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7,9-Diaryl-1,6,8-trioxaspiro[4.5]dec-3-en-2-ones: readily accessible and highly potent anticancer compounds

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Abstract

7,9-diaryl-1,6,8-trioxaspiro[4.5]dec-3-en-2-ones are a recently described group of spirocyclic butenolides that can be generated rapidly and as a single diastereomer through a cascade process between γ -hydroxybutenolides and aromatic aldehydes. The following outlines our findings that these spirocycles are potently cytotoxic and have a dramatic structure-function profile that provides excellent insight into the structural features required for this potency.

Keywords

Cancer; Novel Cytotoxic Scaffolds; Covalent Modifiers; Structure-Function Studies; Cascade Processes

Synthetic methods that readily lead to scaffolds with potent bioactivity can be extremely valuable for studying and developing new lead therapeutics or developing new tools for

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Supplementary Material

Supplementary material, including ¹H and ¹³C data of all new compounds, can be accessed online free of charge at XXXXXXXX.

studying related processes. Recently, while studying the reactivity of γ -hydroxybutenolides, it was discovered that a series of unprecedented spirocyclic diaryl butenolides (1,6,8-trioxaspiro[4.5]dec-3-en-2-ones, Figure 1A) can be obtained readily through an elimination/Prins cascade process (Figure 1, B).ⁱ While the compounds themselves did not have any obvious synthetic utility, the “extended ketal-lactone moiety”, or a ketal with one of its oxygen atoms as part of a second ketal, was reminiscent of many well-known bioactive molecules (Figure 1, C).ⁱⁱ In addition, butenolides have more generally been found to have a broad range of bioactivity,ⁱⁱⁱ and are found in a number of compounds with therapeutic significance including Vioxx, Digoxin and Atractylenolide III. Given the novelty of the scaffold, and the rapid method available to synthesize it, we were interested in assessing the bioactivity. Herein, we disclose for the first time that this scaffold has highly potent anticancer activity. We also provide a preliminary structure-function study that lends insight into the important structural features that lead to this activity.

A series of compounds generated through our synthetic studies were tested for anticancer activity through a 48-hour viability assay as previously reported by the Bradner lab.^{iv} During these studies, it was revealed that the compounds had particularly high potency against the 797 NUT midline carcinoma cell line,^v a rare and highly aggressive epithelial cancer that affects people of all ages.^{vi} The most potent of the analogs tested were those that had electron-withdrawing groups on the phenyl appendage (**1–5**, Figure 2), all of which had potency ranges that bordered on the 20–100 nM range. Furthermore, these molecules appear to be stable in neutral aqueous conditions, as was evidenced by no noticeable hydrolysis of **3** in phosphate buffer (w/ 10% DMSO) at 60°C for up to 1 hr. The positioning of the electron-withdrawing group did not have a dramatic effect on the binding. However, removal of the electron-withdrawing group led to roughly an order of magnitude decrease in potency (**6** Spirocycle **7**, which has an electron-withdrawing substituent on an inherently electron-rich furan, was also less potent than compounds **1–5**. Finally, spirocycle **8**, which had no aromatic groups appended, remained active but with even greater diminished potency. Furthermore, a single methyl group added to the dioxane ring of **3** in a non-selective manner further decreased activity (**9**). The collective data demonstrate that the spirocyclic butenolide core is responsible for the toxicity, and the aromatic appendages add significant potency. Unfortunately, electronically rich aromatic aldehydes, such as 4-anisaldehyde, represent a limitation of the chemistry, and thus spirocycles with these appendages could not be assessed.¹ All compounds were also tested against MJ, a T cell lymphoma cell line,^{vii} to gauge selectivity. In all cases, the spirocycles were moderately less toxic against the MJ cell line.

Interested by the importance of the aromatic groups, we synthesized two new spirocycles. The first was the pentafluorophenyl containing bicycle **10**, which we reasoned would be highly electron withdrawing and may have activity superior to those previously tested. However, while active, the molecule was significantly less potent than **1–5**. In addition, we sought to extend the size of the aromatic groups, and synthesized nitronaphthyl spirocycle **11**. However, this molecule was comparable instead to compound **8**, which lacks aromatic groups. Both of these results collectively demonstrate a size restriction to the aromatic groups.

The significance of the molecules' pseudosymmetry was next gauged through testing of analogs **12** and **13**, which had been made previously from **3** and **6** as a mechanistic hypothesis to test the reversible nature of the cascade process.¹ These two molecules, which only differed in the positioning of the nitro group, had nearly identical activity against the cell line, and were each about half as potent as compound **3** (Figure 3). The same trend also held against the MJ cell line.

This result was important for several reasons. For one, at the onset of our studies we rationalized that the acetals of **6–8** could be less stable than those of **1–5**, since electronics can strongly influence rate of hydrolysis of acetals, and those derived from electron poor aldehydes are generally more stable.^{viii} Thus, we had initially reasoned that the lower potency of compounds **6–8** could be an artifact that might arise due to the more facile hydrolysis of these compounds in the buffer solution. Results from the experiments with **12** and **13** disproves this hypothesis, since if this were the case, **12** would be expected to have similar cytotoxicity to **6**, and **13** would be expected to be similar to **3**. Another piece of insight that this provides us is the realization that neither position on the dioxane dominates the activity.

