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Synthesis, determination of stereochemistry, and evaluation of new bisindole alkaloids from the myxomycete *Arcyria ferruginea*: An approach for Wnt signal inhibitor

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Abstract—To determine the stereochemistry of dihydroarcyriarubin C (**1**), new bisindole alkaloid isolated from the myxomycete *Arcyria ferruginea*, *cis*- (**2**) and *trans*-dihydroarcyriarubin C (**3**) were synthesized. Comparison of their NMR characteristics allowed the *trans* stereochemistry of the natural product to be confirmed. Moreover, the Wnt signal inhibitory activities of **2** and **3** were compared with that of arcyriaflavin C (**4**), which is a natural product containing a bond between C-2 and C-2′. The *cis*-dihydroarcyriarubin C (**2**) showed moderate inhibition of Wnt signal transcription, which suggests that bisindole frameworks might be useful as small-molecule Wnt signal inhibitors.

Bisindole alkaloids have received considerable attention because they have potential for biological activity. Our recent interest in myxomycetes, an unusual group of primitive organisms, has led us to isolate new natural compounds;[1–3] for instance, we have recently isolated a new bisindole alkaloid, dihydroarcyriarubin C (Fig. 1: **1**), together with arcyriaflavin C (**4**),[3] from the myxomycete *Arcyria ferruginea*.[4] However, we had not determined its stereochemistry and bioactivity. Here, we describe the synthesis of two stereoisomers of **1**, *cis*- (**2**) and *trans*-dihydroarcyriarubin C (**3**), and use them to determine its stereochemistry. Moreover, we found the Wnt signal inhibitory activity of *cis*-dihydroarcyriarubin C (**2**) and arcyriaflavin C (**4**).

From a number of possible synthetic approaches to the bisindole compounds, we first attempted a coupling between the appropriate indolyl-glyoxlate and indolylacetamide (Scheme 1).[5] Reduction of 6-methoxyindolyl glyoxylamide (**5**) with $NaBH_4$ followed by dehydroxylation under TMSCl, NaI conditions, gave methyl indolylacetamide (**7**). Methyl indolyl-glyoxlate (**8**), prepared from 6-methoxy indole, reacted with **7** to give bis-indolylmaleimide (**9**). Hydrogenation with palladium on carbon in methanol afforded an 82% yield of the *meso* succimide (**10**). The *dl* isomer (**11**) was readily prepared by reduction with magnesium in refluxing methanol.[6a–d]

To clarify the configuration, the *cis*-isomer (**10**) was treated with magnesium in refluxing methanol to give the thermally stable *trans*-isomer (**11**). The ^1H NMR peak patterns were similar for natural product **1** and the *trans*-isomer (**11**) strongly suggesting that **1** has a *trans* configuration.[7]

Several methods for deprotecting the isomers were examined, but the corresponding bisindole products

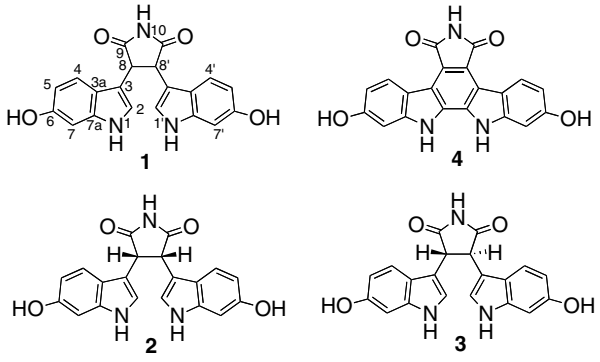

Figure 1. Structures of compounds **1**–**4**.

Keywords: *Arcyria ferruginea*; Bisindole alkaloid; Wnt signal inhibitor.
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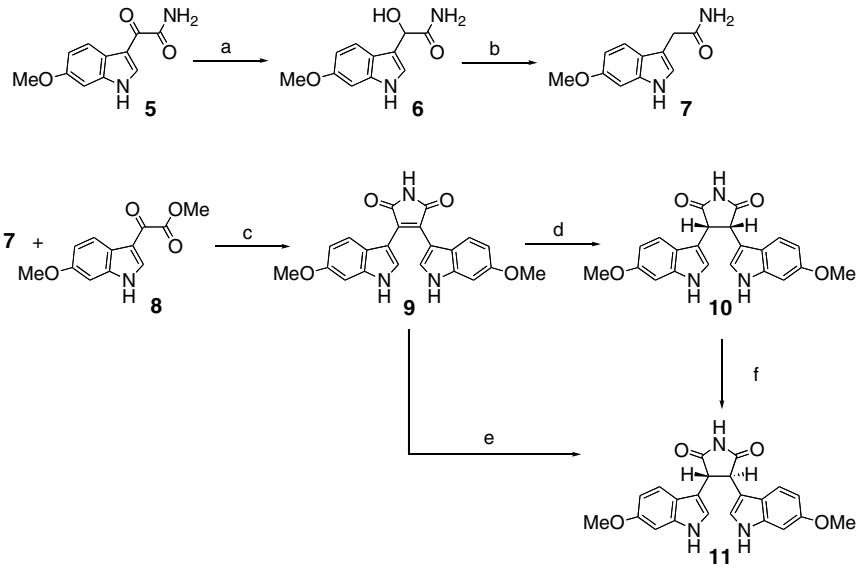


