**: R = 4-OMe; **5**: R = 4-NO_2; **6**: R = 4-Cl; **7**: R = 2-OH; **8**: R = 3-Cl; **9**: R = 2-OMe; **10**: R = 4-Br; **11**: R = 2-Cl; **12**: R = 3-Br; **13**: R = 4-NMe_2; **14**: R = 3,4-OCH_2O-; **15**: R = 3,4-di-OMe; **16**: R = 4-OH; **17**: R = 3-NO_2; **18**: R = 3,4,5-tri-OMe; **19**: R = 4-F

Scheme 1. Synthesis of methyl 2-hydroxy-5-[(5-benzylidene-4-oxo-4,5-dihydro-1,3-thiazol-2-yl)-amino]benzoates **2**–**19**.

Table 1
Physicochemical parameters of minimized tautomers and conformers of compound **1**

	T-1 (*S-trans*)	T-2 (*E*)	T-3 (*S-cis*)	T-4 (*Z*)	T-5 (*Z*)
STR[a]	0.263	0.323	0.228	0.341	0.521
BND[b]	3.473	7.333	3.995	9.757	16.801
QQ[c]	−0.222	−10.567	−1.296	−12.883	1.657
HF[d]	−129.29	−114.58	−129.19	−116.75	171.23
DM[e]	7.559	4.959	3.748	3.943	3.495

[a] Stretching energy.
[b] Bending energy.
[c] Core–core interaction.
[d] Heat of formation.
[e] Dipole moment.

Table 2
Percent reduction in the survival of MCF7 cells after treatment with a single concentration (10 μM, 48 h)

Compound	% of cells remaining survival[b]	SD
DMSO[a]	100.0	8.3
1	97.0	12.0
2	67.8	8.5
3	17.8	4.0
4	26.0	2.9
5	73.9	5.0
6	28.0	4.0
7	16.5	5.0
8	65.9	4.5
9	70.0	6.0
10	19.0	2.4
11	85.0	8.7
12	68.0	6.2
13	16.2	4.8
14	28.0	4.2
15	26.6	4.8
16	93.5	7.0
17	82.2	7.3
18	73.3	11.7
19	86.0	8.2
Doxorubicin[c]	4.0	1.8

[a] Control cells treated with the vehicle, (DMSO) added as 0.1%.
[b] Each value is the average of six readings.
[c] 10 μM doxorubicin is used as an internal reference standard.

A pair of T-1 and T-3 makes strong hydrogen bonds with another pair of T-3 and T-1 in which there is strong hydrogen bonding between the NH of the N-*cis* conformer in a pair and the thiazoline-N of the NH-*trans*, occupies the same plane, with the other pair, providing the crystal lattice.

The molecular modeling of the tested compound was studied for each possible structure as fully optimized at full self-consistent field (SCF) levels by using MOPAC;[28,29] a general molecular orbital package implemented with molecular mechanics software MMX-PC.[30]

The potential antiproliferative activity of all synthesized compounds (**1**–**19**) was preliminarily evaluated in vitro at a single concentration of 10 μM against the breast cancer cell line (MCF-7), using the Sulforhodamine B assay (SRB).[31,32] The effect of this concentration on cell survival is given in Table 2 (see ESI for Experimental details).

These results showed that several compounds exhibited strong growth inhibitory activities against MCF-7, such as **3**, **7**, **10** and **13**. These **c**ompounds were further selected for more detailed investigation to obtain full dose response survival curves using different concentrations (0.001–10 μM) against four different human cancer cell lines from different histological backgrounds (breast MCF-7, colon HCT-118, lung A549 and cervix HeLa).

The effects of the compounds on the proliferation of a human normal fibroblast strain (F180) was also used to test the effects of the compounds on normal cells. Doxorubicin was used in all experiments as the reference standard because it is a wide spectrum anticancer agent that is effective against different types of cancer. From the full dose–response curve, (Figs. 1S–3S in ESI), the respective IC_{50} (concentration of the compound that kills 50% of the cells after 48 h incubation compared to untreated controls) was calculated and used as a parameter for the antiproliferative activity of the compounds.

