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Synthesis and biological evaluation of thiazolidine-2,4-dione and 2,4-thione derivatives as inhibitors of translation initiation

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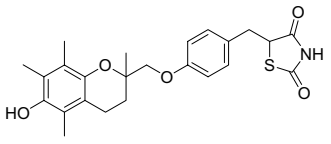
Abstract—In an effort to generate novel translation initiation inhibitors for cancer therapy, a series of 2′-benzyloxy-5′-substituted-5-benzylidene-thiazolidine-2,4-thione and dione derivatives was synthesized and evaluated for activity in translation initiation specific assays. Several candidates of the 5-benzylidene-thiazolidine-2,4-diones (**3c**, **3d**, and **3f**) and -thiones (**2b**, **2e**, and **2j**), inhibit cell growth with low μM GI_{50} mediated by inhibition of translation initiation, which involves partial depletion of intracellular Ca^{2+} stores and strong phosphorylation of eIF2α.

1. Introduction

The regulation of protein synthesis at the level of translation initiation plays a critical role in the regulation of cell growth and tumorigenesis.[1] mRNAs of growth-promoting proteins and oncogenes often have long and complex secondary structure in their 5′ untranslated region (5′UTRs) that make their translation inefficient and highly dependent on the activation of translation initiation factors, such as eIF2α and eIF4E.[2] For this reason, inhibition of translation initiation preferentially reduces the translation of mRNAs coding for growth regulatory proteins and oncogenes, and translation initiation inhibitors are considered promising candidates for anticancer drug development.[3]

In our previous studies we demonstrated that the anticancer effects of troglitazone (TRO) (Fig. 1) are mediated by partial depletion of intracellular Ca^{2+} stores leading to inhibition of translation initiation.[4] Troglitazone releases Ca^{2+} from the endoplasmic reticulum and at the same time inhibits store-operated Ca^{2+} channels thus blocking Ca^{2+} influx through the plasma membrane causing a partial depletion of intracellular Ca^{2+} stores.[4,5] Depletion of intracellular Ca^{2+} stores activates eIF2α kinases resulting in phosphorylation of eIF2α on Ser51 and its concomitant inactivation that limits the availability of the ternary complex eIF2-GTP-Met-tRNA.[6] Since availability of the ternary complex is a rate-limiting step in translation initiation, phosphorylation of eIF2α leads to preferential down regulation of G1 cyclins and other oncogenic proteins, and to cell cycle arrest in G1 phase.[7]



Troglitazone (TRO)

Figure 1.

As part of an ongoing program aimed at identifying novel target-specific small molecules, which act as anticancer agents through inhibition of translation initiation, we now report the synthesis and biological evaluation of a series of thiazolidine-2,4-dione and thione derivatives as potent inhibitors of translation initiation. Compound **1** was identified as a hit by initial screen of commercially available libraries. Interestingly, compound **1** is equipotent to TRO (Fig. 1) in Ca^{2+} releasing activity, induction of eIF2α phosphorylation, and about

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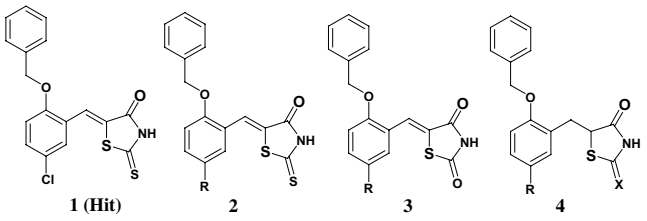


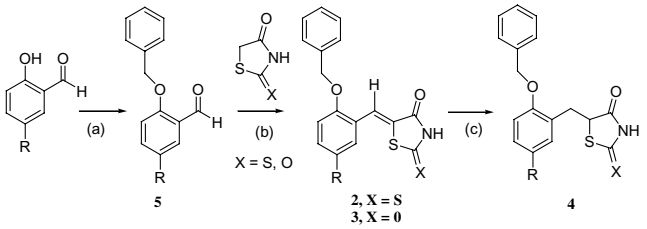
Figure 2. Analogues evaluated as translation initiation inhibitors.

threefold more potent in inhibiting cancer cell proliferation. In addition, TRO and compound **1** share a similar molecular scaffold. We focused our structure–activity relationship studies on substitutions at the 5′-position of the benzenlidene ring linked to the heterocycle (**2**), replacement of the thiazolidine-2,4-thione moiety with the thiazolidine-2,4-dione (**3**) and the reduction of the exocyclic double bond (**4**). We were able to identify some structural features that affect the potency of these compounds as Ca^{2+} depleting translation initiation inhibitors (Fig. 2).

