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Letters

Novel Arylsulfoanilide−Oxindole Hybrid as an Anticancer Agent That Inhibits Translation Initiation

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Abstract: Structure−activity relationship studies of substituted arylsulfoanilides as antiproliferatives, which are mediated by the partial depletion of intracellular Ca^{2+} stores, resulted in the identification of compounds with micromolar activity against lung cancer cells in a growth inhibition assay. Incorporating the substitution pattern of the best arylsulfoanilides onto the 3-phenyloxindole scaffold resulted in a potent arylsulfoanilide−oxindole hybrid, **27**. Compound **27** inhibits cancer cell growth by partial depletion of intracellular Ca^{2+} stores and phosphorylation of eIF2α.

Translation initiation plays a vital role in the expression of oncogenic, prometastatic, and growth regulatory proteins;[1−3] inhibitors of translation initiation make an attractive class of anticancer agents.[4] The key step in translation initiation is the formation of a ternary complex between eukaryotic initiation factor 2 (eIF2), guanosine 5′-triphosphate (GTP), and initiator methionine transfer ribonucleic acid ($tRNA_i^{met}$).[5] At the end of each initiation cycle the GTP in the ternary complex is hydrolyzed to guanosine 5′-diphosphate (GDP) that must be exchanged for GTP by eIF2B to trigger a new round of translation initiation.[6] Phosphorylation of eIF2α on serine 51 increases the affinity of eIF2α for the scarce eIF2B and converts the phosphorylated eIF2α into an inhibitor of the GDP−GTP exchange factor eIF2B, and thereby inhibits translation initiation.[7]

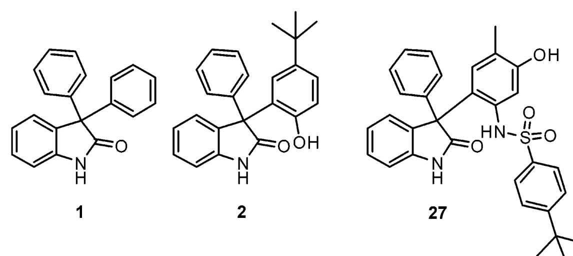

Figure 1. Diaryloxindoles as Ca^{2+} depleting translation initiation inhibitors.

Importantly, the low eIF2B/eIF2α molecular ratio suggests that even partial phosphorylation of eIF2α will suffice to scavenge eIF2B effectively and block translation initiation.[8] Small-molecule translation initiation inhibitors such as clotrimazole (CLT), the n-3 polyunsaturated fatty acid eicosapentaenoic acid (EPA), and troglitazone (TRO) cause phosphorylation of the key translation initiation factor eIF2α, which mediates their growth inhibitory activity in cancer cells and anticancer activity in tumors.[9−11] The anticancer effects of CLT, EPA, and TRO are triggered by the partial depletion of intracellular Ca^{2+} stores and inhibition of restorative Ca^{2+} store-regulated Ca^{2+} influx through the plasma membrane, thereby causing a sustained depletion of the intracellular Ca^{2+} stores.[12,13] This partial Ca^{2+} depletion activates eIF2 kinases (PKR and/or PERK), resulting in phosphorylation of eIF2α on serine 51 leading to its inactivation. Inactivation of eIF2α inhibits primarily ternary-complex-dependent translation initiation, resulting in the preferential down-regulation of growth-promoting proteins such as cyclin D1.[10−12] We have identified this Ca^{2+} depletion mediated translation initiation inhibition in other small molecules including the 3,3-diaryloxindole[14] (Figure 1, **1** and **2**) and TRO analogues.[15]

During a high-throughput screen of the open repository NCI library for translation initiation inhibitors, we encountered several hits containing the sulfonamide functionality. Sulfonamides are an important class of compounds that are reported to have substantial anticancer activity in vitro and in vivo.[16,17] A *p*-substituted

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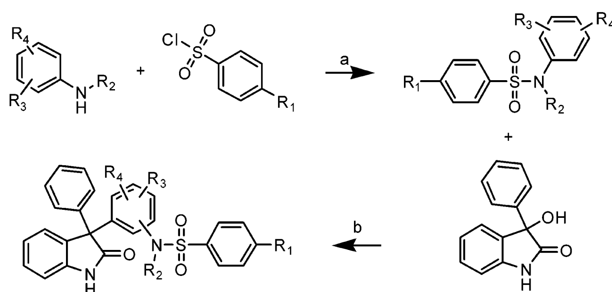
Scheme 1. General Synthetic Approach to Aryl Sulfonanilide−Oxindole Hybrids[a]



