Bioorganic & Medicinal Chemistry Letters 22 (2012) 1439–1444

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and neurite growth evaluation of new analogues of honokiol, a neolignan with potent neurotrophic activity

Vemula Praveen Kumar [a,b], R. Gajendra Reddy [c], Duc Duy Vo [b], Sumana Chakravarty [c], Srivari Chandrasekhar [a,*], René Grée [b,*]

[a] Division of Natural Product Chemistry, Indian Institute of Chemical Technology, Hyderabad 500 607, India
[b] Université de Rennes 1, Laboratoire SCR, CNRS UMR 6226, Avenue du Général Leclerc, 35042 Rennes Cedex, France
[c] Chemical Biology/Division of Natural Product Chemistry, Indian Institute of Chemical Technology, Hyderabad 500 607, India

ARTICLE INFO

Article history:
Received 7 November 2011
Revised 1 December 2011
Accepted 3 December 2011
Available online 9 December 2011

Keywords:
Honokiol
Suzuki–Miyaura cross coupling
Neurotrophic activity
Lignans

ABSTRACT

A versatile synthetic route is reported towards the preparation of new analogues for potent neurotrophic agent biaryl-type lignan honokiol. A focused 24-membered library of derivatives containing five different groups at 5′-position of honokiol has been prepared in fair to good overall yields. Compared to the natural product, or to analogues with a short alkyl chain in this position, these new derivatives have lost most of the neurotrophic activity.

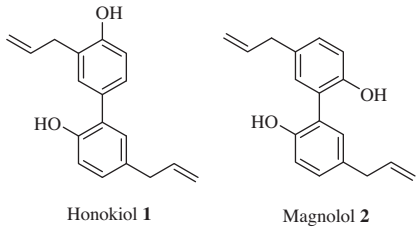
The root and stem bark of the oriental herb *Magnolia officinalis*[1] (also known as *Houpo*) or *Magnoliae obovata*[2] have been used in traditional Chinese and Japanese medicines for treatment of various diseases like flu, anxiety and allergy. Early research on these traditional medicines has identified honokiol **1** and its structural isomer magnolol **2** as the two main biphenyl-type neolignans active compounds (Fig. 1).

The structure of honokiol **1** consists of a biphenyl skeleton with *ortho, para*-C,C-coupling of *para*-allyl and *ortho*-allyl phenols. Honokiol **1** exhibits anticancer,[3] anti-inflammatory,[4] anti-viral,[5] anti-fungal,[6] anti-oxidant[7] and anxiolytic[8,9] properties. Further, it has a neuroprotective effect and promotes neurite outgrowth.[2,8] Due to these biological properties, honokiol attracted a great interest among biologists and chemists. Four total syntheses have been reported till date for honokiol,[9,10] and two for 4′-*O*-methylhonokiol.[11] On the other hand, a few analogues have been already synthesized for biological studies.[5,12,13]

Small molecules, active as neurotrophic agents, appear to be of much interest in the context of fast growing neurodegenerative diseases.[14] Honokiol has demonstrated neurotropic activity at 0.1–1 μM concentrations on the cultures of rat cortical neurons.[9] From a mechanistic point of view, this action seems to be related to activation of extracellular signal-related kinases.[15] A structure–activity relationship (Fig. 2) has been already performed on a few compounds where double bonds were reduced and phenols selectively protected.[12] The results reveals that the 4′-phenol and 5-allyl group are essential for neurotrophic activity. In agreement with this proposal, it was found that 4-*O*-methylhonokiol promotes neurite outgrowth, and this occurs through ERK activation.[16]

As part of our CNS program on the development of novel neurotrophic agents based on natural products, we selected new analogues of honokiol as our target molecules. Based on literature data indicated above, we designed derivatives with various **R** substituents at 5′-allyl position of **1**. Further, we have studied the effect of the substitution of two phenol groups by preparing corresponding methyl ethers.

In this Letter, we describe the synthesis and biological evaluation of a focused library of new analogues of honokiol, with heteroaromatic (pyrimidine, pyridine, pyrazole) **R** groups in position 5′,



Honokiol **1** Magnolol **2**

Figure 1. Structures of honokiol **1** and magnolol **2**.