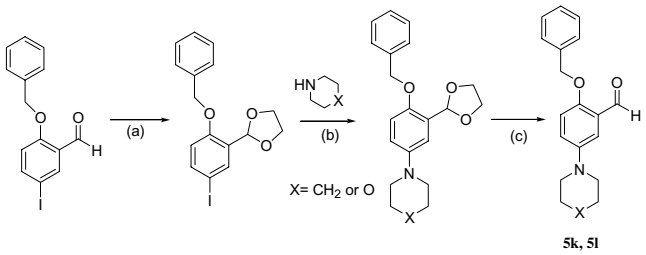
2. Chemistry

The general strategy for the synthesis of 2′-benzyloxy-5′-substituted-5-benzylidene-thiazolidine-2,4-thiones (**2**) and diones (**3**) was to condense different *O*-benzyl-5-substituted salicylaldehydes with rhodanine or thiazoline-2,4-dione. *O*-Benzyl-5-substituted salicylaldehydes (**5a**–**g**, **5i**, and **5j**) were prepared from commercially available 5-substituted salicylaldehydes by alkylating with benzyl bromide in DMF in the presence of potassium carbonate as base. The appropriately substituted 2-benzyloxy-aldehydes (**5**) underwent Knoevenagel condensation with rhodanine or thiazoline-2,4-dione in refluxing toluene in the presence of a catalytic amount of piperidinium acetate.[8] The products (**2** and **3**) were recrystallized from toluene with high purity. The reduction of the exocyclic olefinic bond of the benzylidene-thiazolidine-2,4-dione and thione was achieved with lithium borohydride in a 1:1.1 ratio of THF and pyridine mixture[9] (Scheme 1).

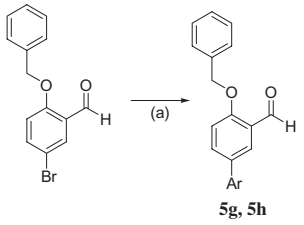
2-Benzyloxy-5-*N*-substituted salicylaldehydes were prepared by Buchwald amination reaction using copper(I) iodide as catalyst with the aldehyde protected as the dioxolane.[10] The protecting group was removed under acidic conditions to yield the free aldehydes (**5k** and **5l**) (Scheme 2).[11]



Scheme 1. Reagents: (a) K_2CO_3, benzyl bromide, DMF; (b) piperidinium acetate, toluene, reflux; (c) $LiBH_4$, THF–pyridine.



Scheme 2. Reagents: (a) ethylene glycol, TsOH, toluene; (b) CuI, K_3PO_4, ethylene glycol, isopropanol; (c) PPTS, acetone.



Scheme 3. Reagents: (a) $ArB(OH)_2$, K_2CO_3, $Pd(PPh_3)_4$.

5-Aryl-salicylaldehydes (**5g** and **5h**) were obtained through Suzuki cross-coupling reactions between *O*-benzyl-5-bromo-salicylaldehyde and the corresponding aryl boronic acids in good yields (Scheme 3).[12]

^1H NMR and LC–MS were used to confirm the structure and purity of all listed compounds. Characteristically, a single 5-methylidene proton was observed in the range of 7.72–7.97 ppm as a singlet, and NH proton was observed in the range of 12.5–13.5 ppm as a broad signal in DMSO-d_6 solution, both observations were consistent with previously reported data in the literature.[9] The absence of 5-CH_2 resonance of thiazlidine-2,4-dione confirmed the structure of the desired products **2** and **3** series.

