Bioorganic & Medicinal Chemistry Letters 25 (2015) 4838–4842

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Structure–activity relationship studies of QS11, a small molecule Wnt synergistic agonist

Manish K. Singh [a,†], Huanyao Gao [a,†], Wei Sun [a,†], Zhiquan Song [a], Robert Schmalzigaug [b], Richard T. Premont [b], Qisheng Zhang [a,*]

[a] Division of Chemical Biology and Medicinal Chemistry, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
[b] Division of Gastroenterology, School of Medicine, Duke University, Durham, NC 27710, USA

ARTICLE INFO

Article history:
Received 24 May 2015
Revised 15 June 2015
Accepted 17 June 2015
Available online 23 June 2015

Keywords:
Wnt/β-catenin signaling
ADP-ribosylation factors
GTPase-activating proteins
QS11
Structure–activity relationship (SAR)

ABSTRACT

Both the Wnt/β-catenin signaling pathway and small GTPases of the ADP-ribosylation factors (ARF) family play important roles in regulating cell development, homeostasis and fate. The previous report of QS11, a small molecule Wnt synergist that binds to ARF GTPase-activating protein 1 (ARFGAP1), suggests a role for ARFGAP1 in the Wnt/β-catenin pathway. However, direct inhibition of enzymatic activity of ARFGAP1 by QS11 has not been established. Whether ARFGAP1 is the only target that contributes to QS11's Wnt synergy is also not clear. Here we present structure–activity relationship (SAR) studies of QS11 analogs in two assays: direct inhibition of enzymatic activity of purified ARFGAP1 protein and cellular activation of the Wnt/β-catenin pathway. The results confirm the direct inhibition of ARFGAP1 by QS11, and also suggest the presence of other potential cellular targets of QS11.

The Wnt/β-catenin signaling pathway is evolutionarily conserved and plays crucial roles in cellular differentiation, proliferation and apoptosis. Aberrant regulation of the pathway has been associated with various diseases including colorectal cancer, bipolar disorder and osteoporosis.[1–3] Consequently, identifying novel Wnt modulators or pathways that cross-talk with the Wnt/β-catenin pathway has potential therapeutic significance.[4–6] Previously, the small molecule QS11 (Fig. 1) was demonstrated to synergize with Wnt proteins to activate β-catenin signaling.[7] This appears to be through binding and inhibiting the ADP-ribosylation factor GTPase-activating protein 1 (ARFGAP1). The close analog QS11-NC did not have effects on either Wnt signaling or ARFGAP1 activity.[7] These results suggest an unexpected role of ARFGAP1 in the Wnt/β-catenin pathway.

ADP ribosylation factors (ARFs) are a family of GTP-binding proteins that are functional in cellular vesicle trafficking and actin remodeling processes,[8,9] and have been associated with various diseases such as invasive breast cancer, colorectal cancer, and autosomal recessive periventricular heterotopia.[10,11] Like other small GTPases, ARFs are activated by guanine nucleotide exchange factors (GEFs) that facilitate the release of GDP and binding of GTP, and deactivated by GAPs that catalyze the hydrolysis of bound GTP

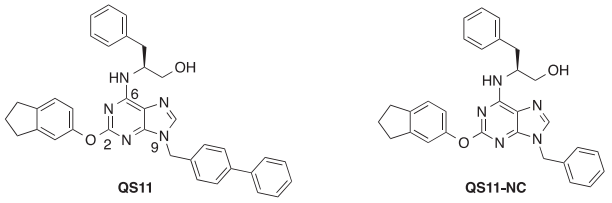
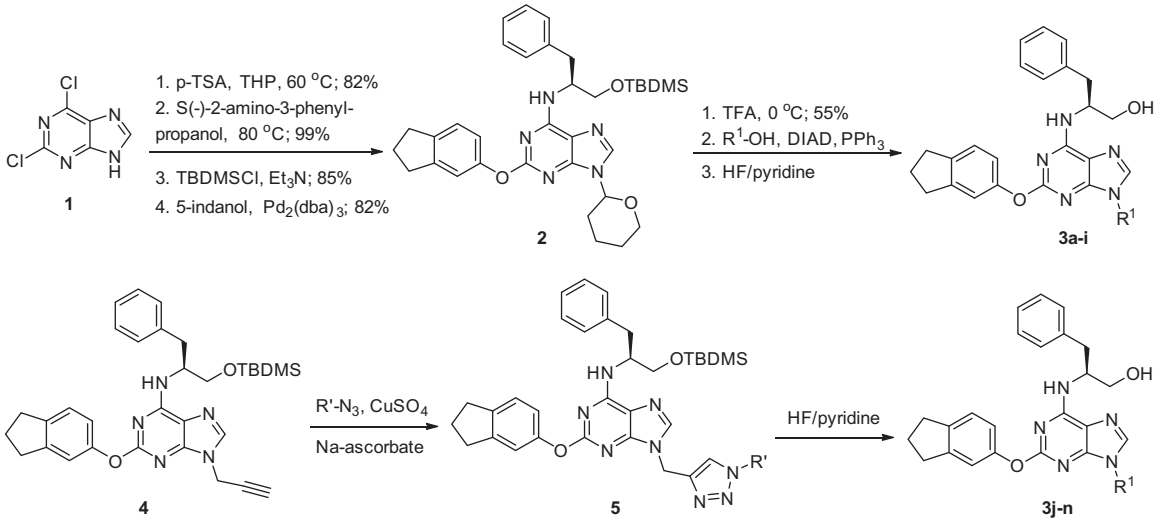


