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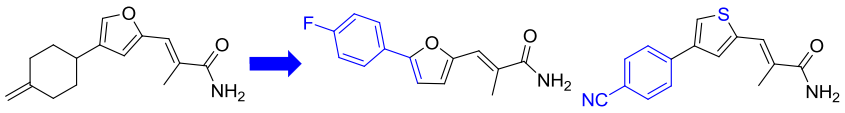

Brasilamide E (**1**) Compound **9b** Compound **12b**

Cell lines		IC$_{50}$ (μM)	
MCF-7	8.47	0.97	0.24
HO8910	18.0	0.99	0.13
NIH3T3	> 50	0.96	> 50

Design and synthesis of natural product derivatives with selective and improved cytotoxicity based on a sesquiterpene scaffold

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Abstract

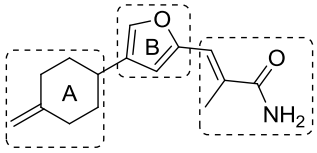
Brasilamide E (**1**) is a bisabolane sesquiterpenoid isolated from the solid-substrate fermentation cultures of a plant endophytic fungus *Paraconiothyrium brasiliense*. The compound specifically inhibited proliferation of the MCF-7 cells, but did not show cytoxicity towards the negative controls HaCaT and NIH3T3 cells (IC_{50} > 50 μM). To improve its potency while maintain selectivity, a total of 27 derivatives of **1** were designed, synthesized, and evaluated for *in vitro* cytotoxicity against six tumor cell lines and the negative control NIH3T3 cells. Among these compounds, compound **12b** showed significantly improved potency against the MCF-7, HeLa, and HO8910 cells with IC_{50} values of 0.13–0.25 μM compared to **1** (IC_{50} 8.47–18.00 μM), and remained nontoxic to the NIH3T3 cells.

Key words: Brasilamide E; Synthesis; Derivatives; Selectivity; Cytotoxicity

A common drawback of the existing anticancer drugs is their toxicity to noncancerous cells, resulting in chemotherapy damage to normal cells and causing various side effects, such as bone marrow suppression, gastrointestinal toxicity, anemia, hair loss, and constipation.[1] Therefore, effective and safe treatments for cancer are still desperately needed.[2] Despite advances in molecular modeling, combinatorial chemistry, and other synthetic chemistry fields, natural products continue to be an important source of drug leads, particularly in the field of anticancer therapy, with over 50% of clinically used drugs are natural products or their derivatives.[3] Terpenoids are the largest and most diverse group of natural products, showing various biological activities including potent *in vitro* and *in vivo* inhibitory effects on proliferation of a variety of human tumor cell lines.[4–8] As the sesquiterpene type of natural products, the bisabolane sesquiterpenoids have been isolated from various sources,[9–11] and a number of which have been identified from fungi as antitumor metabolites.[12–17]

In our previous study, a series of new bisabolane sesquiterpenoids have been isolated from the solid-substrate fermentation culture of *Paraconiothyrium brasiliense*, a fungus endophytic to *Acer truncatum* Bunge.[18–20] Among those, a metabolite with the 4-cyclohexylfuran skeleton, named brasilamide E (**1**, Fig. 1), selectively inhibited proliferation of MCF-7 (human breast cancer) cells, but did not show detectable toxicity towards the HaCaT (human keratinocyte) and NIH3T3 (mouse embryo fibroblast) cells at 50 μM.[19] Excellent selectivity between tumor and noncancerous cells for brasilamide E (**1**) suggested its potential to be an antitumor lead compound. In view of this, structural optimization of **1** was initiated to prepare new derivatives with selectivity and improved potency.

The structure of brasilamide E (**1**) can be dissected into three fragments, the

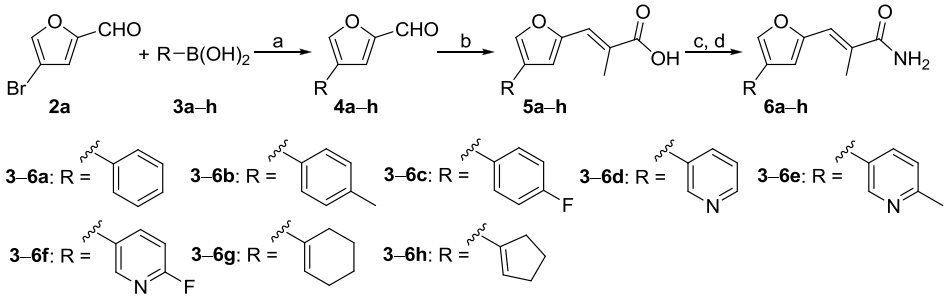


Brasilamide E (**1**)

