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# Synthesis and evaluation of (1*S*)-1,2-dihydro-1-naphthalenol derivatives against PANC-1 cells

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ABSTRACT

Several derivatives of (1*S*)-1,2-dihydro-1-naphthalenol ((*S*)-**7**) have been synthesized and evaluated against human pancreatic adenocarcinoma cell line PANC-1 under nutrient-rich and nutrient-deprived conditions. The *tert*-butyldiphenylsilyl protected homoallylic alcohol (*S*)-**8** displayed cytotoxicity against PANC-1 cells with an $LC_{50}$ value of 11 μM in the absence of essential amino acids, glucose, and serum, while exhibiting no cytotoxicity under nutrient-rich conditions. The observed selective antitumor activity of (*S*)-**8** under nutrient deprived conditions suggests its potential as a promising lead structure for the design of future anti-pancreatic cancer agents.



## Introduction

A recent area of focus in our laboratory has been the design, synthesis, evaluation, and optimization of novel compounds possessing activity against the human pancreatic cancer cell line, PANC-1.[1,2] In parallel with this ongoing research program, we recently published a novel synthetic approach toward the tertiary naphthoquinol core of the natural product spiroxin A.[3] Given the known cytotoxic activity of spiroxin A,[4] we sought to explore potential synergies between these two research areas. To this end, we evaluated the activity of various synthetic intermediates en route to spiroxin A against PANC-1 in an effort to identify a useful pharmacophore.

Pancreatic adenocarcinoma is one of the most lethal human cancers. It is the fourth leading cause of cancer-related deaths in the United States. The American Cancer Society estimated 46,420 new cases and 39,590 deaths in 2014. The 5-year survival rate of pancreatic cancer has remained the lowest (6%) among all cancers.[5] Pancreatic cancer is known for its early metastasis and aggressive invasion of surrounding tissues. Currently, no effective clinical treatment guarantees the complete eradication of pancreatic cancer. In addition, side effects that many cancer patients experience, such as nausea, vomiting, and hair loss, often accompany traditional existing chemotherapies due to the fact that chemotherapeutic cytotoxic drugs commonly target both malignant cells as well as healthy normal cells. Thus, there is an urgent need for effective anticancer drugs that are capable of targeting tumor cells specifically.

A distinctive feature of pancreatic cancer cells is their tolerance to nutrient- and oxygen-deprivation through tumor progression. Izuishi et al. reported that four pancreatic cancer cell lines, including PANC-1, survived for 48 h in the absence of essential amino acids, glucose, and serum, whereas normal fibroblasts died within 24 h under the exact same conditions.[6] Since normal tissues seldom encounter nutrient deprivation, the austerity of pancreatic cancer cells under nutrient-deprived conditions has become a novel and selective biochemical target for cancer therapy. Motivated by this unique characteristic, our laboratory has recently reported novel agents that specifically inhibit the proliferation of pancreatic tumor cells under nutrient-deprived conditions and show no cytotoxicity under nutrient-rich conditions.[1,2]

Spiroxins A–E (**1**–**5**, Fig. 1) belong to a family of natural products possessing a unique octacyclic structure, which consists of two epoxides, five six-membered rings, and a single five-membered ring all interlocking in a framework of only twenty carbons.[4] Of the known spiroxins, only spiroxin A (**1**) has been thoroughly investigated and found to exhibit antitumor activity in nude mice against ovarian carcinoma (59% inhibition after 21 days, 1 mg/kg dose) and cytotoxicity with a mean $IC_{50}$ value of 0.09 μg/mL against a panel of 25 diverse cell lines. This activity may arise in part by single-stranded DNA cleavage; however experiments have

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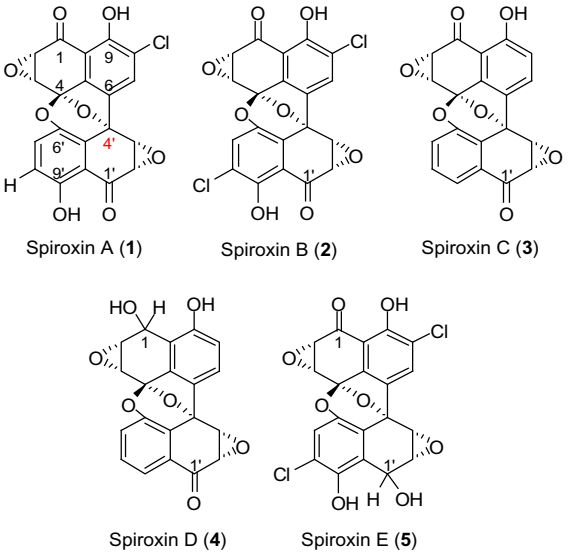



**Figure 1.** Spiroxins A–E.

demonstrated that spiroxin A reacts with 2-mercaptoethanol and dithiothreitol to form conjugates, indicating that its mechanism of action may be more complex.[4] To date, the biological activities of spiroxins B–E (**2**–**5**) have not been reported.