* Corresponding authors.
E-mail address: rene.gree@univ-rennes1.fr (R. Grée).

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doi:10.1016/j.bmcl.2011.12.015

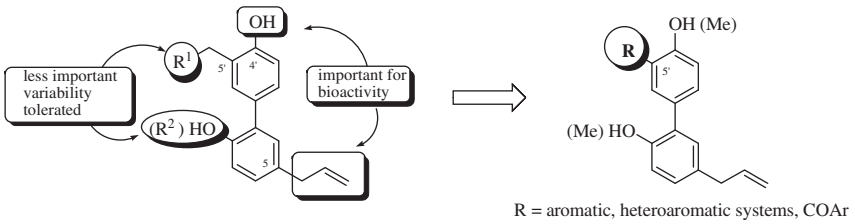


Figure 2. Reported SAR studies and designed analogues of honokiol.

both as phenols and corresponding methylethers (Fig. 2). Further, to explore the effect of a polar carbonyl group in this position, we prepared also derivatives with a benzoyl system (compounds **g**) as well as similar compounds with an acyl triazole group (derivatives **h**) Scheme 3.

As shown in Scheme 1, we developed a convergent and versatile synthetic route to produce designed analogues of **1**, that is, compounds from Series I, II and III depending upon the number of free phenol groups. A Pd-catalyzed Suzuki–Miyaura coupling could be employed to construct biaryls (**3a–h**, **4a–h, 5a–h**) from bromides (**7a–h, 6a–h**) and boronate **10**. The aromatic bromides with various heterocyclic and carbonyl groups at α-position to *O*-methyl/hydroxyl group could be easily obtained from propargyl ketone **8**, which should itself be synthesized from commercially available 5-bromosalicylaldehyde **9**. The boronate **10** could be prepared from 4-allylanisole **11** by a known procedure.[17]

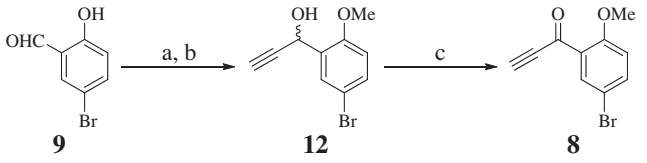
The synthetic route started with the preparation of key intermediate propargyl ketone **8**. The *O*-methylation of 5-bromo salicylaldehyde with dimethylsulfate in acetone in the presence of Na_2CO_3, followed by the reaction with ethynyl magnesium bromide gave propargyl alcohol **12** in good yield over two steps. Oxidation of compound **12** with Jones' reagent gave the desired ketone **8** in 78% yield (Scheme 2).

The stage was set for the preparation of the required intermediates. The propargyl ketone **8** was refluxed in acetonitrile with several amidine hydrochlorides in the presence of Na_2CO_3 to give corresponding pyrimidines **6a–c** in fair to good yields. Then pyrazoles **6d,e** were prepared in excellent yields by refluxing **8** with hydrazines in ethanol for 12 h. The pyridine **6f** was obtained in 78% yield by a Bohlmann–Rahtz reaction,[18] by reacting **8** with ethylacetoacetate, ammonium acetate and zinc bromide in toluene at reflux for 24 h. On the other hand, the Diels–Alder product **6g** was obtained by treating propargyl ketone **8** with 2,3-dimethyl-1, 4-butadiene in toluene. Finally, a click reaction,[19] performed between compound **8** and benzylchloride and sodium azide in water at reflux for 24 h, gave the triazole **6h** in 94% yield (Scheme 3).