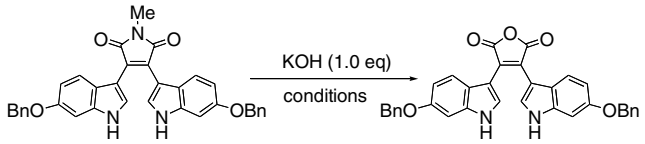
Scheme 1. Reagents and conditions: (a) $NaBH_4$, MeOH, 4 h, 61%; (b) TMSCl, NaI, CH_3CN, 0 °C, 5 h, 35%; (c) KO^{t}Bu, THF, 0 °C to rt, 50%; (d) cat. Pd/C, H_2, MeOH, 48 h, 82%; (e) Mg, MeOH, reflux, 10 h, 56%; (f) Mg, MeOH, reflux, 2 h, 57%.

were not obtained. From the desire to change the protective group and to improve the yield of the coupling of **7** and **8**, the synthetic route was changed to use the reaction of 2,3-dibromo-*N*-methylmaleimide and 4-benzyloxyindole. The compound **12** was synthesized in four steps following the procedure of Ohkubo et al.[8] The conditions for conversion of **12** to the maleic anhydride **13** were examined (Table 1). Compound **12** is quite insoluble in H_2O, so no reaction took place under aqueous conditions (run 1); using an organic solvent (dioxane, ethanol) allowed synthesis of **13** in good yield under reflux conditions (run 3, 4).

After ammonolysis of **13**, compound **14** was reduced to the *cis*-isomer (**2**) using Pd/C under H_2 atmosphere (Scheme 2). Treatment of **14** with Mg gave compound **15** in 84% yield, and then **3** was obtained by reduction. Product **3**, produced by epimerization of **2** under basic conditions, proved to be the thermally stable *trans*-isomer.

NMR data from the natural isolated dihydroarcyriarubin C (**1**) and the synthesized *cis*- (**2**) and *trans*-isomers (**3**) are shown in Table 2. The chemical shifts of **3** were identical to those of **1**; the *cis*- and *trans*-isomers were obviously different. Thus, the stereochemistry of **1** was shown to be *trans*.[9]

Table 1.



12 **13**

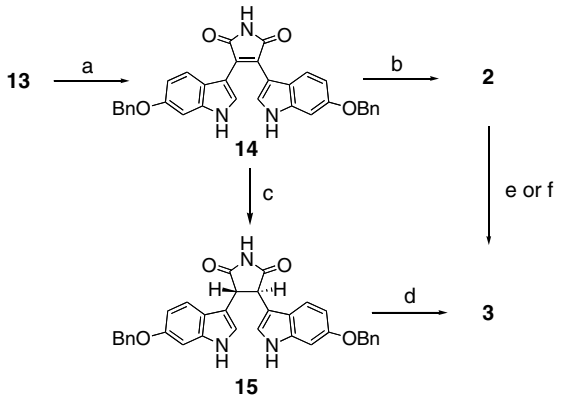
Run[a]	Solvent (v/v)	Temperature (°C)	Time (h)	Yield (%)
1	H_2O	Reflux	10	No reaction
2	Dioxane/H_2O (5:1)	rt	96	0
3	Dioxane/H_2O (5:1)	Reflux	24	84
4	EtOH/H_2O (5:1)	Reflux	4.5	89

[a] All reactions were carried out using 2 M aq KOH.

With synthetic *cis*- and *trans*-dihydroarcyriarubin C in hand, we evaluated their biological activities.[10] Arcyriaflavin C (**4**), which has a C–C bond between C-2 and C-2′, was also synthesized.[6b,8] The Wnt signal transduction pathway has important functions in vertebrate development, and its deregulation is believed to occur early in human colorectal cancer.[11,12] Wnt target genes have recently been found to be highly expressed in colon cancers; some of the genes are likely to contribute to cancer formation.[13] Small-molecule inhibitors of this pathway are desired because it is believed their clinical use would suppress cancer cell growth. Several potent small-molecule inhibitors have been reported;[14a–4d] however, the development of therapies to specifically target the Wnt pathway in cancer cells is still in its infancy.