[a] Reagents and conditions: (a) pyridine, 0 °C, 1−12 h; (b) *p*-TsOH, DCE, 85 °C.

diarylsulfonamide (E7070) that is in clinical trials displays antitumor cell cycle in the G1 phase.[18−20] Taken together, this led us to investigate arylsulfoanilides as calcium-depleting translation initiation inhibitors. Herein, we report a structure−activity relationship study of a series of arylsulfoanilides that led to the synthesis and functional evaluation of a series of arylsulfoanilide−oxindole hybrids (Figure 1, **27**) using translation initiation specific assays.

The general synthetic approach to the arylsulfoanilides and the arylsulfoanilide−oxindole hybrids is outlined in Scheme 1. The appropriately substituted arylamines were coupled to the corresponding sulfonyl chlorides in pyridine at 0 °C.[21,22] The sulfonanilides **3**−**25** synthesized were purified by recrystallization or by flash silica gel chromatography. The different substitution patterns on the two phenyl rings and the nitrogen with various functional groups represent a wide range of electronic and steric effects. A bioassay guided iterative approach using two cell-based assays was undertaken in the study of the arylsulfoanilide compounds. The structure−function relationship study identified potent arylsulfoanilides as Ca^{2+} depleting anticancer agents. The substitution pattern of potent arylsulfoanilides was used to generate the arylsulfoanilide−oxindole hybrids. An electrophilic substitution of the anilide portion of the appropriately substituted sulfonanilides on the 3-hydroxy-3-phenyl-1,3-dihydroindol-2-one (Scheme 1) was carried out in the presence of *p*-toluenesulfonic acid (*p*-TsOH) to yield the arylsulfoanilide−oxindole hybrids.

All compounds were screened for their ability to partially deplete intracellular Ca^{2+} stores using Fura-2 AM loaded cells (NIH 3T3) and cell growth inhibition in a human lung cancer cell line (A549).[23,24] To formally demonstrate that the most active arylsulfoanilide−oxindole hybrid, **27**, targets ternary-complex-dependent translation initiation, **27** was further evaluated in translation initiation specific functional assays, namely, phosphorylation of eIF2α and a dual luciferase ternary complex assay.[25] Additionally, the growth inhibitory activity of the hit, lead, and lead-optimized diaryloxindoles **1**, **2**, and **27**, respectively, was assessed against two cancer cell lines (A549 and DU145) using the sulforhodamine B (SRB) assay.[24]

In our efforts to develop novel mechanism-specific antiproliferatives, we explored the SAR of arylsulfoanilides and arylsulfoanilide−oxindole hybrids as anticancer agents mediated by partial depletion of intracellular stores. The results from the growth inhibition and Ca^{2+} depletion assays of a series of arylsulfoanilides are summarized in Table 1.

Table 1. Arylsulfoanilide Analogues Assayed for Partial Depletion Intracellular Ca^{2+} Stores and Growth Inhibition of Lung Cancer Cells (A549)

compd	R_1	R_2	R_3	R_4	GI_{50} ± <10% (μM)	Ca^{2+} depletion assay[a]
1					13	0.87
2					3	1.10
3	H	H	H	H	>80	0
4	Br	H	H	H	30	0.38
5	I	H	H	H	23	0.87
6	*t*-Bu	H	H	H	16	1.44
7	Ph	H	H	H	17	1.37
8	OMe	H	H	H	16	0.63
9	NO_2	H	H	H	52	0.13
10	*t*-Bu	Me	H	H	30	0.14
11	*t*-Bu	H	2-OH	H	13	1.17
12	*t*-Bu	H	3-OH	H	12	0.55
13	*t*-Bu	H	4-OH	H	14	0.33
14	*t*-Bu	H	2-Me	H	>80	0.49
15	*t*-Bu	H	3-Me	H	80	0.95
16	*t*-Bu	H	4-Me	H	11	1.11
17	*t*-Bu	H	2-OH	4-Me	8	1.44
18	*t*-Bu	H	3-OH	4-Me	6	1.12
19	NO_2	H	3-OH	4Me	29	0.14
20	OMe	H	3-OH	4-Me	51	0.12
21	NHAc	H	3-OH	4-Me	48	0.19
22	Br	H	3-OH	4-Me	20	0.27
23	I	H	3-OH	4-Me	15	0.45
24	Ph	H	3-OH	4-Me	9	0.72
25	OPh	H	3-OH	4-Me	7	1.10

[a] Ratio of the peak heights of the compound normalized to the peak of CLT as a reference measured in the same batch of cells. The data presented are the average of two runs with a variability of <10%.