Figure 1. Structure of brasilamide E (**1**), a bisabolane sesquiterpene from the plant endophytic fungus *Paraconiothyrium brasiliense*

methylenecyclohexane (ring A), furan (ring B), and *E*-methylacrylamide moieties (Fig. 1). Modifications were focused on the 4-cyclohexylfuran portion (rings A and B) of the structure. A total of 27 derivatives (**6a**–**h**, **9a**–**j**, and **12a**–**I**; Schemes 1–3) were designed, synthesized, and evaluated for their *in vitro* cytotoxicity against a panel of six human tumor cell lines, MCF-7 (breast cancer), HeLa (cervical carcinoma), HO8910 (ovarian cancer), A549 (lung cancer), T24 (bladder carcinoma), and BGC-823 (gastric cancer), with NIH3T3 (mouse embryo fibroblast) cell line as the negative control.

Since some aromatic bisabolane sesquiterpenes have been reported to modulate different levels of cellular growth and apoptosis,[21–23] the methylenecyclohexane unit (ring A) in **1** was first replaced by substituted phenyl rings (**6a**–**c**; Scheme 1). In addition, ring A was also replaced by substituted pyridines (**6d**–**f**) and cycloalkenes (**6g** and **h**), respectively, to find clues for structure–activity relationship. The key intermediates **4a**–**h** were synthesized via Suzuki cross-coupling reactions, in which 4-bromofuran-2-carbaldehyde **2a** was individually coupled with phenyl, and pyridyl or alkenyl-boronic acids **3a**–**h** using $Pd(Ph_3P)_4$ as catalyst in the presence of Cs_2CO_3 (Scheme 1).[24] Subsequently, intermediates **4a**–**h** reacted with methylmalonic acid in the presence of catalytic amount of piperidine via Knoevenagel condensation to afford



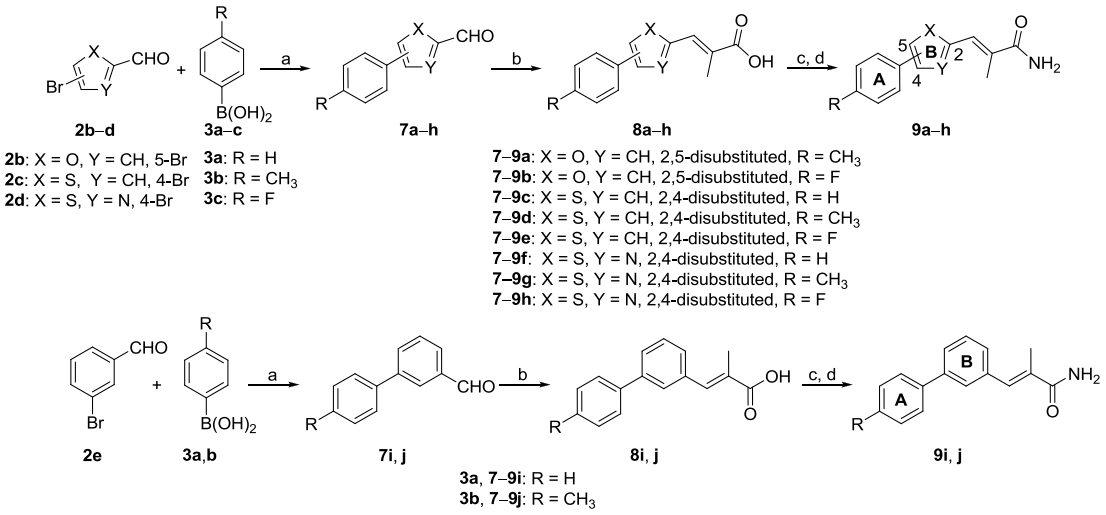
Scheme 1. Synthetic routes for compounds **6a**–**h**. Reagents and conditions: (a) $Pd(Ph_3P)_4$, Cs_2CO_3, 1,4-dioxane/water (2:1), reflux, 4–6 h; (b) methylmalonic acid, piperidine cat., pyridine, reflux, 3 h; (c) $SOCl_2$, DMF cat., THF, reflux, 2 h; (d) $NH_3 \cdot H_2O$, THF, 0 °C, 2 h.