Instead, we offer two possible reasons for this result. One possibility is that the two aromatic groups work in concert with one another, and thus **12** and **13** have similar potency because they each share the same aromatic appendages. The other possibility is that only a single aromatic group dominates the binding event, but that the pseudosymmetry of **3** and **6** makes each enantiomeric form roughly equivalent in potency. This hypothesis is perhaps best explained by thinking of one of the nitroaryl groups of **3** being responsible for the potency for one enantiomer, and the other nitroaryl group being responsible for the other enantiomer (see Figure 4 for illustrative aid 3). When only one nitroaryl group exists (**12** and **13**), one would expect that only one enantiomeric form would be as potent as **3**, and thus a decrease in potency by approximately a factor of two would be expected. Methods for accessing enantiomerically enriched **12** and **13**, currently a restriction of the chemistry, are necessary for determining this latter hypothesis, and efforts toward these studies are currently underway.

The importance of the electrophilic alkene of the butenolide was next tested. α,β -Unsaturated carbonyls are a common structural component in many covalent enzyme inhibitors,^{ix} and thus we reasoned that testing a reduced version of the molecules were necessary to assess the possibility that our compounds could be covalent enzyme inhibitors. Thus, compound **14** was synthesized through the reduction of **4** through the use of Pd/C under a hydrogen atmosphere (Scheme 1). At concentrations of up to 100 μ M, **14** showed no activity, supporting the covalent inhibitor hypothesis.

We next set out to assess whether the cytotoxicity was general to other cell types. Thus, compounds **3**, **5**, and **6** were tested against representative Gram-negative bacteria (*Escherichia coli*) and Gram-positive bacteria (*Bacillus subtilis*), in addition to *Mycobacterium smegmatis* (Figure 5). Growth inhibition in all cases were either not seen, or only at very high concentrations ($IC_{50} > 200 \mu$ M). In addition, in those cases in which some antibacterial activity was seen, such as against *B. subtilis*, there was not a strong preference

for those electron-poor bearing aryl groups (**3** and **5**) versus the phenyl containing spirocycle **6**, as had been seen previously with NMC-797 and MJ cell lines. Thus, it appears that the cytotoxicity may be selective for mammalian cells.

In summary, we disclose our findings that readily synthesizable spirocyclic butenolides have potent cytotoxic activity against the cancer cell lines NMC797 and MJ, with modest selectivity for NMC797 over MJ cells. The cytotoxicity is most potent when the aromatic appendages are electronically poor, although significant size restrictions exist. Furthermore, the positioning of the aromatic groups does not appear to be critical. The alkene is essential for cytotoxicity, which suggests that the molecules' cytotoxic properties could be due to covalent modifications. The cytotoxicity does not translate to the bacterial cell lines tested, which suggests that the activity may be mammalian cell-specific. A more thorough profiling of the compounds against alternative cell lines will provide some insight into possible mechanisms of action, as well as a better understanding into the selectivity and therapeutic potential of the compounds. These studies are currently underway.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(**A**) Previously unprecedented spirocyclic structure, and (**B**) a cascade process between γ -hydroxybutenolides and aldehydes that rapidly generates this compound. (**C**) Known, bioactive molecules with similar “extended ketal-lactone”, highlighted in red.

Figure 2.

Preliminary structure function studies against NMC-797 Nut Midline carcinoma cell line and MJ T cell lymphoma cell line in 48 hour viability assays. IC_{50} are reported along with 95% confidence interval from triplicate experiment. All molecules are racemic.

Figure 3.

Anticancer activity of **12** and **13**, which have the same aromatic appendages, but differ in the positioning of these groups. These molecules were made previously by treating **3** and **6** with phenylacetylene and 3-nitrophenylacetylene in the presence of triflic acid.¹ IC_{50} are reported along with 95% confidence interval from triplicate experiment

Figure 4.

Illustrative figures to help explain one of our hypotheses for the potency of racemic **12** and **13**, which are each approximately half the potency of **3**. Shown are the six enantiomers of **3**, **12**, and **13** binding to a generic representation of an enzyme pocket. The more potent conformations have the nitroaryl in the pocket, whereas the less potent have a phenyl group.

	(3)	(5)	(6)
<i>E. coli</i>	IC ₅₀ = > 500 μ M ^a MIC ₉₅ = > 500 μ M ^b	IC ₅₀ = > 500 μ M MIC ₉₅ = > 500 μ M	IC ₅₀ = > 500 μ M MIC ₉₅ = > 500 μ M
<i>B. subtilis</i>	IC ₅₀ = 216 ($\pm$ 10) μ M MIC ₉₅ = 425 ($\pm$ 7) μ M	IC ₅₀ = 408 ($\pm$ 6) μ M MIC ₉₅ = > 500 μ M	IC ₅₀ = 224 ($\pm$ 4) μ M MIC ₉₅ = > 500 μ M
<i>M. smegmatis</i>	IC ₅₀ = > 500 μ M MIC ₉₅ = > 500 μ M	IC ₅₀ = > 500 μ M MIC ₉₅ = > 500 μ M	IC ₅₀ = 313 ($\pm$ 17) μ M MIC ₉₅ = > 500 μ M

Figure 5.

Activity of select spirocycles against bacterial species representing Gram-negative and Gram positive, as well as *Mycobacterium* sp. ^aIC₅₀ ($\pm$ S.E.M., $n = 6$), half maximal inhibitory concentration; ^bMIC₉₅ ($\pm$ S.E.M., $n = 6$), minimal inhibitory concentration.

Scheme 1.

Reduction of alkene of **4** to generate compound **14**, along with activity against NMC-797 and MJ cell lines. IC₅₀ are reported as a 95% confidence interval from triplicate experiment. Molecules are racemic.