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Synthesis, cytotoxicity, and antiviral activities of new neolignans related to honokiol and magnolol

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Abstract—A series of new bisphenol derivatives bearing allylic moieties were synthesized as potential analogs of honokiol and/or magnolol. Certain compounds exhibited specific anti-proliferation activity against SVR cells and moderate anti-HIV-1 activity in primary human lymphocytes. Compound **5h** was the most potent compound and its anti-tumor activity was evaluated in vivo.
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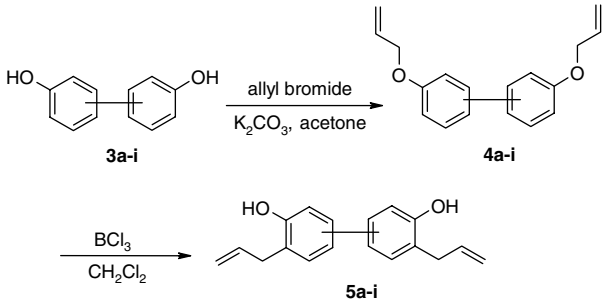
In the fight against diseases and body disorders, plant extracts were exploited heavily by ancient civilizations. Thus, Chinese medicine has developed over a period of several thousand years many preparations of which are used in the treatment of a wide variety of clinical diseases. It is only in the last century that efforts have been made to isolate and identify specific active chemicals from these 'cocktails'. *Saiboku-to*, a mixture of natural products that contains magnolia bark, has been historically used for clinical depression, anxiety as well for thrombotic stroke. The principal active components of the mixture appear to be the neolignans magnolol **1** and honokiol **2**.[1] These two compounds demonstrated various biological properties including anti-oxidant and antidepressant activities.[2] Recent studies in our laboratory also indicate that honokiol induces apoptosis in tumor cells,[3,4] inhibits angiogenesis,[5] and has weak in vitro anti-HIV-1 activity.[6] This combination of biological properties justifies further studies in order to increase the potency of these lead compounds and to understand their mechanism of action. Even though, until now, only few analogs have been reported in the literature, it appears that honokiol's and magnolol's potent activities are attributed to the presence of hydroxyl and allylic groups on a biphenolic moiety.[7] Thus, to clarify a structure–activity relationship and to improve the potent activity of these two compounds, some simple allylated biphenol analogs and some 'flexible' allylated biphenolic derivatives varying a linker between the two aromatic rings were prepared. Herein, we report the preparation of various compounds **5a**–**i** and **10**, their antiviral and anti-proliferative activities in vitro, and the in vivo activity of compound **5h**.

The syntheses of **5a**–**i** were accomplished with phenolic *O*-allylation of compounds **3a**–**i** followed by Claisen rearrangement. Therefore, biphenol derivatives **3a**–**i** were treated with excess of allyl bromide in the presence of potassium carbonate to afford corresponding bis(allyloxy)-biphenyls **4a**–**i**. The following Claisen rearrangement of **4a**–**i** was then performed in dichloromethane



Scheme 1.

Keywords: Antiviral; Anti-tumoral; Honokiol; Magnolol; Neolignans.
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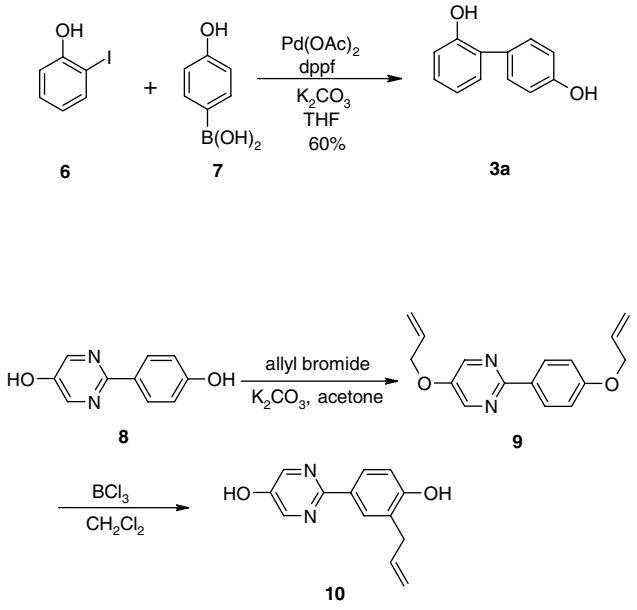
using 1.5 equiv of a 1 M solution of BCl_3 to give the desired 3,3′-bis-allylbiphenol **5a–i** (Scheme 1).