the corresponding *E*-methacrylic acids **5a**–**h**.[25] Finally, treatment of **5a**–**h** with thionyl chloride followed by reaction with freezing ammonia gave the corresponding products **6a**–**h** (Scheme 1), according to a described procedure with minor modifications.[26] Compounds **6a**–**h** were tested for their cytotoxic effects using the Cell Counting Kit-8 assays, and the results are summarized in Table 1. In these compounds, the phenyl derivatives **6a**–**c** were more potent against the MCF-7 cells, showing IC_{50} values of 3.34, 1.63, and 2.21 μM, respectively, compared to that of 8.47 μM for **1**. In addition, compound **6a** was slightly more potent than **1** (IC_{50} 3.28 vs 5.01 μM) towards the HeLa cells, while **6c** showed weak cytotoxicity against the A549 cells, with an IC_{50} value of 14.91 μM compared to that of greater than 50 μM for **1**. In general, the pyridine derivatives **6d**–**f** were less potent than **1** against MCF-7, HeLa, and HO8910 cells, showing IC_{50} values ranging from 8.64 to greater than 50 μM (Table 1). The cyclohexene derivative **6g** showed potency (IC_{50} 6.58 μM) against MCF-7 cells comparable to **1**, but was inactive against the HeLa and

Table 1. Cytotoxicity of **1**, **6a**–**h**, **9a**–**j**, and **12a**–**i** against six human tumor cell lines and mouse embryo fibroblast cells

Compound	C log*P*	IC_{50} [a] (μM)						
		MCF-7	HeLa	HO8910	A549	T24	BGC-823	NIH3T3
1		8.47 ± 0.36	5.01 ± 1.24	18.00 ± 0.24	>50	>50	>50	>50
6a	2.952	3.34 ± 0.51	3.28 ± 0.30	42.35 ± 2.72	>50	>50	>50	>50
6b	3.451	1.63 ± 0.13	12.00 ± 0.35	24.22 ± 1.50	>50	>50	>50	>50
6c	3.095	2.21 ± 0.06	11.81 ± 0.65	>50	14.91 ± 4.78	>50	>50	>50
6d	1.455	13.16 ± 0.58	>50	8.64 ± 1.68	6.62 ± 0.72	>50	>50	>50
6e	1.954	10.16 ± 1.78	>50	>50	>50	>50	>50	>50
6f	1.682	14.10 ± 3.42	>50	30.03 ± 4.29	>50	>50	>50	>50
6g	3.380	6.58 ± 0.53	>50	>50	>50	>50	>50	>50
6h	2.821	15.00 ± 0.72	17.60 ± 1.92	>50	16.62 ± 1.26	>50	>50	49.29 ± 5.00
9a	3.661	>50	4.59 ± 0.75	1.66 ± 0.34	>50	>50	>50	>50
9b	3.305	0.97 ± 0.12	2.03 ± 0.08	0.99 ± 0.03	1.65 ± 0.01	3.32 ± 0.33	0.94 ± 0.16	0.96 ± 0.11
9c	3.422	2.68 ± 0.38	12.65 ± 2.76	>50	>50	>50	>50	>50
9d	3.921	>50	>50	>50	>50	>50	>50	>50
9e	3.573	1.44 ± 0.07	20.84 ± 0.74	>50	>50	>50	>50	>50
9f	2.330	2.38 ± 0.34	2.77 ± 0.15	3.78 ± 0.21	>50	>50	>50	>50
9g	2.829	>50	24.96 ± 1.87	>50	>50	>50	>50	>50
9h	2.480	3.30 ± 0.89	5.40 ± 0.35	4.45 ± 0.35	13.21 ± 0.98	>50	>50	>50
9i	3.776	6.54 ± 1.60	15.69 ± 3.67	12.60 ± 0.80	>50	>50	>50	>50
9j	4.275	6.71 ± 0.13	24.80 ± 2.37	26.79 ± 1.33	5.10 ± 0.75	7.65 ± 0.98	>50	>50
12a	3.621	4.92 ± 0.53	19.44 ± 0.58	15.21 ± 0.52	>50	>50	>50	>50
12b	2.875	0.24 ± 0.05	0.25 ± 0.02	0.13 ± 0.02	>50	>50	>50	>50
12c	1.820	>50	>50	>50	>50	>50	>50	>50
12d	3.183	1.21 ± 0.19	2.90 ± 0.40	2.38 ± 0.17	3.50 ± 0.42	>50	>50	>50
12e	4.320	>50	>50	>50	>50	>50	>50	>50
12f	4.143	17.11 ± 0.73	>50	>50	>50	>50	>50	>50
12g	3.366	>50	>50	>50	>50	>50	>50	>50
12h	3.403	3.05 ± 0.91	>50	>50	>50	>50	>50	>50
12i	4.146	>50	>50	>50	>50	>50	>50	>50
Cisplatin[b]	–	46.70 ± 1.35	14.70 ± 0.93	17.34 ± 0.97	15.97 ± 0.68	1.86 ± 0.18	2.80 ± 0.30	5.79 ± 0.63