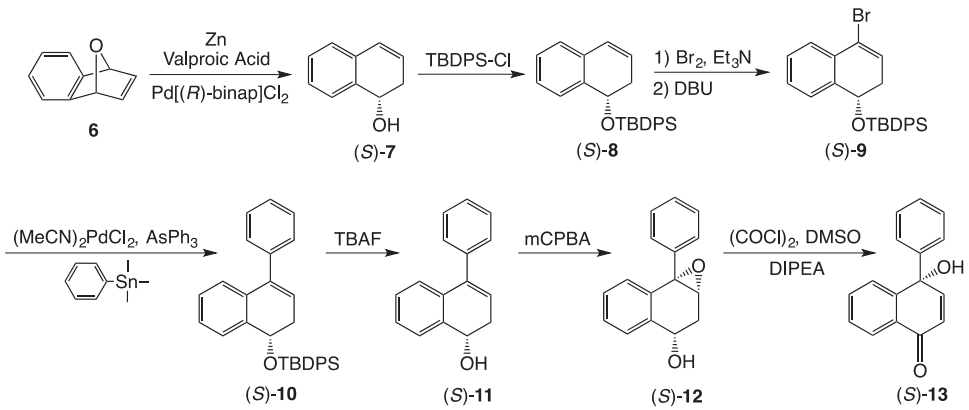
In 2011, our laboratory reported a novel catalytic asymmetric approach to the core structure of spiroxin A via a tandem oxidation/ring-opening sequence (Scheme 1).[3] Intrigued by the antitumor activity of spiroxin A and with ready access to multiple synthetic intermediates, we decided to examine whether structural components of the core tertiary naphthoquinol might themselves possess useful biological activity. Herein, we present the evaluation of structural components of spiroxin A against PANC-1 cells under both nutrient-deprived and nutrient-rich conditions, and the identification of a lead pharmacophore for future structure–activity relationship (SAR) studies.

## Results and discussion

Five compounds (**6**, (*S*)-**7**, (*S*)-**8**, (*S*)-**10**, (*S*)-**11**, Scheme 1) were available in sufficient quantity to be tested in vitro for their cytotoxic activity against PANC-1 cells under both nutrient-rich and nutrient-deprived conditions.[7] (*S*)-**7** was obtained by a stereoselective ring-opening of the cyclic ether **6**. The protection of (*S*)-**7** was accomplished using *tert*-butyldiphenylsilyl chloride and imidazole. Following the generation of a bromide intermediate (*S*)-**9**, the vinylarene (*S*)-**10** was obtained via a Stille cross-coupling using trimethyl(phenyl)stannane. Deprotection of (*S*)-**10** with tetrabutylammonium fluoride produced the alcohol (*S*)-**11**.[3]

As previously described, pancreatic tumor cells are known to be significantly more resilient than normal human cells under nutrient-deprived conditions. Therefore, we expect an ideal selective anti-austerity agent to induce cell death only under nutrient-deprived conditions conferred by the absence of essential amino acids, glucose, and serum. The survival of PANC-1 cells under nutrient-deprived and nutrient-rich conditions within 24 h following exposure to compounds **6**, (*S*)-**7**, (*S*)-**8**, (*S*)-**10**, and (*S*)-**11** is shown in Figure 2. In this study, compounds **6**, (*S*)-**7**, and (*S*)-**10** showed no cytotoxicity in either medium condition, even at 100 μM. Compound (*S*)-**11** also did not show appreciable cytotoxicity. It induced 30% cell death only at 100 μM, exhibiting no cytotoxicity at low concentrations. We were pleased to discover that compound (*S*)-**8**, exhibited preferential cytotoxicity under nutrient-deprived conditions, with an $LC_{50}$ of 11 μM. In this investigation, 100 μM of (*S*)-**8** induced 88% cell death under nutrient-deprived conditions, whereas no cytotoxicity was observed under nutrient-rich conditions (Fig. 2).