Figure 1. Chemical structures of QS11 and QS11-NC.

to GDP.[12] Different from other small GTPases, guanine nucleotide binding of ARFs is accompanied by conformational changes at its unique myristoylated N-terminal helix and by membrane association/dissociation.[13–16] The mechanism of QS11 has therefore been proposed as activating cellular ARFs through inhibiting ARFGAP1, and QS11 has been successfully employed as ARFGAP inhibitors in a few studies in cellular environments.[17–19] This hypothesis has been supported by other recent explorations of the role of ARFs for the Wnt/β-catenin signaling pathway. For example, Kim and co-workers showed that ARF-GTP level transiently increased upon stimulation with Wnt in a frizzled (Fzd), dishevelled, and LRP6-dependent manner.[20] In addition, the activation of ARF1 was essential for Wnt-mediated synthesis of PtdIns(4,5)P_2, which regulates the aggregation, phosphorylation and endocytosis of LRP6. Grossmann and coworkers further showed that in melanoma

* Corresponding author.
E-mail address: qszhang@unc.edu (Q. Zhang).
† These authors contribute equally to this work.

http://dx.doi.org/10.1016/j.bmcl.2015.06.062
0960-894X/

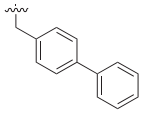
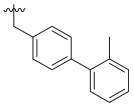
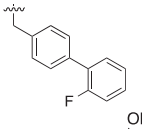
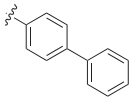
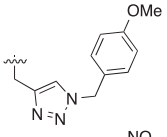
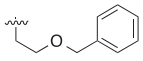
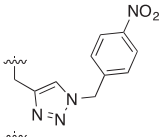
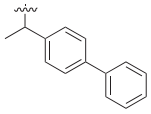
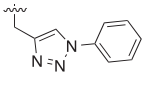
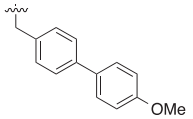
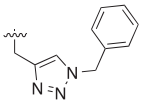
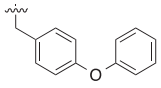
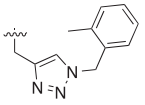


Scheme 1. Synthesis of **QS11** analogs with modifications at the N9 position.

cells, ARF6 was activated via Fzd4-LRP6, which led to dissociation of β-catenin from membrane-bound N-cadherin and subsequently enhanced β-catenin-mediated gene transcription and cell invasion.[21] Despite these positive connections, the direct inhibition of ARFGAP1 or any other GAP by QS11 has not been established. In addition, whether ARFGAP1 is the only major target of QS11 that contributes to its Wnt synergy remains unclear.

We synthesized QS11 derivatives and tested their activity in two assays that measure their capacity as ARFGAP1 inhibitors and as Wnt synergists for three reasons: (1) to confirm direct inhibition of ARFGAP activity by QS11; (2) to improve QS11's potency and physical properties such as solubility; and (3) to compare the SAR of the two sets of assay data. The assays were carried out using modifications to protocols previously described

Table 1
SAR on QS11 analogues with modifications at the N-9 position

Compound	R^1	EC_{50}[a] (μM)	Activity[b] (%)	Compound	R^1	EC_{50}[a] (μM)	Activity[b] (%)
3a (**QS11**)		1.5	10 ± 6/33 ± 12	**3h**		>100	41 ± 3/56 ± 11
3b (**QS11-NC**)		>100	113 ± 15/117/12	**3i**		>100	47 ± 18/81 ± 13
3c		>100	32 ± 9/50 ± 22	**3j**		>100	75 ± 5/98 ± 7
3d		>100	109 ± 4/106 ± 11	**3k**		>100	45 ± 2/58 ± 4
3e		2.6	36 ± 5/50 ± 7	**3l**		>100	57 ± 10/105 ± 11
3f		>100	34 ± 4/42 ± 4	**3m**		>100	62 ± 6/93 ± 7
3g		>100	40 ± 2/42 ± 1	**3n**		>100	27 ± 4/50 ± 2

[a] EC_{50} in TOPFlash reporter assay in μM.
[b] Percent remaining activity (RA) of ARFGAP1 with 20 μM and 10 μM of QS11 analogues in [γ-^{32}P]GTP assay. Number expressed as mean ± standard deviation.