To explore the size and electronic effects of a substituent at the *p*-position on the partial depletion of intracellular Ca^{2+} stores and growth inhibitory activity, commercially available *p*-substituted sulfonyl chlorides were condensed with aniline to generate the first set of arylsulfoanilides (**3**−**9**). Both electron-donating and -withdrawing groups at the *p*-position contributed to enhanced growth inhibition and partial depletion of intracellular Ca^{2+} stores activity (cf. **6**, **8**, and **9** to **3**). However, the improvement in the biological activity contributed by the electron-withdrawing group (**9**) is marginal compared to the contribution from electron-donating groups (**6** and **8**).

The biological data also show that the 4-position substituted with a hydrophobic group (cf. **5**−**7** to **3** and **9**) results in better depletion of intracellular Ca^{2+} stores and growth inhibitory activity. From this series of compounds a *tert*-butyl group at the 4-position is the most active arylsulfoanilide compound in both assays. Unexpectedly, we observed that substituting the nitrogen of the sulfonanilide group (**6** and **10**) resulted in an order of magnitude loss of activity in the Ca^{2+} depletion assay and a 50% loss of activity in the growth inhibition assay. This suggests that the −NH of sulfonamide group is critical for its biological activity.

Although **6** and **8** are equipotent in the growth inhibition assay, **6** (which includes a *p*-*tert*-butyl substitution) was more potent than **8** (which includes a

p-methoxy substitution) in the Ca^{2+} depletion assay. Therefore, the next set of compounds (**11**−**16**) were generated by condensing *p*-*tert*-butylphenylsulfonyl chloride with *o*-, *m*-, and *p*-hydroxylanilines (**11**−**13**) and *o*-, *m*-, and *p*-tolylamines (**14**−**16**). These compounds addressed the electronics and the positional effects on the arylamino ring while maintaining the best substituent, *p*-*tert*-Bu, on the arylsulfonyl ring. The positional effect of the hydroxyl group (**11**−**13**) was minimal with regard to the growth inhibition and showed progressive improvement: *o*-OH (**11**) > *m*-OH (**12**) > *p*-OH (**13**) in the Ca^{2+} depletion assay. Interestingly, the reverse order of potency was true with the methyl substitutions (**14**−**16**), namely, the *p*-Me (**16**) > *m*-Me (**15**) > *o*-Me (**14**) in the growth inhibition and the calcium depletion assays. On the basis of these results we hypothesized that doubly substituting the arylamino ring with an *o*- or *m*-hydroxy and *p*-methyl groups (**17** and **18**) while maintaining the *p*-*tert*-butyl substitution on the arylsulfonyl ring would result in a more active Ca^{2+} depleting anticancer agent. As anticipated, **17** and **18** turned out to be low micromolar Ca^{2+} depleting antiproliferatives, suggesting that the substitutions on the arylamino ring result in an additive effect.

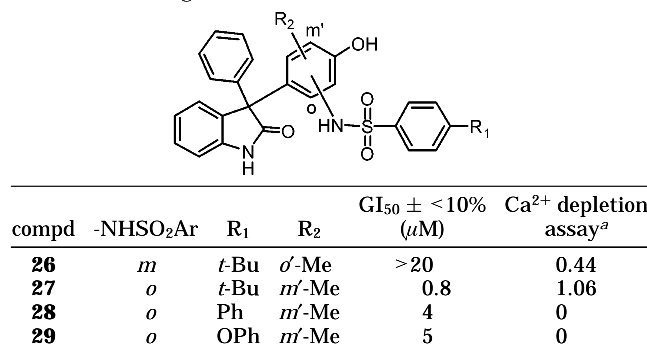
We generated the next series of compounds (**19**−**25**), maintaining its substitution pattern of **18**, by condensing 5-amino-*o*-cresol with a series of *p*-substituted phenylsulfonyl chlorides. Upon testing this series in the biological assays, we identified two additional low micromolar compounds (**24** and **25**) that were good Ca^{2+} depletors. Again, substitution with large hydrophobic substituents in the *p*-position of the arylsulfonyl ring was favorable.

