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Novel tertiary sulfonamides as potent anti-cancer agents

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ABSTRACT

For adult women in the United States, breast cancer is the most prevalent form of cancer. Compounds that target dysregulated signal transduction can be efficacious anti-cancer therapies. A prominent signaling pathway frequently dysregulated in breast cancer cells is the Wingless-related integration site (Wnt) pathway. The purpose of the work was to optimize a "hit" from a screening campaign. 76,000 compounds were tested in a Wnt transcription assay and revealed potent and reproducible "hit," compound **1**. Medicinal chemistry optimization of **1** led to more potent and drug-like molecules, **19**, **24** and **25** (i.e., Wnt pathway IC_{50} values = 11, 18 and 7 nM, respectively). The principal results showed compounds **19**, **24** and **25** were potent anti-proliferative agents in breast cancer cell lines, MCF-7 (i.e., IC_{50} values = 10, 7 and 4 nM, respectively) and MDA-MB 231 (i.e., IC_{50} values = 13, 13 and 16 nM, respectively). Compound **19** synergized anti-proliferation with chemotherapeutic Doxorubicin in vitro. A major conclusion was that compound **19** enhanced anti-proliferation of Doxorubicin in vitro and in a xenograft animal model of breast cancer.

1. Introduction

In the United States, breast cancer is the most prevalent form of cancer and second most prevalent cause of death for adult women.[1,2] Common anti-breast cancer therapeutic modalities typically use a combination of surgery, radiation and chemotherapeutics. Currently used chemotherapies include anthracyclines and taxanes (e.g., Doxorubicin and paclitaxel) and are usually used in combination with other chemotherapeutics including fluorouracil and cyclophosphamide.[3]

Therapies that target protein components of dysregulated signal transduction pathways can be efficacious anti-cancer therapies with minimal adverse effects.[4] Prominent among dysfunctional breast cancer signal transduction pathways is the Wingless-related integration site (Wnt) pathway.[5] Wnt is named after Int1, a mouse mammary proto-oncogene and Wingless (Wg), a gene from the Drosophila essential for wing development.[6] In adult women, genes for a dysfunctional Wnt pathway have been established in causal association with breast cancer.[7] Currently, targeting the Wnt pathway has been pursued for drug development, but only a few small molecules that modulate the Wnt pathway are approved for treatments, (i.e., Pyrvinium, Celecoxib and Sulindac).[8]

In addition to the Wnt pathway, dysregulation of the tumor protein p53 (p53) pathway is linked to the complexity of cancer biology.[9] Therapies that have targeted p53 have focused on restoration of functional activity of p53, but increases in endogenous p53 causes problems with cell adhesion and maintenance of tissue structure.[10,11]

Previously, a screen of 76,000 small molecules[12] was conducted for inhibition of Wnt transcription.[13,14] On the basis of results from this screen, one selective and reproducible "hit", (i.e., compound **1**) was identified.[15,16] In contrast to other Wnt inhibitors described in the literature that inhibit signaling at the Axin level,[13,14,17] **1** was shown to inhibit Wnt signaling far downstream in the pathway.[15] Herein, we describe the medicinal chemistry and development of potent Wnt inhibitors derived from "hit" **1**. The compounds elaborated were evaluated for Wnt inhibition, inhibition of breast cancer cell proliferation and induction of p53 transcription in vitro. Compound **1** was optimized for both potency of inhibition of Wnt and for improvement in physicochemical properties. Based on the results for Wnt inhibition and inhibition of breast cancer cell proliferation, a structure–activity relationship (SAR) arose for the compounds examined. The results led to

Abbreviations: Wnt, Wingless-related integration site gene; p53, tumor protein p53; Pd/C, palladium on carbon; HCl, hydrochloric acid; (ER+), estrogen receptor-positive; (ER−), estrogen receptor-negative; (PR−), progesterone receptor-negative; (HER2−), human epidermal growth factor receptor 2-negative; (DMEM), Dulbecco's Modified Eagle's cell culture medium; (FBS), fetal bovine serum

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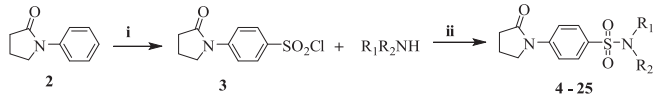