[a] IC_{50} values were averaged from at least three independent experiments. [b] Positive control.



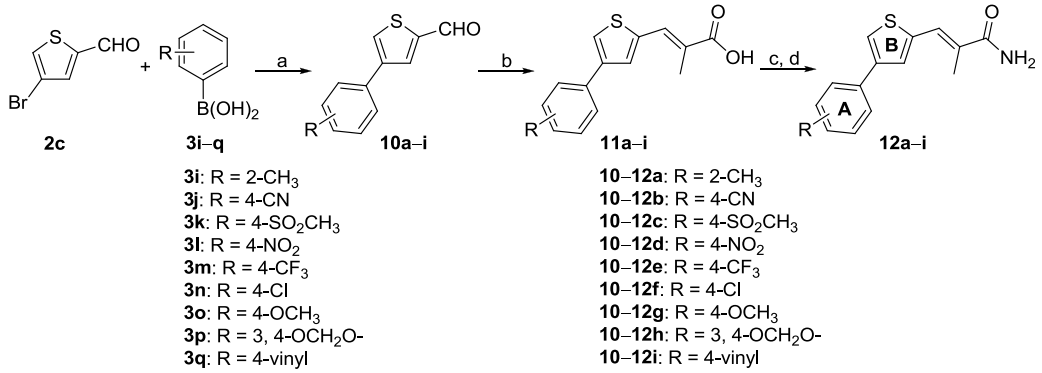
Scheme 2. Synthetic routes for compounds **9a**–**j**. Reagents and conditions: (a) $Pd(Ph_3P)_4$, Cs_2CO_3, 1,4-dioxane/water (2:1), reflux, 4–6 h; (b) methylmalonic acid, piperidine cat., pyridine, reflux, 3 h; (c) $SOCl_2$, DMF cat., THF, reflux, 2 h; (d) $NH_3 \cdot H_2O$, THF, 0 °C, 2 h.

HO8910 cells, while the cyclopentene derivative **6h** was less potent against the MCF-7, HeLa, and HO8910 cells. However, compound **6h** was cytotoxic to A549 and NIH3T3 cells, with IC_{50} values of 16.62 and 49.29 μM, respectively, compared to those of greater than 50 μM for **1**. The above results implied that the more bulky cyclohexene derivative **6g** showed better selectivity than **6h**.

Since the phenyl derivatives **6a**–**c** maintained selectivity and showed improved potency, ring A remained to be phenyl rings in modification of the ring B portion of **1**. It was reported that thiophene, thiazole, and phenyl derivatives showed inhibitory effects against several human tumor cell lines.[27,28] Therefore, compounds **9a**–**j** (Scheme 2) were designed and synthesized by varying the substitution patterns of the furan ring (**9a**, **b**), and replacing the furan ring with a thiophene (**9c**–**e**), thiazole (**9f**–**h**) or an phenyl ring (**9i**, **j**). Procedures similar to those used in preparation of **6a**–**h**