On the basis of our previous studies, we knew that **2** was a potent Ca^{2+} depleting translation initiation inhibitor and that there was no activity-enhancing effect by functionalizing the other two aromatic rings.[14] Therefore, we hypothesized that incorporating the structural features of the most active (GI_{50} < 10 μM) arylsulfoanilide Ca^{2+} depletors (**17**, **18**, **24**, and **25**) into the 3-aryl-3-phenyl-1,3-dihydroindol-2-one scaffold would result in potent Ca^{2+} depleting translation initiation inhibitors. To test this hypothesis, we synthesized four arylsulfoanilide−oxindole hybrids (**26**−**29**) by an electrophillic substitution in the presence of *p*-toluenesulfonic acid. The biological data for this series are summarized in Table 2. Indeed, incorporation of the (3-hydroxy-4-methyl)-4′-*tert*-butylbenzenesulfonanilide (**18**) resulted in a submicromolar **27** in a growth inhibition assay with good Ca^{2+} depletion activity. Additionally, moving the sulfonanilide moeity to the meta position (**26**) results in reduced growth inhibitory (GI_{50} > 20 μM) and Ca^{2+} depletion activity, suggesting that the orientation of the *p*-*tert*-butylphenylsulfonanilide group is important for the activity. It is also interesting to note that the −Ph (**28**) and −OPh (**29**) substitutions at R_1 do not possess any Ca^{2+} depletion activity, suggesting that their moderate growth inhibitory activity may not be mediated by the Ca^{2+} depleting translation initiation inhibitory pathway.

A comparison of the hit **1**, lead **2**, and the lead-optimized **27** diaryloxindoles in functional assays involving the translation initiation cascade (viz., eIF2α phosphorylation and the ternary complex assay (TC)) is summarized in Table 3. The data from these assays are normalized to the hit **1**. Going from **1** to **27**, we observe a 2-fold increase in the phosphorylation and about a 5-fold increase in the ternary complex assay, which correlates well with an order of magnitude improvement in the growth inhibition of lung (A549) and prostate (DU145) cancer cells lines.

In conclusion, we used two cell-based assays to screen a focused library of arylsulfoanilides. Structure−function analysis led to the identification of key substitution patterns necessary for partial depletion of intracellular Ca^{2+} stores mediated growth inhibition. Incorporating these structural features into the diaryloxindole scaffold resulted in a submicromolar mechanism specific translation initiation inhibitor **27** that is an order of magnitude more potent in growth inhibition assay than the hit **1** in two different cancer cell lines. We are currently conducting preclinical studies to evaluate the efficacy of **27** in animal models of experimental cancer.

Table 2. Arylsulfoanilide−Oxindole Hybrids Assayed for Partial Depletion Intracellular Ca^{2+} Stores and Growth Inhibition of Lung Cancer Cells (A549)



compd	-NHSO2Ar	R1	R2	GI_{50} ± <10% (μM)	Ca^{2+} depletion assay[a]
26	*m*	*t*-Bu	*o*′-Me	>20	0.44
27	*o*	*t*-Bu	*m*′-Me	0.8	1.06
28	*o*	Ph	*m*′-Me	4	0
29	*o*	OPh	*m*′-Me	5	0

[a] Ratio of the peak heights of the compound normalized to the peak of CLT as a reference measured in the same batch of cells. The data presented are the average of two runs with a variability of <10%.

Table 3. Comparison of Hit **1**, Lead **2**, and Lead-Optimized **27** in Cell-Based Translation Initiation Specific Assays

compd	eIF2α-P	TC[a]	GI_{50} (μM) A549	GI_{50} (μM) DU145
1	1.00	1.00	13.12 ± 0.73	12.51 ± 0.99
2	2.01	2.15	2.71 ± 0.15	2.49 ± 0.21
27	2.14	4.98	0.7 ± 0.13	1.08 ± 0.09

[a] For details of the ternary complex assay (TC), see Supporting Information.

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Supporting Information Available: Experimental details for the biological assays and for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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