Bioorganic & Medicinal Chemistry Letters 26 (2016) 3529–3532

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and evaluation of (+)-decursin derivatives as inhibitors of the Wnt/β-catenin pathway

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ARTICLE INFO

Article history:
Received 4 November 2015
Revised 7 June 2016
Accepted 10 June 2016
Available online 11 June 2016

Keywords:
Cinnamoyl decursin
Phenylpropionyl decursin
Wnt/β-catenin pathway
Prostate cancer
Protein degradation

ABSTRACT

We synthesized (+)-decursin derivatives substituted with cinnamoyl- and phenyl propionyl groups originating from (+)-CGK062 and screened them using a cell-based assay to detect relative luciferase reporter activity. Of this series, compound **8b**, in which a 3-acetoxy cinnamoyl group was introduced, most potently inhibited (97.0%) the Wnt/β-catenin pathway. Specifically, compound **8b** dose-dependently inhibited Wnt3a-induced expression of the β-catenin response transcription (CRT) and increased β-catenin degradation in HEK293 reporter cells. Furthermore, compound **8b** suppressed expression of the downstream β-catenin target genes cyclin D1 and c-myc and suppressed PC3 cell growth in a concentration-dependent manner.

The Wnt/β-catenin signaling pathway is associated with embryonic development and regulates cell proliferation and differentiation. Abnormal activity of this pathway and increased β-catenin-dependent transcription induces tumor development including colon and prostate cancer.[1–3]

6,7-Dihydroxycoumarin (**1**, Fig. 1), also called esculetin, was recently identified as a potential Wnt/β-catenin pathway inhibitor. This compound suppresses growth of human colon cancer cells by disrupting formation of the β-catenin-Tcf complex.[4] We also found that (+)-decursin (**2**, Fig. 1) and (+)-CGK062 (**3**, Fig. 1), which have a coumarin-ring skeleton similar to esculetin, inhibit Wnt/β-catenin pathway activity.[5–7] In particular, (+)-CGK062, in which a 3,4-dihydroxy cinnamoyl group was introduced, potently inhibits (99.5%) the Wnt/β-catenin pathway and dose-dependently antagonizes Wnt3a-induced β-catenin response transcription (CRT). In addition, 100 mg/kg (+)-CGK062 inhibits PC3 tumor growth and 50 mg/kg (+)-CGK062 inhibits >80% of subcutaneously established PC3 xenograft tumor growth in athymic nude mice. This compound induces no observable signs of toxicity in mice.

In this study, we synthesized (+)-decursin derivatives with various cinnamoyl and phenyl propionyl groups using (+)-CGK062 as the lead compound. These compounds were screened using a cell-based assay to detect relative luciferase reporter activity.

Semi-synthesis of the (+)-decursin derivatives is outlined in Scheme 1. (+)-Decursinol (**4**) was the starting material obtained by hydrolyzing an ethanol extract of *Angelica gigas* root with alkaline solvent.[8] Esterification by EDC-mediated coupling provided non-substituted cinnamoyl-, halo-substituted cinnamoyl-, nitro-substituted cinnamoyl- and methoxy-cinnamoyl-decursin derivatives **5a–d** and **6a–i**. Demethylation of $(OCH_3)_n$-cinnamoyl decursin derivatives **6a–d** and **6h** was accomplished using 1 M BBr_3 solution, leading to **7a–e**. The $(OH)_n$-cinnamoyl decursin derivatives **3** (CGK062) and **7a–e** were acetylated using acetyl chloride to obtain good yields of the $(OAc)_n$-cinnamoyl decursin derivatives **8a–f**.[9–16] We synthesized (+)-decursin derivatives by introducing various substituted phenyl propionyl groups. The reaction conditions for these compounds were identical to the conditions used for compounds **6–8**.

We used a cell-based screening system to identify decursin derivatives that inhibit the Wnt/β-catenin pathway. HEK293 reporter cells were stably transfected with TOPFlash and the hFz-1 expression plasmid. TOPFlash reporter activity was monitored using a microplate reader after adding Wnt3a-conditioned

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http://dx.doi.org/10.1016/j.bmcl.2016.06.029
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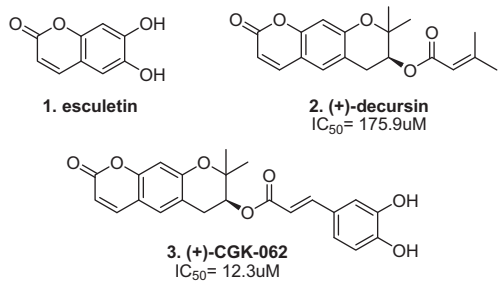


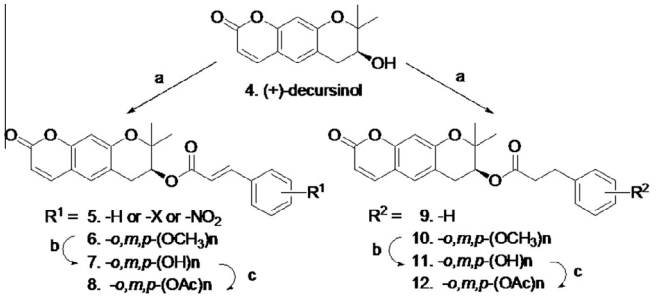
Figure 1. Inhibitors of the Wnt/β-catenin pathway.

medium (Wnt3a-CM) to each decursin derivative.[17–22] The controls were assayed in the presence or absence of Wnt3a-CM.