Results of the survival assay (Table 3 and Figs. 1S–3S in ESI) showed that some compounds have an IC_{50} comparable to doxorubicin on some tested cancer cell lines. Compounds **3** (IC_{50} = 0.085 ± 0.01 μM) and **13** (IC_{50} = 0.31 ± 0.02 μM), for example, have an IC_{50} comparable to doxorubicin on the Cervix (HeLa) and colon (HCT-116) cancer cell lines, respectively. Compound **10** (IC_{50} = 0.07 ± 0.09 μM) has the same IC_{50} of doxorubicin on MCF-7.

The lung cancer cell line (A549) was more resistant to the four tested compounds than doxorubicin. Generally, the four compounds were less effective on the lung adenocarcinoma cell line (A549) than the other three cancer cell lines. This is in line with the fact that non-small cell lung cancer cells (like the A549) are known to be resistant to chemotherapy.[32]

The most interesting result was that the four compounds were less toxic to the normal fibroblast strain (F180) than the doxorubicin. The IC_{50} of compound **10**, for example, was about 28 times higher than doxorubicin's IC_{50} on the normal fibroblasts which may indicate that these compounds are safer than doxorubicin.

Based on the results presented in Tables 2 and 3, some preliminary structure–activity relationship aspects can be deduced. This preliminary SAR study has focused on the effect of substituent at benzylidene ring on the antitumor activity. Incorporation of unsubstituted benzylidene moiety has rendered the compound **2** with moderate activity. The data reveal that the activity is related to the substituent at the benzylidene ring. Since fluorine has a size similar to those of hydrogen, it is introduced as an isosteric to it. Fluorine substituent at the 4-position (compound **19**), resulted in a partial loss of the activity. In contrast, introduction of chlorine or bromine atom (more bulky than fluorine) at this position (**6** and **10**) has caused a remarkable enhancement in the antitumor activity. Interestingly, moving the chlorine from the 4- and 3-positions to the 2-position has sharply decreased the antitumor activity. Accordingly, the activities of the halogenated members were decreased in the order of 4-Br > 4-Cl > 3-Br ≈ 3-Cl > 2-Cl > 4-F for the MCF-7 cells. This finding suggests that the incorporation of

Table 3
IC_{50} values of compounds **3**, **7**, **10** and **13** against MCF-7, HCT-116, Hela, A549 and F118 as determined based on sulforhodamine assay

Compd	IC_{50}[a] (μM)				
	MCF-7	HCT-116	Hela	A549	F180
3	0.41 ± 0.06	0.45 ± 0.06	0.085 ± 0.01	2.2 ± 0.28	1.05 ± 0.18
7	0.5 ± 0.07	3.44 ± 0.03	1.39 ± 0.01	>10	>10
10	0.07 ± 0.00	5.58 ± 0.04	3.48 ± 0.15	8.3 ± 0.62	10 ± 0.85
13	0.63 ± 0.04	0.31 ± 0.02	0.18 ± 0.02	2.4 ± 0.11	0.94 ± 0.08
Doxorubicin	0.07 ± 0.00	0.38 ± 0.02	0.1 ± 0.01	0.45 ± 0.05	0.35 ± 0.02

[a] Concentration required to decrease viability to 50% of control untreated cells; values are the mean ± SD for $n = 3$ determinations each in triplicates.

halogens into 4-position may be advantageous. Interestingly, the introduction of 4-methyl or 4-dimethylamino (electron-donating group) at the 4-position of benzylidene has enhanced the activity against the four tested cancer cell lines; compounds **3** and **13**, respectively. These compounds have represented the most active derivatives against the breast cancer cell line MCF-7. The introduction of 4-methoxy (**4**) decreased the activity while 4-hydroxy (**16**) showed a significant drop in activity. Surprisingly, 2-hydroxy derivative (**7**) showed promising activity and was one of the most active compounds.