3. Biology

The biological activities of compound series **2**–**4** were evaluated for intracellular Ca^{2+} depletion (by measuring the net change in the cytosolic Ca^{2+}), phosphorylation of eIF2α and inhibition of cancer cell growth, as previously described.[6a,13]

Since the Ca^{2+} depletion assay is carried out in Ca^{2+} free medium and in the presence of EGTA that sequesters any trace of extracellular Ca^{2+}, the increase in cytosolic Ca^{2+} reflects its release from intracellular stores rather than an influx from the extracellular milieu. The intracellular Ca^{2+} release assay was measured in Fluo-4 loaded 3T3 cells at 37 °C. The assay results are summarized in Table 1, the '+' and '–' notations indicate the release and no release of Ca^{2+}, respectively. Exemplary calcium release curves are given for compounds **3f** and **2f** depicting a Ca^{2+} releasing, '+', and an inactive Ca^{2+} releasing '–' compounds, respectively (Fig. 3).

Table 1. Activity of thiazolidine-2,4-thione (**2**) and 2,4-dione (**3**) in the Ca^{2+} release assay, phosphorylation of eIF2α and growth inhibition assay

Compounds R[a]=	Ca^{2+} release[b]	Phosphorylation of eIF2α[c]	GI_{50} (μM)[d]	Compounds R[a]=	Ca^{2+} release[b]	Phosphorylation of eIF2α[c]	GI_{50} (μM)[d]
H (**2a**)	+	1.17 ± 0.05	13 ± 1	H (**3a**)	+	0.92 ± 0.30	35 ± 7
Fluoro (**2b**)	+	5.02 ± 1.1	9 ± 2	Fluoro (**3b**)	+	0.72 ± 0.28	4 ± 1
Chloro (**1**)	+	1.76 ± 0.48	5 ± 1	Chloro (**3c**)	+	4.52 ± 1.81	5 ± 1
Bromo (**2d**)	+	1.40 ± 0.04	7 ± 2	Bromo (**3d**)	+	4.05 ± 1.5	5 ± 1
Methyl (**2e**)	+	4.15 ± 1.55	6 ± 1	Methyl (**3e**)	+	1.94 ± 0.53	8 ± 1
t-Butyl (**2f**)	–	1.97 ± 0.35	7 ± 3	*t*-Butyl (**3f**)	+	6.98 ± 1.35	2 ± 1
Phenyl (**2g**)	–	2.35 ± 0.42	4 ± 1	Phenyl (**3g**)	–	0.76 ± 0.22	2 ± 0
p-*t*-Butyl phenyl (**2h**)	–	1.37 ± 0.37	2 ± 1	*p*-*t*-Butyl phenyl (**3h**)	–	1.16 ± 0.39	2 ± 1
Methoxy (**2i**)	–	0.948 ± 0.06	7 ± 2	Methoxy (**3i**)	+	0.98 ± 0.33	14 ± 2
Nitro (**2j**)	+	3.49 ± 0.15	5 ± 1	Nitro (**3j**)	–	0.89 ± 0.13	14 ± 2
Piperidine (**2k**)	–	0.75 ± 0.26	3 ± 2				
Morpholine (**2l**)	–	0.92 ± 0.30	5 ± 1				
Chloro, X = S, (**4c**)	–	1.25 ± 0.25	29 ± 5	*t*-Butyl, X = O, (**4f**)	+	0.79 ± 0.12	12 ± 2
				Troglitazone (TRO)	+	2.12 ± 0.24	15 ± 2

[a] R group is substitution on the benzylidene ring.

[b] Release of Ca^{2+} from intracellular stores was observed using Fluo-4 loaded cells (3×10^6/well). The + and – notations indicate release and no release, respectively. In this assay Clotrimazole, a known Ca^{2+} releaser, was used as a positive control. This assay was done in triplicate.