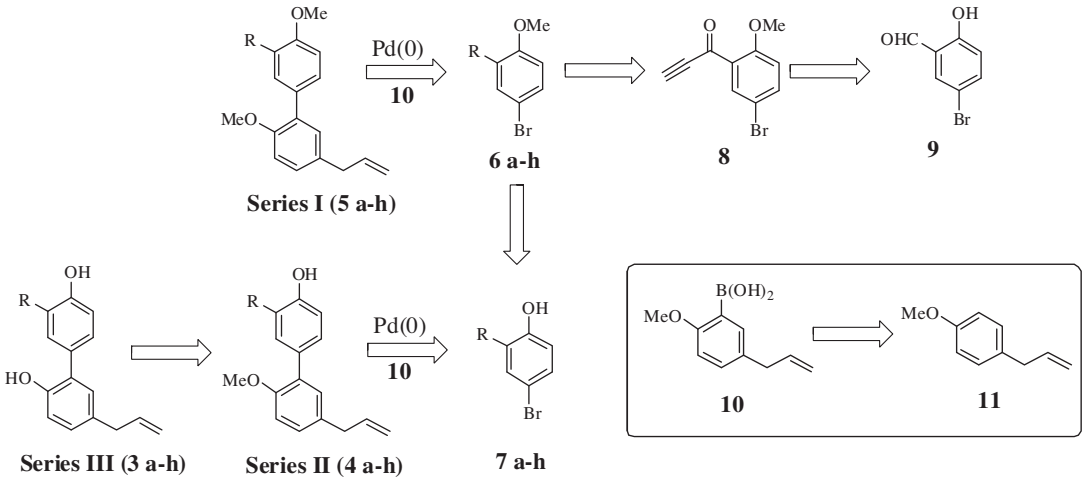
The second series of intermediates, the demethylated compounds **7a–h**, were prepared in good yields by heating compounds **6a–h** with borontrichloride–dimethylsulfide complex in dichloroethane at reflux for 12–24 h (Scheme 3).

Next, the cross coupling partner boronic acid **10** was prepared from 4-allyl anisole **11** in a one step process of lithiation followed by reaction with trialkylborate (Scheme 4).[17]

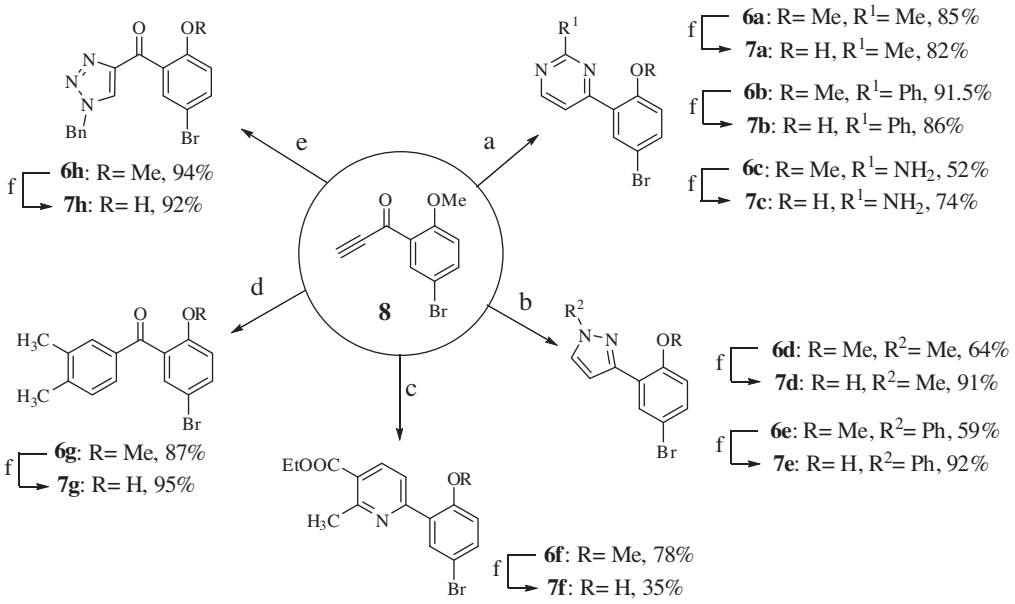
The series I of biaryl compounds **5a–h** have both phenol functions protected as methyl ethers. They were synthesized using Suzuki–Miyaura coupling reactions.[20] The $Pd(PPh_3)_4$ catalyzed cross coupling reaction between aryl bromides **6a–h** and aryl boronic acid **10** in 1,2-DME-H_2O (8:2) in the presence of Na_2CO_3 at reflux temperature for 16 h gave **5a–h** in 76–92% yield (Scheme 5, Table 1). In the case of **6f** (Table 1, entry 6) these reaction conditions gave a lower yield, possibly due to base hydrolysis of ester group. Finally, by changing solvent to benzene and base to potassium carbonate, the biaryl derivative **5f** was isolated in 82% yield.