Results are shown in Tables 1 and 2. The (+)-decursin derivatives with a cinnamoyl group were more potent inhibitors of the Wnt/β-catenin pathway than (+)-decursin derivatives with a phenyl propionyl group. This result indicates that the double bond between the ester and phenyl groups is very important for Wnt/β-catenin pathway inhibitory activity. The position of the cinnamoyl group substituent on the decursin derivative was a significant factor for inhibiting the Wnt/β-catenin pathway. *Ortho*- or *meta*-substituted cinnamoyl decursin derivatives **5b**, **5c**, **6a**, **6b**, **7a**, **7b**, **7d**, **8a**, **8b**, **8d**, **8e**, and **11d** were potent inhibitors of the Wnt/β-catenin pathway.

In particular, (+)-decursin derivative **8b**, with a 3-acetoxy cinnamoyl group, exhibited strong inhibitory activity, similar to that of CGK062 which had two phenolic OH group. To our best knowledge, because the proportion of poly phenolic groups of CGK-062 excreted in the free state was very low due to conjugation phenol groups with glucuronic acid and sulfuric acid in vivo, compound **8b** with acetyl group instead of phenolic groups had the advantage compared to CGK-062. (+)-Decursin derivatives with a $(OH)_n$-cinnamoyl group (**3**, **7a**–**e**) or a $(OAc)_n$-cinnamoyl (**8a**–**f**) group strongly inhibited the Wnt/β-catenin pathway.

We further characterized the effects of compound **8b**, 3-(3-acetoxy-phenyl)-acrylic acid, and 8,8-dimethyl-2-oxo-6,7-dihydro-*2H,8H*-pyrano[3,2-*g*]chromen-7yl-ester on the Wnt/β-catenin pathway. Treating HEK293 reporter cells with varying concentrations of compound **8b** dose-dependently decreased CRT induced by Wnt3a (IC_{50} = 9.85 μM) (Fig. 2A). A western blot analysis with an anti-β-catenin antibody was performed to examine whether compound **8b** affected intracellular β-catenin levels in HEK293 cells, as CRT is regulated by intracellular β-catenin.[23] β-Catenin level increased consistently upon treatment with Wnt3a-CM, which was downregulated by compound **8b** (Fig. 2B), consistent with its inhibitory effect on Wnt3a-stimulated CRT, and suggesting



Scheme 1. Reagents and conditions: (a) cinnamic acid or phenyl propionic acid, EDC, 4-DMAP, dry MC, (b) 1 M BBr3 solution, dry MC, (c) acetyl chloride, pyridine, dry MC.

Table 1
Inhibitory percentage of the Wnt/β-catenin pathway for (+)-decursin derivatives, introduced cinnamoyl group

Compd No.	R^1	% of inhibition[a]
2	(+)-Decursin	79.4
3	(+)-CGK062	99.5
5a	–H	81.1
5b	–3-F	93.6
5c	–3-Br	84.8
5d	–4-Br	40.1
5e	–4-NO_2	34.3
6a	–2-OCH_3	88.3
6b	–3-OCH_3	87.5
6c	–4-OCH_3	54.8
6d	–2,3-$(OCH_3)_2$	57.8
6e	–2,4-$(OCH_3)_2$	52.8
6f	–2,5-$(OCH_3)_2$	65.3
6g	–3,4-$(OCH_3)_2$	42.3
6h	–3,4,5-$(OCH_3)_3$	46.9
6i	–2,4,5-$(OCH_3)_3$	59.6
7a	–2-OH	94.2
7b	–3-OH	93.3
7c	–4-OH	78.1
7d	–2,3-$(OH)_2$	95.7
7e	–3,4,5-$(OH)_3$	75.3
8a	–2-OAc	95.9
8b	–3-OAc	97.0
8c	–4-OAc	79.6
8d	–2,3-$(OAc)_2$	89.7
8e	–3,4-$(OAc)_2$	94.0
8f	–3,4,5-$(OAc)_3$	67.4

[a] These compounds were tested in concentration of 20 μM.

Table 2
Inhibitory percentage of the Wnt/β-catenin pathway for (+)-decursin derivatives, introduced phenyl propionyl group

Compd No.	R^2	% of inhibition[a]
2	(+)-Decursin	79.4
3	(+)-CGK062	99.5
9	–H	10.6
10a	–2-OCH_3	50.4
10b	–3-OCH_3	49.9
10c	–4-OCH_3	41.5
10d	–2,3-$(OCH_3)_2$	13.5
10e	–2,4-$(OCH_3)_2$	18.8
10f	–2,5-$(OCH_3)_2$	19.9
10g	–3,4-$(OCH_3)_2$	25.1
11a	–2-OH	10.0
11b	–3-OH	42.0
11c	–4-OH	32.8
11d	–2,3-$(OH)_2$	81.4
11e	–3,4-$(OH)_2$	68.0
12a	–2-OAc	23.1
12b	–3-OAc	27.5
12c	–4-OAc	12.0
12d	–2,3-$(OAc)_2$	77.7
12e	–3,4-$(OAc)_2$	46.4

[a] These compounds were tested in concentration of 20 μM.

that compound **8b** attenuates the Wnt/β-catenin pathway by lowering β-catenin protein levels.[24]

The Wnt/β-catenin pathway is activated by aberrant upregulation of intracellular β-catenin in PC3 prostate cancer cells. Thus, we carried out a western blot analysis to determine cytoplasmic β-catenin levels in compound **8b**-treated PC3 cells.[25] As shown in Figure 3A, compound **8b** dose-dependently downregulated cytoplasmic β-catenin. Next, we examined its effect on the expression of β-catenin-dependent genes in PC3 cells. After incubating with increasing concentrations of compound **8b**, expression of cyclin D1 and c-myc, established β-catenin target genes, was quantified by western blot.[24,26] As expected, we found a significant reduction in cyclin D1 and c-myc protein levels (Fig. 3B).