Scheme 1. General synthesis of pyrrolidinone phenylsulfonamides **4–25**. Reagents and conditions: i) chlorosulfonic acid, 25 °C, 15 h, ii) triethylamine, acetonitrile, 130 °C, 30 min.

synthesis of significantly more potent Wnt inhibitors with much improved anti-cancer and physicochemical properties. From SAR optimization of **1**, several compounds emerged (i.e., **19**, **24** and **25**) that showed increased Wnt potency and improved physicochemical properties. Compounds **19**, **24** and **25** were shown to be effective inhibitors of breast cancer cell (i.e., MCF-7 and MDA-MB 231) proliferation. In the presence of Doxorubicin, compound **19** showed increased inhibition of proliferation of breast cancer cells in vitro and in in vivo xenograft studies. Compounds **19**, **24** and **25** are new, potent small molecules with potent anti-breast cancer functional activity.

2. Results and discussion

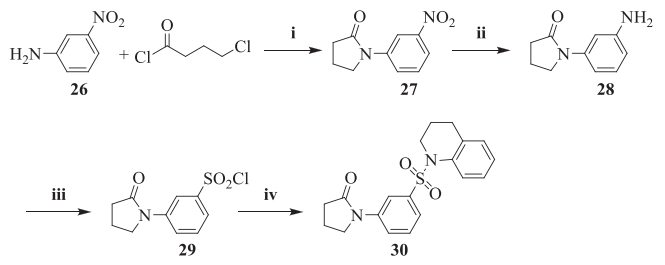
2.1. Chemical synthesis

Sulfonamides **4–25** were prepared using the synthetic route described in Scheme 1. In a typical synthesis, 1-phenylpyrrolidinone, **2**, was directly converted to the *para*-substituted sulfonyl chloride **3** using chlorosulfonic acid. Generally, the crude solid material was isolated in sufficient purity to use without further purification. Sulfonamides **4–25** were prepared by addition of the requisite amine to sulfonyl chloride **3**.

For the synthesis of *meta*-substituted sulfonamide **30**, a modified synthesis was used that followed literature procedures[18,19] (Scheme 2). 3-Nitroaniline, **26**, was converted to γ-lactam **27** by treatment with 4-chlorobutyryl chloride that was added slowly by addition funnel. In a second step, the crude reaction product was added to a freshly prepared solution of sodium ethoxide in ethanol to afford cyclization and produced a yellow-orange solid, **27**. Hydrogenation of **27** in the presence of Pd/C gave aniline **28**. Aniline **28** was converted to a diazonium salt with sodium nitrite under acidic conditions followed by addition of sulfur dioxide gas and copper (II) chloride dihydrate to afford **29**. Sulfonyl chloride **29** was combined with the requisite amine to provide the *meta*-substituted sulfonamide **30** in a similar manner as described in Scheme 1.

The synthesis of aryl-substituted compounds (e.g., **34**) was done beginning with synthesis of key intermediate **32** (i.e., 2-methylphenyl pyrrolidinone) according to a literature method[19] (Scheme 3). Aryl-substituted compound **34** was prepared by combining the sulfonyl chloride with an amine in a similar manner as described in Scheme 1.

Analogs were synthesized to examine the effect of substituents on the heterocyclic ring of the pyrrolidine moiety shown in Region **I** (Fig. 1). For example, to prepare 1,2,4-triazole analog **36** (Scheme 4a), phenylhydrazine hydrochloride and formamide were combined in the absence of solvent in a microwave vial and heated at 175 °C to afford 1-phenyl-1,2,4-triazole, **35**.[20] Intermediate **35** was directly converted to *para*-substituted sulfonyl chloride by treatment with chlorosulfonic acid. Likewise, dihydropyrazole analog **38** (Scheme 4b), was prepared. Phenylhydrazine and dibromopropane were combined in the absence of solvent in a microwave vial and heated at 175 °C to provide the dihydropyrazole **37** following a literature procedure.[21,22] Intermediate **37** was converted to its *para*-substituted sulfonyl chloride by treatment with chlorosulfonic acid. Sulfonamides **36** and **38** were prepared by combining sulfonyl chlorides formed from **35** and **37** with 1,2,3,4-tetrahydroisoquinoline according to the same procedure shown in Scheme 1.