The biphenols **3b–i** were commercially available but, biaryl **3a** was synthesized through a Pd-catalyzed Suzuki-Miyaura reaction (Scheme 2). Thus the coupling between 2-iodophenol **6** and the phenol 4-boronic acid **7** was achieved by using $Pd(OAc)_2$ (10 mol%), dppf (diphenylphosphino ferrocene) (10 mol%) in presence of K_2CO_3 (3.0 equiv) in THF at room temperature.

Interestingly, the allylation/Claisen procedure applied to the 2-(4-hydroxyphenyl)-5-pyrimidinol **8** did not give the expected diallylated derivative, but produced the monoallylated compound **10** (Scheme 3). In fact, if the migration of the allyl group is generally the main product of the Claisen reaction,[8] the cleavage can be the dominant reaction mode in particular with electron deficient rings such as the case of pyrimidine.

Cytotoxic activities. The anti-tumoral activities of compounds **5a–i** and **10** were determined by measuring their effect on the survival and proliferation of the immortalized endothelial cell line SVR.[9] Briefly, SVR cells (10^4) were plated in 24-well dishes. The next day, the medium was replaced with fresh medium containing the inhibitors or controls. Cells were incubated at 37 °C for 72 h,[10] and cell number was determined in triplicate using a Coulter Counter (Hialeah, FL). Immortalized and K-Ras transformed rat epithelial cells (RIEpZip and RIEpZipK-Ras12V) were maintained at 37 °C, 5% CO_2, in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (RIE).[11,12] Cells were plated at 10^5/well in six-well plates. Cells were treated with either vehicle (⩽0.05% DMSO in medium) or increasing concentrations (10 and 15 μg/ml) of honokiol analogs (from a 10 mg/ml stock) and observed for morphology changes after 24 h.



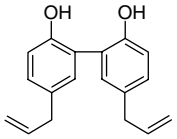
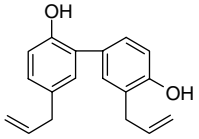
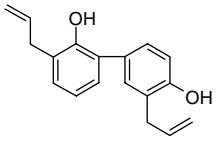
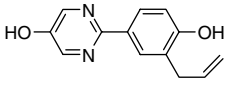
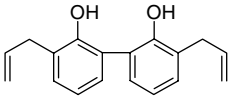
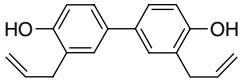
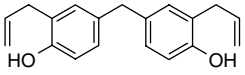
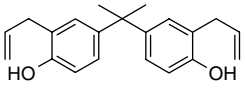
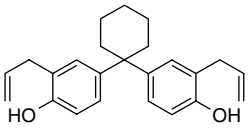
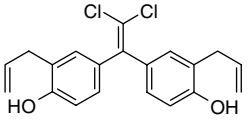
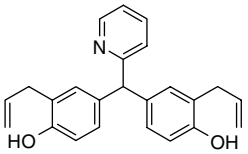
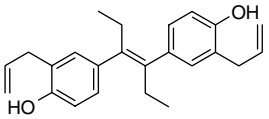
Scheme 3.