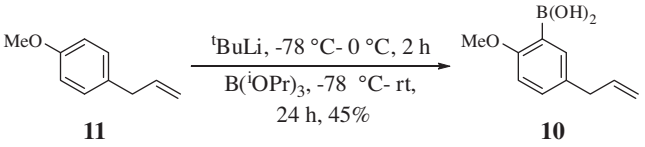
Scheme 2. Synthesis of propargylic ketone **8**. Reagents and conditions: (a) Me_2SO_4, Na_2CO_3, acetone, reflux, 3 h, 92%; (b) ethynylMgBr, THF, rt, 16 h, 72%; (c) Jones reagent, acetone, 0 °C, 78%.



Scheme 1. Retrosynthesis of honokiol analogues.



Scheme 3. Reagents and conditions: (a) $R^1C = NH(NH_2)\cdot HCl$, sodium carbonate, acetonitrile, reflux, 8 h; (b) R^2NHNH_2, 4 Å MS, EtOH, reflux, 12 h; (c) ethylacetoacetate, ammonium acetate, $ZnBr_2$, toluene, reflux, 24 h; (d) 2,3-dimethyl-1,3-butadiene, toluene, 80 °C, 24 h; (e) benzylchloride, sodium azide, water, reflux, 24 h; (f) 2 equiv BCl_3·DMS (2 M solution in dichloromethane), dichloroethane, 24 h.



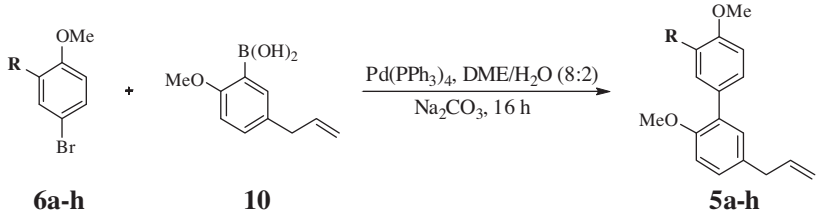
Scheme 4. Synthesis of boronic acid **10**.

The next compounds have either one phenol and one methoxy group (series II) or two phenol groups (series III). The first derivatives **4a–h** were obtained in 62–89% yield by Suzuki–Miyaura cross coupling reactions between aryl bromides **7a–h** and aryl boronic acid **10** in the presence of $Pd(PPh_3)_4$ (Scheme 6, Table 2).

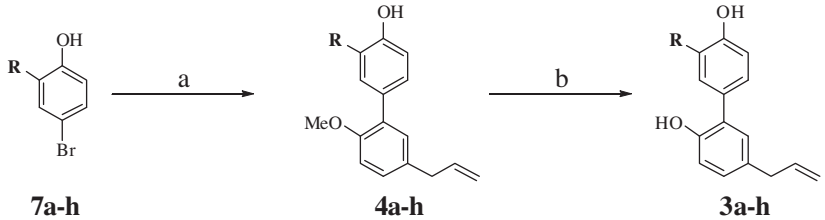
The second series of molecules **3a–h** were obtained in 48–95% yields from adducts **4a–h** through demethylation with borontrichloride–dimethyl sulfide complex in dichloroethane at reflux (Scheme 6, Table 2).

For biological evaluation,[21,22,23] neurotrophic and cell viability assays have been performed for all new compounds, by comparison with honokiol, and the results were analyzed series wise. In view of cell viability tests (data not shown) three compounds were selected to report (see Fig. 3 and Supplementary data for details).

For immunocytochemistry, after 48 h in culture with treatment, cells were fixed with 4% paraformaldehyde at room temperature for 15 min, and incubated in blocking buffer (2% bovine serum albumin + 0.3% Triton X-100 in PBS) for 2 h at room temperature. Primary antibody against beta III tubulin (1:300,

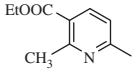
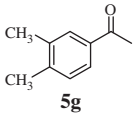
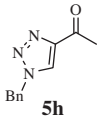


Scheme 5. Synthesis of bisethers analogues **5a–h**. For the nature of **R** groups and yields see Table 1.



