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Development of 6*H*-Chromeno[3,4-*c*]pyrido[3',2':4,5]thieno[2,3-*e*]pyridazin-6-ones as Par-4 Secretagogues

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Abstract

Nitrosation and cyclization of 4-(3-aminothieno[2,3-*b*]pyridine-2-yl)-2*H*-chromen-2-ones **1** afforded substituted 6*H*-chromeno[3,4-*c*]pyrido[3',2':4,5]thieno[2,3-*e*]pyridazin-6-ones **2** that inhibited the intermediary filament protein, vimentin, at low micromolar concentrations. This inhibition promoted the secretion of Prostate Apoptosis Response-4 protein (Par-4), which selectively triggered apoptosis in prostate cancer cells such as CWR22Rv1, LNCaP-derivative C4-2B, PC-3 and its aggressive analog, PC-3 MM2.

Keywords

Prostate Apoptosis Response-4 protein; Par-4; vimentin; prostate cancer; coumarin; pyridazine

Therapy-resistant prostate cancer cells, such as CWR22Rv1, LNCaP-derivative C4-2B, PC-3 and its aggressive analog, PC-3 MM2, are susceptible to apoptosis by the naturally occurring tumor suppressor, Prostate Apoptosis Response-4 protein (Par-4).^{1, 2} Par-4 binds to Glucose-regulated Protein-78 receptor (GRP78) found exclusively on cancer

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cells and triggers apoptosis.² Unfortunately, normal cells, even though they far outnumber cancer cells, sequester endogenous Par-4 and export it only at low levels. Although the administration of exogenous, recombinant Par-4 would provide an alternative to an endogenous source, Par-4 produced by mammalian cells was at least twenty-times more potent in inducing cancer cell apoptosis³ than recombinant Par-4 made in *E. coli*. Consequently, we focused attention on finding “small molecule” secretagogues that would disrupt sequestration and promote endogenous Par-4 secretion from normal cells.

We recently reported⁴ that the *in vitro* or *in vivo* administration of 3-arylquinolines at nanomolar concentrations promoted high levels of Par-4 secretion by inhibiting the sequestering agent, the intermediary filament protein, vimentin (Figure 1). This finding stimulated our interest in Par-4 secretagogues because of vimentin's role in the epithelial-mesenchymal transition (EMT) during which cancer cells lose their cell-cell adhesion and metastasize.^{5–8} Within three years of either surgery or hormone treatment, a significant number of prostate cancer patients exhibit castration-resistant tumors that ultimately metastasize to bone, lung, liver, pleura or adrenal glands,^{9, 10} resist further chemotherapeutic treatment, and have fatal outcomes. Consequently, we sought to develop compounds that promote secretion of Par-4 from normal cells by inhibiting the sequestration of Par-4 by vimentin (Figure 1).

We explored the synthesis of substituted quinolines, quinolones and coumarins, particularly those bearing aryl or heteroaryl rings that were either attached or fused to these heterocyclic platforms. In the course of these studies, we sought coumarins bearing fused, heterocyclic rings, and we now report the synthesis of 6*H*-chromeno[3,4-*c*]pyrido[3',2':4,5]thieno[2,3-*e*]pyridazin-6-ones **2** in which a pyridazine ring is fused to the coumarin framework. We selected the pyridazine ring based on its “privileged” position in drug development^{11, 12} and the multiple options for pyridazine synthesis¹³ that include: [1] hydrazine condensations with 1,4-dicarbonyl compounds; [2] Diels-Alder reaction of acetylenes with 1,2,4,5-tetrazines; [3] oxidation of 1,4-diamines; and [4] nitrosation of *ortho*-substituted anilines.

As shown in Scheme 1, we utilized a nitrosation of 4-(3-aminothieno[2,3-*b*]pyridin-2-yl)coumarins¹⁴ **1** to construct the desired pyridazines **2**, which represented a new heterocyclic system. The brief treatment of **1a–1e** with *n*-butyl nitrite in formic acid at 25°C afforded the fused pyridazines **2a–2e** in good yield. Similar nitrosations of anilines and cyclization of the intermediate diazonium ions afford pyridazines,^{15–18} albeit not involving capture of a diazonium formate by the 3-position of coumarins (Scheme 2). Subsequent aminomethylation reactions of the pyridazine **2c** or **2e** using bis(*N,N*dialkylamino) methanes in refluxing 2-methoxyethanol afforded the analogs **3** or **4**, respectively, as shown in Scheme 1, which were required for testing purposes. A similar aminomethylation with paraformaldehyde and primary amines led to the analogs **5a** and **5b** with a fused tetrahydro-1,3-oxazine ring but none of the bis-Mannich adducts seen in other systems.¹⁹ Once again, refluxing 2-methoxyethanol was required to solubilize **2c**, and other procedures using methanol, isopropanol or dioxane were less effective in this case.^{19–24}

Biological testing of the 6*H*-chromeno[3,4-*c*]pyrido[3',2':4,5]thieno[2,3-*e*]pyridazin-6-one **5a** induced the secretion of Par-4 from mouse embryonic fibroblast cells (MEFs) in a dose-

dependent fashion (Supplementary data) and resulted in apoptosis in prostate cancer PC-3 and DU145 cells but not, as expected, in normal fibroblasts (Figure 2). Co-cultures of cancer cells with Par-4 wild type MEFs, but not with Par-4-null MEFs, that were treated with **5a** produced apoptosis of the cancer cells, indicating that Par-4 secretion was essential for apoptosis (Supplementary data). In summary, we report the synthesis of pyridazine-substituted coumarins involving an interesting nitrosation-cyclization to afford a new family of fused heterocycles that promote secretion of the natural tumor suppressor, Par-4, and apoptosis in cancer cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Secretagogues that inhibit vimentin and promote Par-4 release and secretion.

Figure 2.

Compound **5a** induced dose-dependent apoptosis in PC-3 and DU145 cancer cells but not in normal MEF cells. Legend: V = vehicle, MEF = mouse embryonic fibroblast.

Scheme 3.

Synthesis of substituted 5*H*-chromeno[3,4-*c*]pyridazin-5-ones **2–5**. Legend: *a*, $n\text{-C}_4\text{H}_9\text{ONO}$, HCOOH , 25°C ; 5 min; *b*, $(\text{R}_4\text{R}_5\text{N})_2\text{CH}_2$, $\text{CH}_3\text{OCH}_2\text{CH}_2\text{OH}$, 130°C , 4 h; *c*, $\text{R}_6\text{CH}_2\text{NH}_2$, $(\text{CH}_2\text{O})_n$, $\text{CH}_3\text{OCH}_2\text{CH}_2\text{OH}$, 130°C , 4 h.

Scheme 2. Nitrosation and cyclization of 4-(3-aminothieno[2,3-*b*]pyridine-2-yl)-2*H*-chromen-2-ones **1**.