were applied to synthesize these compounds. The 2,5-disubstituted furan derivative **9b** showed potent inhibitory effect on proliferation of all six human tumor cell lines (IC_{50} 0.94–3.32 μM) and NIH3T3 cells (IC_{50} 0.96 μM) (Table 1), indicating that **9b** increase potency, but lacks the desired selectivity when compared to the 2,4-disubstituted furan derivative **6c**. The thiophene derivatives **9c**–**e** showed similar potency to the thiazole derivatives **9f**–**h**, while the phenyl derivatives **9i** and **9j** were less potent against the MCF-7, HeLa, and HO8910 cells (Table 1). Different from compound **1**, the thiazole derivative **9h** was weakly cytotoxic to A549 cells, with an IC_{50} value of 13.21 μM, while the phenyl derivative **9j** was cytotoxic to both A549 and T24 cells, showing IC_{50} values of 5.10 and 7.65 μM, respectively. The thiophene derivatives **9c** and **9e** selectively inhibited proliferation of the MCF-7 and HeLa cells (Table 1).

Subsequently, the effects of substituents on ring A of the thiophene derivatives were investigated. Similarly to compounds **6a**–**h** and **9a**–**j**, the thiophene derivatives **12a**–**i** bearing various substituents on the phenyl ring (ring A) were prepared according to procedures described above (Scheme 3). Notably, compounds **12b** and **12d**, which were substituted with the electron withdrawing 4-cyano and 4-nitro groups, respectively, showed improved potency compared to **1**, when maintained selectivity between certain tumor cells and the negative control NIH3T3 cells (Table 1). Compound **12d** also showed cytotoxicity against the A549 cells with an IC_{50} value of 3.50 μM. The 2-methyl substituted **12a** showed significantly improved potency against the MCF-7, HeLa, and HO8910 cells with IC_{50} values of 4.92–19.44 μM, compared to the 4-methyl substituted **9d** (IC_{50} > 50 μM), implying that the position for substitution on the phenyl ring affected the potency. The 4-chloro substituted derivative **12f** was obviously much less potent than the 4-fluoro



Scheme 3. Synthetic routes for compounds **12a**–**i**. Reagents and conditions: (a) $Pd(Ph_3P)_4$, Cs_2CO_3, 1,4-dioxane/water (2:1), reflux, 4–6 h; (b) methylmalonic acid, piperidine cat., pyridine, reflux, 3 h; (c) $SOCl_2$, DMF cat., THF, reflux, 2 h; (d) $NH_3 \cdot H_2O$, THF, 0 °C, 2 h.

substituted **9e** against the MCF-7 cells (IC_{50} 17.11 vs 1.44 μM). In fact, not all the derivatives with the electron withdrawing groups improved potency, as found in compounds **12c** and **12e**, which have strong electron withdrawing groups trifluoromethyl and methylsulfonyl, respectively, were actually inactive. The log*P* values of the synthesized compounds were calculated by ChemBioDraw Ultra 12.0.2 (Table 1). It was found that the log*P* values for the active thiophene derivatives (**9c**, **9e**, **12a**, **12b**, **12d**, and **12h**) range from 2.875 to 3.621, whereas those for **12c** and **12e** were 1.820 and 4.320, respectively. It is likely that only the thiophene derivatives having both the electron withdrawing groups and suitable log*P* values showed the desired cytotoxicity.

In summary, a total of 27 natural product derivatives were designed and synthesized based on a bisabolane sesquiterpene scaffold. Compounds **9b** and **12b** showed significantly improved potency with IC_{50} values ranging from 0.13 to 3.32 μM, and compound **12b** maintained selectivity between three human tumors cell lines

(MCF-7, HeLa, and HO8910) and the negative control NIH3T3 cells, suggesting that it is the most promising derivative as a lead compound for further investigation. Results from this work suggested that replacement of the methylenecyclohexane ring with substituted phenyl rings in brasilamide E (**1**) increased its activity and maintained selectivity. While the substituents and substitution pattern on ring B appeared to affect the selectivity. The presence of electron withdrawing groups on the phenyl ring, plus suitable log*P* values appear to be essential for most of the 4-phenylthiophene derivatives to maintain their selectivity. These results provide valuable information for further optimization of **1** as an antitumor lead compound. Continuing studies to further optimize the activity profile and to elucidate the mechanism of action are underway and the results will be reported in due course.

Acknowledgments

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Supplementary data

Supplementary data (detailed experimental procedures for the synthesis, cytotoxic activity assay, and the spectral data of the prepared compounds) associated with this article can be found, in the online version, at

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