The preferential cytotoxicity of (*S*)-**8** suggests a future structure–activity relationship (SAR) study to further investigate which structural components of (*S*)-**8** are responsible for its activity. The significant difference in cytotoxicity between (*S*)-**8** and (*S*)-**7** indicates that the presence of the *tert*-butyldiphenylsilyl (TBDPS) protecting group may play an important role in the selective cytotoxic activity observed for (*S*)-**8**. Our results, however, show that the TBDPS group alone is insufficient to solely confer selective cytotoxicity to this series of compounds. For example, the inactivity of (*S*)-**10**, which also contains a TBDPS protecting group, suggests that cytotoxic activity is not necessarily directly related to the structure of the TBDPS group. Given these results, we explored the role of hydrophobicity in the activity observed for this series of compounds. To this end, the octanol–water partition coefficients of all five compounds (log*P*) were calculated and are listed in Table 1. Log*P* is important for predicting cell membrane penetration[8] and studies have utilized log*P* values to estimate biological activities of structurally-related compounds. For example, Fratello et al. reported a good correlation between the cytotoxicity of halogenated benzenes and their log*P* values[9], whereas Sasaki et al. re-evaluated the tumor-specific cytotoxicity of mitomycin C, bleomycin, and peplomycin based on their log*P* values.[10] Studies have shown excellent correlation between ChemDraw-estimated *C*log*P* values and experimentally measured log*P* values.[8,11] In this study, *C*log*P* estimations by ChemDraw® 8.0 (PerkinElmer, Cambridge, MA, USA) and the experimentally measured values