[c] Drug (5 μM) induced phosphorylation of eIF2α was determined by Western blot analysis using phospho-specific eIF2α antibody [Rabbit Pan Anti-eIF2α (ps51)] and mouse monoclonal eIF2α antibody. Results were expressed as a ratio of the phosphorylated eIF2α to total eIF2α, normalized to DMSO. The results were shown as averages ± SD from three determinations, using TRO and DMSO as the positive and negative controls, respectively.

[d] GI_{50} was measured using SRB assay. The values indicate the concentration needed to inhibit 50% of cell proliferation. The results were shown as averages ± SD from three determinations, using TRO and DMSO as the positive and negative controls, respectively.

Depletion of intracellular Ca^{2+} stores causes the activation of eIF2α kinases (eIF2α-k) such as PKR and PERK leading to the phosphorylation of eIF2α on Ser51. Drug-induced phosphorylation of eIF2α was evaluated by Western blot analysis of cell extracts with anti-phosphoserine 51-eIF2α specific antibodies.[6a,14] The quantification of the phosphorylation of eIF2α was determined by an Odyssey infrared imaging system and was expressed as a ratio of intensity of the phosphorylated eIF2α to the intensity of the total eIF2α, normalized to DMSO. The results from these experiments are summarized in Table 1.

Compounds were further tested for cell growth inhibition in a human lung cancer cell line (A549). The sulforhodamine B (SRB) assay,[15] which specifically measures the inhibition of cell proliferation as a function of the protein content of the cell, was used to estimate the GI_{50}'s of the compounds.

4. Results and discussion

Although growth inhibition of cancer cells is the ultimate criteria for anti-neoplastic activity, our current study is aimed at identifying compounds achieving this end result in a mechanism specific manner, namely, Ca^{2+} depletion-mediated inhibition of translation initiation. Therefore, the Ca^{2+} releasing activity and the phosphorylation of eIF2α are prerequisites for selecting compounds for further development.

Structure–activity relationship (SAR) studies of substituents on C-5 in the benzylidene or benzyl rings demonstrate the important role of this position for the Ca^{2+} release activity of the compounds. In both the thiazolidine-2,4-thione (**2**) and thiazolidine-2,4-dione (**3**) series, the absence of substitution at C-5 results in the least potent analogs, **2a** and **3a**, respectively. In the thiazolidine-2,4-thione series small lipophilic substituents such as methyl (**2e**), fluoro (**2b**), chloro (**1**, original hit compound) and bromo (**2d**) released Ca^{2+} from intracellular stores, caused phosphorylation of eIF2α, especially compounds **2b**, **2e**, and were good inhibitors of cancer

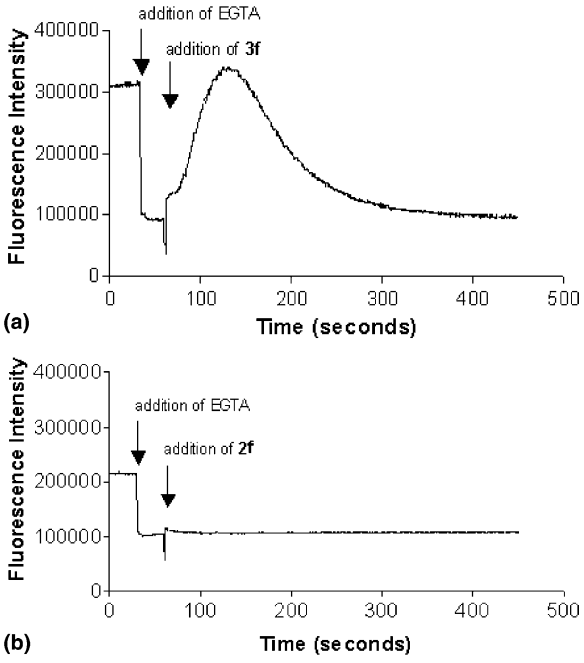


Figure 3. Typical Ca^{2+} curves for calcium releasing ('+') and non-releasing ('–') analogs following pretreatment with EGTA: (a) treatment with 80 μM of compound **3f**; (b) treatment with 80 μM of compound **2f**.