Based on this results, we can deduce that the substitution pattern on the benzylidene moiety is a crucial element for the antitumor activity. The incorporation of electron donating groups, as the 4-dimethylamino or 4-methyl group, is highly favorable and resulted in compounds with promising activity; **3** and **13**. Moreover, substitution with bulky electron withdrawing groups as the 4-bromine has greatly enhanced the activity; **10**. 4-Hydroxylation is completely unfavorable while 2-hydroxylation provided the most active compound; **7**. This may be due to intramolecular interaction with the endocyclic N and/or S.

In the present study, nineteen 5-aminosalicylates incorporating thiazolin-4-ones (**1**–**19**) were synthesized and their geometrical and rotational isomerism were studied by X-ray crystal study and computational method. The compounds were evaluated for their antiproliferative activities against four human cancer cell lines (MCF-7, HCT-118, HeLa and A549). From the structure activity relationships (SARs) we can deduce that the benzylidene moiety is essential for activity. The introduction of an electron donating or withdrawing bulky group at the 4-position of benzylidene plays an important role in enhancing the antitumor activities. It was found that compounds with a 4-methyl (**3**), 2-hydroxy (**7**), 4-bromo (**10**), *p*-dimethylamino (**13**) at benzylidene ring have promising activity. These four compounds showed anti-proliferative activity comparable to doxorubicin against MCF-7, HCT-118 and HeLa cells with lower cytotoxicity to the normal fibroblasts. Accordingly, they are potential anticancer drug leads and worth further detailed investigations to further optimize their structure and detect their mechanism of action and their effects on animal/xenograft models to evaluate in vivo bioavailability and chemotherapeutic potential.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.02.073.

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25. (a) *Synthesis of methyl 2-hydroxy-5-[(4,5-dihydro-4-oxo-1,3-thiazol-2-yl)-amino] benzoate (**1**).* A solution of methyl 5-chloroacetamidosalicylate (2.3 g, 9.5 mmol) and ammonium thiocyanate (1.5 g, 19.7 mmol) in 40 ml ethanol was refluxed for 2 h and allowed to stand overnight. The mixture was evaporated and the residue was washed with water and the recrystallized from ethanol/water to give the target compound (2.13 g, 85% yield); mp = 208–209 °C; IR (KBr): 3250, 3085, 2915, 2770, 1671, 1628, 1595, 1568, 1511, 1479, 1433, 1328, 1273, 1207, 824, 732. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.71, 11.12 (2*s, 1H; NH), 10.40, 10.34 (2*s, 1H; OH), 8.15, 7.39 (2*d, *J*(H,H) = 2.8 Hz, 1H; ArH-6), 7.76, 7.19 (2*dd, *J*(H,H) = 9.0, 2.8 Hz, 1H; ArH-4), 7.03–6.98 (m, 1H; ArH-3), 4.00, 3.96 (2*d, *J*(H,H) = 15.8 Hz, 2H; CH_2), 3.90, 3.88 (2*s, 3H; OCH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ = 187.99, 177.89, 168.64, 157.11, 156.79, 130.80, 129.96, 128.03, 122.16, 121.43, 118.44, 117.97, 113.29, 113.01, 52.63, 52.58, 38.47, 35.01. HRMS calcd for $C_{11}H_{11}N_2O_4S$ $(M+H)^+$, 267.0440; found, 267.0452; (b) *General procedure for synthesis of methyl 2-hydroxy-5-[(5-benzylidene-4-oxo-4,5-dihydro-1,3-thiazol-2-yl)-amino]benzoate (**2**–**19**).* To a well-stirred solution of compound **1** (0.3 g, 1.13 mmol) in acetic acid (10 mL) buffered with sodium acetate (0.18 g, 2.2 mmol), the appropriate arylaldehyde (1.6 mmol) was added. The solution was refluxed overnight till the completion of the reaction as monitoring by TLC, and then poured into ice-cold water. The precipitate was filtered, washed with water, and the resulting crude product was purified by recrystallization from dioxane.
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