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Design, synthesis and prostate cancer cell-based studies of analogs of the Rho/MKL1 transcriptional pathway inhibitor, CCG-1423

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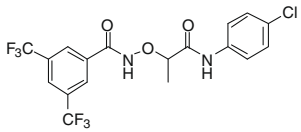
ABSTRACT

We recently identified bis(amide) CCG-1423 (**1**) as a novel inhibitor of RhoA/C-mediated gene transcription that is capable of inhibiting invasion of PC-3 prostate cancer cells in a Matrigel model of metastasis. An initial structure–activity relationship study focusing on bioisosteric replacement of the amides and conformational restriction identified two compounds, **4g** and **8**, with improved selectivity for inhibition of RhoA/C-mediated gene transcription and attenuated cytotoxicity relative to **1**. Both compounds were also capable of inhibiting cell invasion with equal efficacy to **1** but with less attendant cytotoxicity.

Cancer metastasis is a tremendous medical problem responsible for thousands of deaths every year.[1] Metastases arise when dysregulation of one or more cellular processes allow malignant cells to escape the confines of the tissue of origin and establish themselves in alternate sites. Signaling through RhoA/C is important for invasion and metastasis of many cancers.[2–4] In addition to the well-known role of RhoA/C in cytoskeletal function, there is a less well understood downstream action on gene transcription.[5,6] The pathway involved in this has recently been elucidated and several components are related to cancer pathogenesis. The mitogenic G protein coupled receptor ligands bombesin, thrombin, and lysophosphatidic acid (LPA) and their receptors are well-known mitogens and stimulate tumor invasion. The novel Gα12 family of heterotrimeric G proteins (G_{12} and G_{13}) activates RhoA and RhoC through guanine exchange factors such as leukemia-associated RhoGEF (LARG). Most relevant to the present work on Rho-transcriptional mechanisms are the megakaryoblastic leukemia transcriptional co-activator proteins (MKL1 & 2) which cooperate with the transcription factor, serum response factor (SRF), to increase expression of a number of genes potentially related to cancer progression and metastasis.[5,6] Exciting recent knockout and siRNA data have shown a key in vivo role for RhoC in breast cancer metastasis[7] and for MKL1 and SRF in melanoma and breast cancer metastases.[8] These studies provide important support for the idea that Rho signaling *and specifically Rho-regulated gene transcription* may be exciting targets for cancer therapy.

We recently identified a compound CCG-1423 (**1**) that blocks SRE-Luciferase gene transcription in response to activation of RhoA and RhoC signaling pathways.[9] Consistent with its role as a Rho/SRF pathway inhibitor, **1** potently (<1 μM) inhibited LPA-induced DNA synthesis in PC-3 prostate cancer cells. It also inhibited the growth of RhoC-overexpressing melanoma lines (A375M2 and SK-Mel-147) at nanomolar concentrations, but was less active on related cell lines (A375 and SK-Mel-28) that express lower levels of RhoC. Compound **1** inhibited Rho-dependent invasion by PC-3 prostate cancer cells, whereas it did not affect the $G\alpha_i$-dependent invasion by the SKOV-3 ovarian cancer cell line. Thus, based on its profile, **1** is a promising lead compound for the development of novel pharmacologic tools to disrupt transcriptional responses of the Rho pathway in cancer.



1 (CCG-1423)

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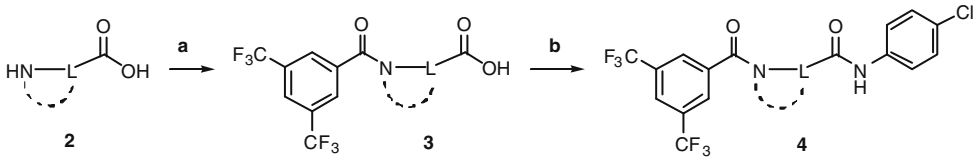
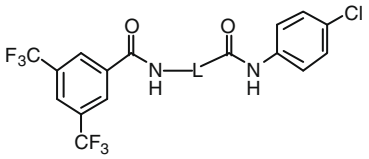


Scheme 1. Reagents and conditions: (a) 3,5-bis(CF_3)PhCOCl, aq NaOH, rt, overnight; or 3,5-bis(CF_3)PhCOCl, triethylamine (TEA), CH_2Cl_2; (b) 4-ClPhNH_2, EDC, HOBt, DIPEA, THF, rt, overnight.