Scheme 6. Reagents and conditions: (a) **10**, $Pd(PPh_3)_4$, 1,2-DME-H_2O (8:2), Na_2CO_3, reflux, 16 h; (b) BCl_3·DMS (2 M in dicloromethane), DCE, reflux.

Table 1

S. No.	R	Yield[a] (%)
1	 **5a**	89
2	 **5b**	84
3	 **5c**	76
4	 **5d**	92
5	 **5e**	90
6	 **5f**	82[b]
7	 **5g**	85
8	 **5h**	84

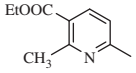
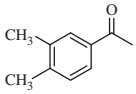
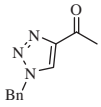
[a] Isolated yields after column chromatography.
[b] Reaction carried out in benzene with K_2CO_3 as a base.

Millipore) was used to visualize neurons. Samples were incubated with primary antibody in blocking buffer overnight at 4 °C. Samples were washed with PBST (PBS with 0.1% Tween20) and incubated with goat anti-mouse IgG conjugated to AlexaFlour 488 (1:400, Molecular Probs). Images were captured using an Olympus IX70 (Olympus America, Melville, NY) inverted microscope and with an Optronics MagnaFire (Goleta, CA) digital color camera. Appropriate composite figures were produced using Adobe Photoshop (Fig. 4).

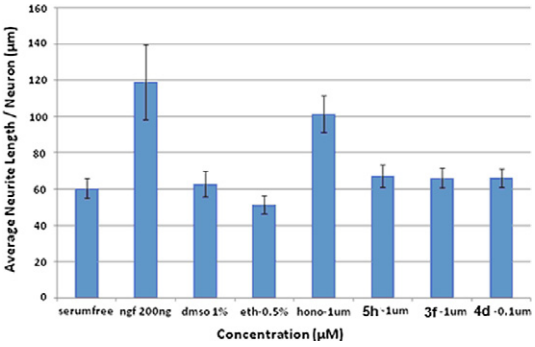
We found only three compounds (dihydroxypyridine **3f**, dimethoxytriazole **5h** and monomethoxymonohydroxymethyl pyrazole **4d**) having some neurotrophic effects at 0.1–1.0 μM concentrations as shown below.

In summary, a versatile strategy was developed for the synthesis of honokiol analogues through Suzuki–Miyaura cross coupling reactions as key steps. In this paper we synthesized, in fair to good overall yields, a focused library of 24 honokiol analogues with different types of substituents at 5′ position. Compared to the natural product, or to analogues with a short alkyl chain in this position, these new derivatives have lost most of the neurotrophic activity. Synthesis and biological evaluation of new analogues of honokiol and other polyphenol-type derivatives are under active study in our groups and corresponding results will be reported in due course.

Table 2

S. No.	R	Product	Yield[a] (%)	Product	Time	Yield[a] (%)
1		**4a**	82	**3a**	24	82
2		**4b**	79	**3b**	28	87
3		**4c**	72	**3c**	36	91[c]
4		**4d**	89	**3d**	18	95
5		**4e**	85	**3e**	18	94
6		**4f**	62[b]	**3f**	36	48
7		**4g**	86	**3g**	12	93
8		**4h**	81	**3h**	18	69

[a] Yields calculated after column chromatography.
[b] Reaction carried out in benzene with K_2CO_3 as a base.
[c] Reaction quenched with ammonium hydroxide solution.



Treatment	Average neurite length per neuron(μm)	Percent change from control
Control	60.27±5.5	-
DMSO	62.97±7.02	2.7
NGF	119.06±20.84	58.79
honokiol	101.30±10.08	41.03
3f	66.05±5.43	5.78
5h	67.14±6.87	6.87
4d	66.16±5.02	5.89

Figure 3. Morphometric analysis of neurite outgrowth of differentiated Neuro2a cells cultured for 48 h after treatment with NGF [200 ng/mL], DMSO [1%], EtOH [0.5%], honokiol, **3f** & **5h** [1 μm] and **4d** [0.1 μm]. The tabulated and bargraph data were expressed as mean ± SEM where $n = 60$ and compared with control, however, none of them are found to be having a significant neurotrophic effect.