**Scheme 1.** Synthesis of enantioenriched tertiary naphthoquinol (*S*)-**13**.

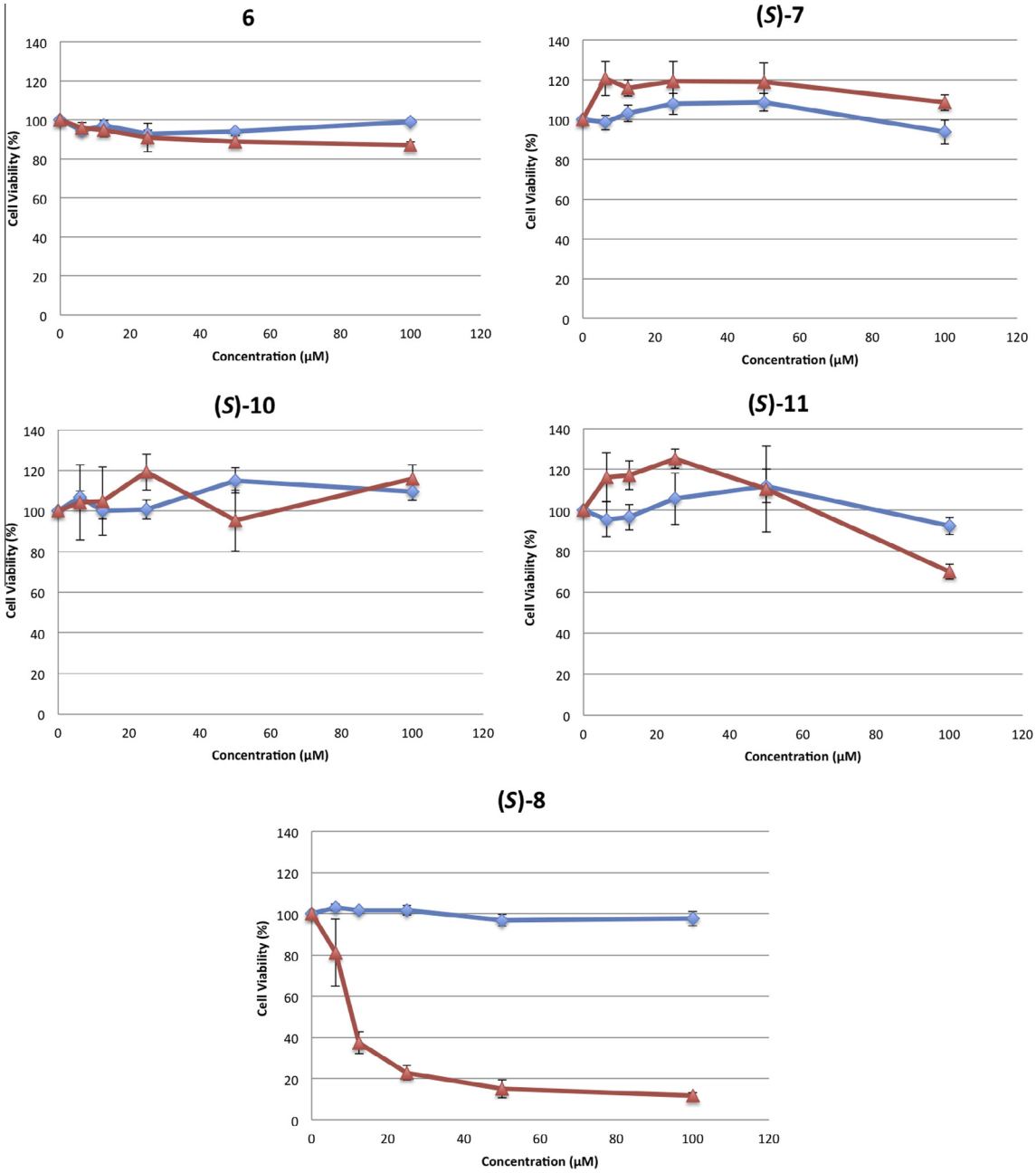



**Figure 2.** Survival of PANC-1 cells under nutrient-deprived conditions (red) within 24 h. PANC-1 cell survival under nutrient-rich conditions (blue) was examined as control. All cell viabilities are means of ±SEM, $n = 3$. Replicate experiments were performed and similar values were obtained. Concentrations of compounds investigated were 6.25, 12.5, 25, 50, and 100 μM.

**Table 1**
*C*Log*P* values of all compounds tested

| Compound | *C*Log*P* |
|---|---|
| **6** | 1.66 |
| (*S*)-**7** | 1.78 |
| (*S*)-**11** | 3.33 |
| (*S*)-**8** | 7.94 |
| (*S*)-**10** | 9.50 |

The *C*Log*P* values were obtained from ChemBioDraw® 13.0.

demonstrated excellent correlations ($r^2 = 0.97$).[11] The log*P* values examined in our study were calculated using ChemBioDraw® 13.0 (PerkinElmer, Cambridge, MA, USA). In the absence of the TBDPS protecting group, the *C*log*P* values of compounds **6**, (*S*)-**7**, and (*S*)-**11** are relatively low compared to that of (*S*)-**8**, suggesting a possible correlation between the increase in hydrophobicity and cell cytotoxicity. However, the TBDPS protected vinylarene (*S*)-**10**, possessing the highest *C*log*P* value of 9.5, did not exhibit any cytotoxic activity. This suggests that other factors are likely contributing to the activity of this compound series and further exploration of the SAR will be reported in due course.

In conclusion, derivatives of (1*S*)-1,2-dihydro-1-naphthalenol ((*S*)-**7**) were successfully synthesized and evaluated against PANC-1 cells under nutrient-rich and nutrient-deprived conditions. Among all compounds tested, the *tert*-butyldiphenylsilyl ether (*S*)-**8** induced 50% cell death at concentration of 11 μM selectively under nutrient-deprived conditions, while exhibiting no

cytotoxicity against PANC-1 cells under nutrient-rich conditions. Current efforts are ongoing to investigate the effect of altering various structural components of (*S*)-**8** on its preferential pancreatic antitumor activity.

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7. *In vitro cytotoxicity assay:* PANC-1 cells were seeded in 96-well plates at a density of $2.5 \times 10^4$ cells per well and incubated in a fresh Dulbecco's modified Eagle's medium (DMEM, Sigma Aldrich) at 37 °C, 5% $CO_2$ for 24 h. After rinsing with PBS, cells were subjected to the addition of either nutrient rich media (NRM: complete media with serum) or nutrient deprived media (NDM: media lacking amino acids, glucose, and serum). Serially diluted solutions of coumarin compounds (5.5% DMSO in NDM) were added to the cells up to a final concentration of 100 μM, followed by a 24 h incubation at 37 °C, 5% $CO_2$. Cell morphology was monitored under an inverted microscope. Cytotoxicity was assessed on PBS washed cells by the addition of fresh DMEM containing 10% WST-8 cell counting reagent (Dojindo Molecular Technologies). Following a 3 h incubation at 37 °C, 5% $CO_2$, absorbance values were measured with a plate reader at 450 nm, and cell viability was calculated using the equation % cell viability = $[\{Abs_{test\ sample} - Abs_{blank}\}/\{Abs_{control} - Abs_{blank}\}]*100$.
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