Despite its favorable effects on cancer cell function, **1** did exhibit some modest acute cellular toxicity toward PC-3 cells at 24 h as evidenced by some non-specific inhibition of gene expression (TK-*Renilla*) and a parallel decrease in a WST-1 cell viability readout. Consequently, we undertook initial molecular modifications of **1** with the goal of improving its potency and/or selectivity and attenuating its cytotoxicity. Three structural features of the lead were identified as potential areas of concern. First, the N–O bond in the tether between the two carboxamides is susceptible to reductive cleavage by thiols, thereby giving **1** the potential to non-selectively modify cysteine-containing proteins or to be cleaved by glutathione. Second, the two carboxamides could be expected to limit potency by impeding cell penetration.[10,11] Finally, the relatively flexible nature of the tether between the two aromatic rings is likely not optimal for achieving both potency and selectivity.[12] Our initial strategy to modify **1** thus included: removal of the N–O bond, bioisosteric replacement of the amides,[13] and conformational restriction[14] of the tether between the aromatic rings. A limited survey of aromatic substitution was also undertaken to clarify the role of the lipophilic substituents.

The synthetic routes to new analogs of **1** are presented in Schemes 1–6. A preparation of bis(amides) **4** that was general for a variety of amino acids **2** (acyclic or cyclic, Tables 1 and 4) is summarized in Scheme 1. Acylation with bis(trifluoromethyl)benzoyl chloride, either under Schotten–Baumann conditions or under anhydrous conditions, afforded the mono(amides) **3** in good yields. Final amidation with 4-chloroaniline was then effected with *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole (HOBt) to afford bis(amides) **4**. *N*-Methylanilide **4e**, benzylamide **4f**, and indoline amide **4p** were made under similar conditions using *N*-methyl-4-chloroaniline, 4-chlorobenzylamine or 5-chloroindoline, respectively, in the final amidation step. Applying the same chemistry, bis(amides) **5a–g** of aminoxyacetic acid (Table 2) with various aromatic substitution

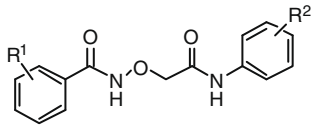
Table 1
Effects of tether length and composition on transcription and cytotoxicity in transfected PC-3 cells[a]



Compd	L	IC_{50} SRE.L[b] (μM)	% inh SRE.L[b] (10, 100 μM)	% inh pRL-TK[c] (10, 100 μM)	% inh WST-1[d] (10, 100 μM)
1	–OCH(CH_3)–	1.5	74, ND	48, ND	44, ND
5a	–OCH_2–	4.7	71, 100	53, 89	42, 91
4a	–CH_2CH_2–	38	38, 64	0, 22	0, 10
4b	–CH_2–	33	45, 85	15, 25	0, 30
4c	–$CH_2CH_2CH_2$–	21	37, 79	5, 42	0, 12
4d	–$CH_2CH_2CH_2CH_2$–	>100			

[a] For assay descriptions, see Ref. 20. All values are mean of ⩾3 experiments, each run in triplicate.
[b] Inhibition of Rho-pathway selective serum response element–luciferase reporter.
[c] Inhibition of control pRL-thymidine kinase *Renilla* luciferase reporter.
[d] Inhibition of mitochondrial metabolism of WST-1.

Table 2
Effects of aromatic substitution on transcription and cytotoxicity in transfected PC-3 cells[a]



Compd	R^1	R^2	IC_{50} SRE.L (μM)	% inh SRE.L (10, 100 μM)	% inh pRL-TK (10, 100 μM)	% inh WST-1 (10, 100 μM)
5a	3,5-Bis(CF_3)	4-Cl	4.7	71, 100	53, 89	42, 91
5b	3,5-Bis(CF_3)	3-Cl	5.9	65, 100	51, 89	49, 97
5c	3,5-Bis(CF_3)	4-H	36	13, 65	33, 59	0, 37
5d	3-CF_3	4-Cl	27	25, 86	5, 19	0, 58
5e	4-CF_3	4-Cl	29	26, 91	6, 0	0, 56
5f	4-H	4-Cl	>100			
5g	4-Cl	3,5-Bis(CF_3)	8.6	58, 100	19, 87	11, 96

[a] Assays defined in Table 1.