Scheme 2. Synthesis of *meta*-substituted phenylpyrrolidinone sulfonamide **30**. Reagents and conditions: i) sodium phosphate dibasic, chloroform, 25 °C, 15 h; sodium ethoxide, ethanol, 25 °C, 15 h, ii) H_2, Pd/C, ethyl acetate, 25 °C, 15 h, iii) sodium nitrite, acetic acid/HCl, acetonitrile 0 °C, 1 h; $SO_{2(g)}$, copper (II) chloride dihydrate, 0–25 °C, 15 h, iv) 1,2,3,4-tetrahydroquinoline, triethylamine, acetonitrile, 130 °C, 30 min.

Pyrrolidine analogs (e.g., compound **39**) were prepared by direct reduction of pyrrolidinone **1** with zinc / triethoxysilane[23] as shown in Scheme 5.

3. Biological studies

3.1. Effect of sulfonamides on the Wnt pathway

A diverse set of 76,000 compounds[24] were tested for inhibition of Wnt transcription in a cell-based assay in HEK293 cells, where cells were transfected with a Wnt luciferase plasmid to over-express Wnt.[25] From a screen for inhibition of Wnt, one reproducible "hit" was found (i.e., compound **1**, IC_{50} = 25 nM). Counterscreens showed none of the "hit" compounds examined inhibited luciferase. Based on the structure of "hit" **1**, synthetic analogs **4–25**, **30**, **34**, **36**, **38** and **39** were synthesized and tested for inhibition of Wnt transcription. Each compound was tested in a dose-response study (i.e., 0–5 μM, nine concentrations, in triplicate) to determine an IC_{50} for inhibition of Wnt transcription. To systematically determine a structure-activity relationship (SAR) for inhibition of Wnt transcription and quantify the effect of modification of **1**, the molecule was conceptually divided into three exploratory regions: (**I**) the pyrrolidinone region, (red), (**II**) the central aryl region, (black) and (**III**) the 1,2,3,4-tetrahydroquinoline region, (blue). The 1,2,3,4-tetrahydroquinoline ring (**III**) was further conceptually divided into **A** and **B** rings (Fig. 1). Initially, a small group of approximately 20 compounds directed at structural modifications of the 1,2,3,4-tetrahydroquinoline portion of **1** (i.e., Region **III**) were synthesized and tested for inhibition of Wnt transcription (Table 1). The results of these studies showed that a sulfonyl-1,2,3,4-tetrahydroquinoline moiety was essential to maintain potency. For example, compared to **1**, ring-opening and ring contraction of the **A** ring of Region **III** (i.e., **4** and **5**, respectively, IC_{50} > 5000 nM) resulted in loss of potency. Various other unsaturated ring-systems (e.g., **6**, IC_{50} > 5000 nM) or saturated ring-systems (e.g., **7** and **8**, IC_{50} > 5000 nM, respectively) showed that other ring systems in Region **III** of the molecule decreased potency. To observe the effect of the regio orientation of the 1,2,3,4-tetrahydroquinoline nitrogen in ring system **III**, analog **9** was prepared and tested. Compared to **1**, 1,2,3,4-tetrahydroisoquinoline analog **9** gave decreased potency (IC_{50} = 634 nM). This indicated that the position of the nitrogen atom in the 1,2,3,4-tetrahydroquinoline ring system was essential to maintain maximal potency. The data for **4–9**, Table 1, showed the 1,2,3,4-tetrahydroquinoline moiety was necessary to maintain potency. Finally, to explore the effect of the sulfonamide moiety on inhibition of Wnt transcription, the analogous amide analog of **1** was prepared (i.e., **10**). Replacement of the sulfonamide moiety with an amide moiety resulted in loss of potency (i.e., **10** had an IC_{50} > 5000 nM). This data showed that the sulfonamide moiety of **1** was essential for the potency of the molecule.

To further examine the SAR around region **III**, substituted 1,2,3,4-tetrahydroquinolines were prepared (i.e., compounds **11–16**). The effect of small substituents on the aryl portion of the 1,2,3,4-tetrahydroquinoline ring of **1** on inhibition of Wnt transcription showed that