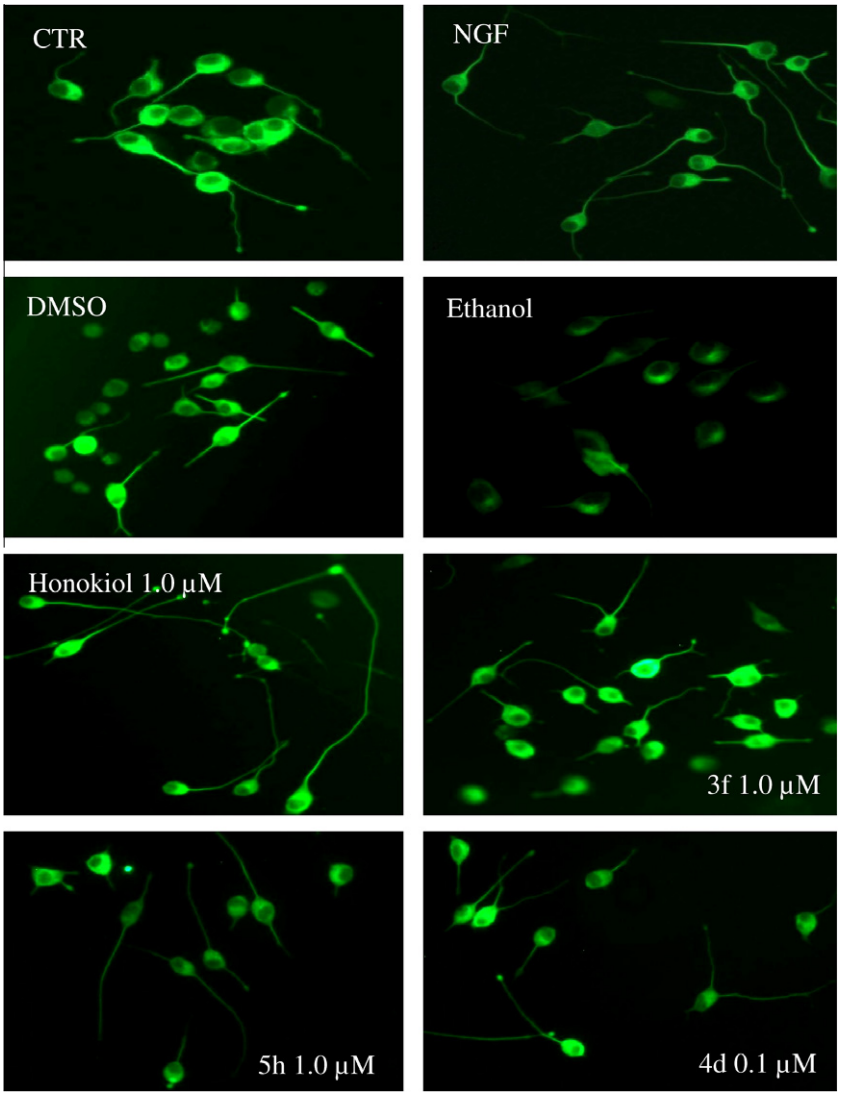


Figure 4. Immunocytochemical images, stained with beta III tubulin, showing optimum changes in neurite outgrowth of differentiated Neuro2a cells after treatment with NGF [200 ng/mL], DMSO [1%], ethanol [0.5%], honokiol, **3f** & **5h** [1 μm] and **4d** [0.1 μm] after 48 hours.

Acknowledgments

This research has been performed as part of the Indo-French '*Joint Laboratory for Sustainable Chemistry at Interfaces*'. We thank CNRS, MESR, CSIR for support of this research. V.P.K. thanks for financial assistance the CSIR, New Delhi in India and French Embassy (Delhi) for support through the Sandwich thesis Program. V.D.D. thanks Laboratoires Servier and Société de Chimie Thérapeutique for a fellowship. R.G.R. thanks CSIR for Junior Research Fellowship and S.C. likes to acknowledge the financial assistance of Department of Biotechnology (DBT), India, in part, under Ramalingaswami re-entry Fellowship.

Supplementary data

Supplementary data (synthetic procedures and analytical data for new compounds, biological tests) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.12.015.

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