We examined the Wnt signal inhibitory activity of our synthesized compounds using the luciferase reporter gene assay. Wnt signaling activates gene transcription by forming a complex between DNA-binding proteins of the Tcf/LEF family and β-catenin. SuperTOP-Flash, a β-catenin-responsive reporter plasmid with multiple TCF-binding sites (CCTTTGATC), was activated in cells.[15,16] SuperFOP-Flash has eight mutated TCF-binding sites (CCTTTGGCC), so a selective inhibitor would not show enhanced transcription in SuperFOP-Flash-transfected cells; thus, it provides a negative control for the assay and the TOP/FOP-Flash reporter activation ratio provides a measure of selective Wnt signal inhibition.

The results are shown in Figure 2, along with cell viability in order that a decrease in cell number should not resemble inhibition. The *cis*-isomer (**2**) exhibited moderate dose-dependent Wnt signal inhibition, whereas the



Scheme 2. Reagents and conditions: (a) NH_4OAc, 140 °C, 3 h, 97%; (b) cat. Pd/C, H_2, EtOH, 12 h, 95%; (c) Mg, MeOH, reflux, 6 h, 84%; (d) cat. Pd/C, H_2, EtOH, 5 h, 98%; (e) Mg, MeOH, reflux, 7 h, 81%; (f) DBU, THF, rt to reflux, 15 h, 93%.

trans-isomer (**3**) had no effect. Interestingly, arcyriaflavin C (**4**) also showed dose-dependent inhibition between 12.5 and 50 μM, although the Wnt signal activation effect seems to occur at low concentration. There is no reported potent inhibitor with bisindole framework, we believe that these results are interesting for a search of Wnt signal inhibitor from many known or new indole compounds. A large number of bisindole compounds have been reported as kinase inhibitors. We also reported kinase inhibitory activities of bisindole alkaloids from a myxomycete.[2] Wnt signal pathway includes several kinases such as GSK3β which phosphorylates β-catenin for degradation. Because these kinase inhibition may lead to activation of β-catenin–TCF transcription, these bisindole compounds might inhibit a β-catenin–TCF protein interaction. A protein level mechanistic study is in progress.

In summary, the configuration of the new bisindole compound dihydroarcyriarubin C (**1**), which had previously been isolated by our group, was determined to be *trans* by comparison of its NMR data with that of its synthesized *cis*- (**2**) and *trans*-isomers (**3**). The *cis*-dihydroarcyriarubin C (**2**) showed moderate inhibition of Wnt signal transcription. Thus, we suggest that a bisindole framework would be included as a candidate for small-molecule Wnt signal inhibitor.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.05.033.

Table 2. ^1H and ^{13}C NMR data of compounds **1**–**3** (in acetone-d_6)

	Dihydroarcyriarubin C (**1**)		**2** (*cis*)		**3** (*trans*)	
	δ_H (Hz)	δ_c[a]	δ_H (Hz)	δ_c	δ_H (Hz)	δ_c
1,1′	9.88 br s		9.48 br s		9.88 br s	
2,2′	7.15 d (2.2)	122.3	6.60 d (2.0)	123.4	7.16 d (2.4)	122.6
3,3′		111.9		109.7		112.0
3a,3a′		121.0		122.2		121.0
4,4′	7.25 d (8.5)	120.1	7.28 d (8.8)	120.0	7.26 d (8.4)	120.1
5,5′	6.61 dd (2.2, 8.5)	110.2	6.49 dd (2.0, 8.8)	109.8	6.61 dd (2.4, 8.4)	110.4
6,6′		154.7		154.0		139.1
7,7′	6.85 d (2.2)	97.7	6.76 d (2.0)	97.3	6.85 d (2.4)	97.7
7,7a′		139.1		138.0		139.1
8,8′	4.44 s	48.8	4.90 s	46.3	4.45 s	48.9
9,9′		178.5		179.2		178.3
6,6′-OH	7.91 br s		7.73 s		7.91 br s	
10-NH	9.51 br s		10.28 br s		10.29 br s	

[a] The ^{13}C NMR chemical shifts of natural dihydroarcyriarubin C (**1**) were reinvestigated and revised as shown here.

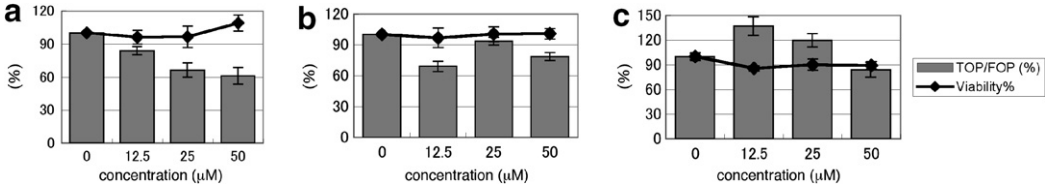

Figure 2. Wnt signal inhibitory activity. (a) *cis*-dihydroarcyriarubin C (**2**); (b) *trans*-dihydroarcyriarubin C (**3**); (c) arcyriaflavin C (**4**).

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