Modifying the position of allyl and hydroxyl groups around the simple biphenyl skeleton, as with compounds **5b** and **5c**, reduced the cellular growth (Table 1), but did not demonstrate any activity enhancement compared with honokiol (57% and 56% inhibition at 15 mg/ml, respectively, for **5b** and **5c**, compared to 85% inhibition for honokiol). The mono-allylated compound **10** was almost completely inactive in all cell lines. Introduction of a linker between the two phenyl rings, making larger, but also more 'flexible' molecules, is an interesting way to determine the influence of the size of the compound on the target. Initial results indicated that introduction of a one-carbon bridge bearing a dimethyl (**5e**) or a pyridine group (**5h**) significantly decreased cytotoxicity in SVR cells. On the other hand, the presence of groups such as methylene (**5d**), methylenecyclohexyl (**5f**) or dichloroalkene (**5g**) provided similar anti-proliferative activities, but without selectivity compared to normal human lymphocytes [peripheral blood mononuclear (PBM) cells]. Compound **5i** was quite potent and showed an excellent anti-proliferative response in SVR cells (68% and 89% inhibition at 10 and 15 μg/mL) and an IC_{50} of 3.4 μM in human T-cell lymphoma cells (CEM). At the same time, no toxicity was detected in normal human PBM cells. This selectivity made this compound a good candidate for further evaluation in a small animal model. To determine whether compound **5i** exhibits anti-tumor activity in vivo, SVR cells were injected into flank of 6-week-old nude male mice. When tumors became visible at approximately 1 week after inoculation, mice received 3 mg/day compound **5i** or vehicle control intraperitoneally. Treatment up to 100 mg/kg for 30 days did not inhibit the tumor growth, but also did not result in any toxicity compared with vehicle control.

Antiviral activities. Since honokiol **2** itself is a modest antiviral agent against HIV-1, the activities of analogs **5a–i** and **10** were also evaluated and the results expressed as a median effective concentration or EC_{50} (Table 1). The antiviral[13] and cytotoxicity[14] assays were performed as previously described. Compared to honokiol **2**, compounds **5a–d**, **5f–h**, and **10** appeared less potent against HIV-1 in human PBM cells and only compounds **5e** and **5i** showed moderate activities close to honokiol's activity (EC_{50} of 4.1 and 9.5 μM, respectively, compared to 3.3 μM for honokiol).

In conclusion, the discovery of the anti-proliferative potency of compounds **5d**, **5f**, **5g**, and especially **5i** provides new insights into the synthesis and the evaluation of new biphenyl-linked compounds. As a result of the above studies, further modifications between the two phenol moieties would be envisaged in order to generate more potent and selective analogs with improved in vivo activity.

Table 1. Inhibition of SVR proliferation, anti-HIV-1 activity, and cytotoxicity against human PBM, CEM, and Vero cells

Compound	Structure	Inhibition of SVR cells at 10 μg/mL[a]	Inhibition of SVR cells at 15 μg/mL[a]	Anti-HIV-1 activity in PBM cells (μM)		Cytotoxicity (IC_{50}, μM) in		
				EC_{50}	EC_{90}	PBM	CEM	Vero
1		60%	77%	69.3	>100	38.6	99.5	50.6
2		60%	85%	3.3	22.7	16.1	10.9	22.5
5a		74%	81%	23.4	81	36.7	17.9	11.4
10		4.4%	10%	>100	>100	31.9	>100	>100
5b		41%	57%	34.8	63.0	45.9	10.4	19.8
5c		45%	56%	19.4	66.4	>100	23.0	83.2
5d		75%	74%	13.6	35.1	33.7	15.1	1.9
5e		33%	57%	4.1	47.3	>100	5.8	13
5f		63%	79%	36.5	67.2	55.4	11.0	12.5
5g		54%	76%	14.3	48.8	43.6	17.1	12.1
5h		25%	46%	35.0	67.2	16.8	16.5	14.8
5i		68%	89%	9.5	30.9	>100	3.4	3.7

[a] To convert from μg/mL to μmol/L (μM), concentration has to be multiplied by an average of 3.2.

Acknowledgments

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