cell growth (5–9 μM). Substitution of the 5-position with larger lipophilic groups such as *t*-butyl (**2f**), phenyl (**2g**), and *p*-*t*-butylphenyl (**2h**) resulted in compounds that were unable to release Ca^{2+} from intracellular stores, while they still remained potent inducers of eIF2α phosphorylation. The dissociation between Ca^{2+} depletion activity and phosphorylation of eIF2α may suggest an alternative Ca^{2+}-independent mechanism leading to the phosphorylation of eIF2α. The different time courses in which the two assays are conducted (minutes for the Ca^{2+} release and $\geqslant 2$ h for the eIF2α phosphorylation assay) could also explain this discrepancy. For example, compounds with slow Ca^{2+} release kinetics may still deplete the intracellular stores and induce phosphorylation of eIF2α without causing a detectable change in net cytosolic Ca^{2+}. Interestingly, while strong electronegative substituents such as nitro (**2j**) retained the activities in all assays, the electron-donating substituents (methoxy **2f**, piperidine **2k**, and morpholine **2l**) caused loss of activity in both Ca^{2+} depletion and phosphorylation of eIF2α assays. The overall trend in the 5-benzylidene-thiazolidine-2,4-thione series (**2b**–**e** and **2j**) pointed to halogens, nitro and methyl substituents at the 5′ position to have comparable and favorable biological profiles as Ca^{2+} depleting inhibitors of translation initiation.

There is some overlap in SAR between the thiazolidine-2,4-thiones **2** described above and the thiazolidine-2,4-diones **3**. Substitution at the 5′-position with halogens and methyl (**3b**–**e**) results in fully bioactive compounds with a biological profile similar to that of the corresponding thiazolidine-2,4-thione analogs (**2b**–**e**) and substitution with phenyl, *p*-*t*-butyl phenyl and methoxy (**3g**–**i**) results in compromised biological activities similar to the effect of these substituents in the thiazolidine-2,4-thione series (**2g**–**i**).

The impact on biological activities of the 5′-NO_2 (**2j** and **3j**) and 5′-*t*-butyl (**2f** and **3f**) in the two series is quite reversed. While **3f**, the *t*-butyl containing analog, displays good activities in all three assays, with the strongest eIF2α phosphorylation activity, **2f** does not release Ca^{2+} from intracellular stores. On the other hand, while **3j**, the NO_2 containing analog, is devoid of Ca^{2+} release and eIF2α phosphorylation activities the corresponding **2j** is fully active in all three assays.

The significance of the exocyclic olefinic bond at the 5-position was addressed by comparing the most potent compound (**3f**) in the 5-benzylidene-thiazolidine-2,4-dione series and lead compound (**1**) in the 5-benzylidene-thiazolidine-2,4-thione series with the homologous 5-benzyl analog **4f** and **4c** in which the exocyclic olefinic bonds were reduced. In this respect, **4f** is a mimic of TRO. Evidently, while being as potent as **3f** in the induction of Ca^{2+} release, **4f** is inactive in the phosphorylation of eIF2α and sixfold less potent than **3f** in inhibiting cell growth. On the other hand, **4c** has completely lost Ca^{2+} depletion activity and is an order of magnitude less potent in the growth inhibition assay, $GI_{50} = 2$ and 29 μM, respectively. We conclude that unlike TRO, the conjugation of the thiazolidine-2,4-dione to the phenyl ring via the exocyclic methylidene linkage has biological advantages over the less rigidified system containing a 5-benzyl substitution.

In summary, following our original observation that TRO acts as Ca^{2+} depleting translation initiation inhibitor, we have identified compound **1** with structural features and biological profile similar to TRO. We carried out a limited SAR study, and identified some of the structural features contributing to increase of potency. Additionally, several 5-benzylidene-thiazolidine-2,4-diones (**3c**, **3d**, and **3f**), and -thiones (**2b**, **2e**, and **2j**) show intracellular Ca^{2+} release, strong phosphorylation of eIF2α, and low μM GI_{50}. These candidates will be used as leads for further optimization as Ca^{2+} depleting translation initiation inhibitors and putative anticancer agents.

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