Design and Synthesis of Brazilin-Like Compounds

Chengxue Pan, Xianghui Zeng, Yifu Guan, Xiangliu Jiang, Liang Li, Hongbin Zhang*

Key Laboratory of Medicinal Chemistry for Natural Resource (Yunnan University), Ministry of Education, School of Chemical Science and Technology, Yunnan University, Kunming, Yunnan 650091, P. R. of China
Fax +86(871)5035538; E-mail: zhanghb@ynu.edu.cn

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Abstract: A general and flexible synthetic route, which leads to the synthesis of brazilin-like compounds, was developed. The aza-brazilin derivatives show strong anticancer activities in MTT assay towards a number of human cancer cell lines including HT29, A549, HL60, and K562.

Key words: synthetic strategy, bioactive derivatives, anticancer, natural-product-like, brazilin

The dried heartwood of *Caesalpinia sappan* L. (Leguminosae) has long been used in traditional Chinese medicine as an analgesic, emmenagogue, hemostatic, and anti-inflammatory agent. Brazilin (**1**), a tetracyclic homoisoflavanoid indicated in Figure 1, is the major component isolated from the heartwood of *Caesalpinia sappan*.[1] Previous biological studies showed that brazilin improved rheological abnormalities in diabetes,[2] demonstrated antiplatelet activity,[3] modulated immune function,[4] exhibited antiinflammatory activities,[5] and protected oxidative injury.[6] Recent reports have demonstrated that brazilin possesses antitumor activity and acts as a micromolar telomerase inhibitor and produces DNA nicks.[7]

The brazilin skeleton is quite interesting in terms of its diverse biological activities and strongly attracts our attention. Natural products and its mimics are important resources for the development of new pharmaceuticals.[8] As proposed in a review by Tietze,[9] natural product hybrids are an efficient and promising approach towards the generation of lead compounds. The structural framework of brazilin can be viewed as an indane motif merged with a dihydrobenzopyranyl unit from the view point of structure-unit hybrid. We were inspired to establish a general and flexible synthetic strategy leading to the synthesis of brazilin-like compounds. We postulated that recombination of an indane structure unit with a dihydrocoumarin motif, a dihydroquinolin-2-one ring, or a tetrahydroquinoline unit would result in the brazilin-like framework, and finally lead to new types of biologically active agents.

In seeking an efficient and flexible strategy towards the synthesis of brazilin-like compounds, we drafted a new synthetic route as indicated in Scheme 1. It was expected that the indanone intermediate **7** could serve as a platform for approaching molecular diversity.

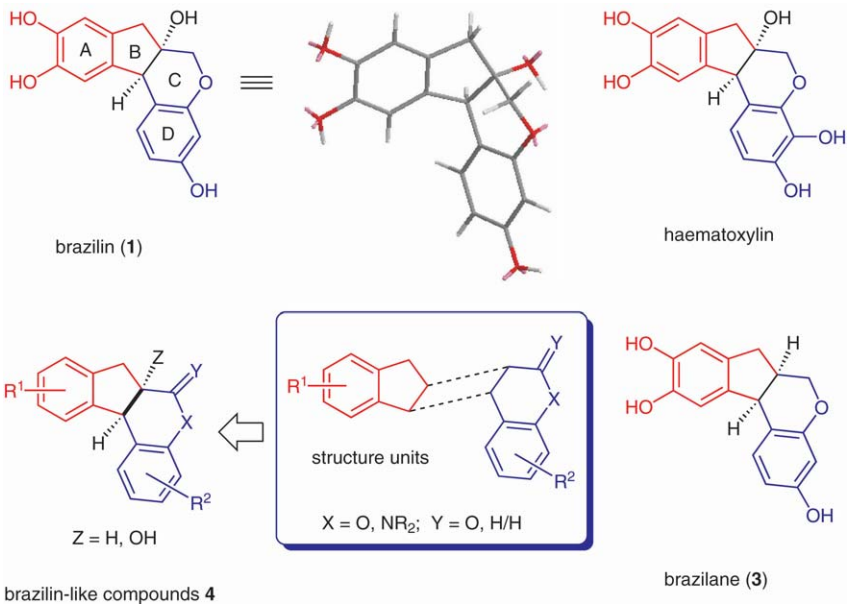

Figure 1 Brazilin-related natural products and its structure units

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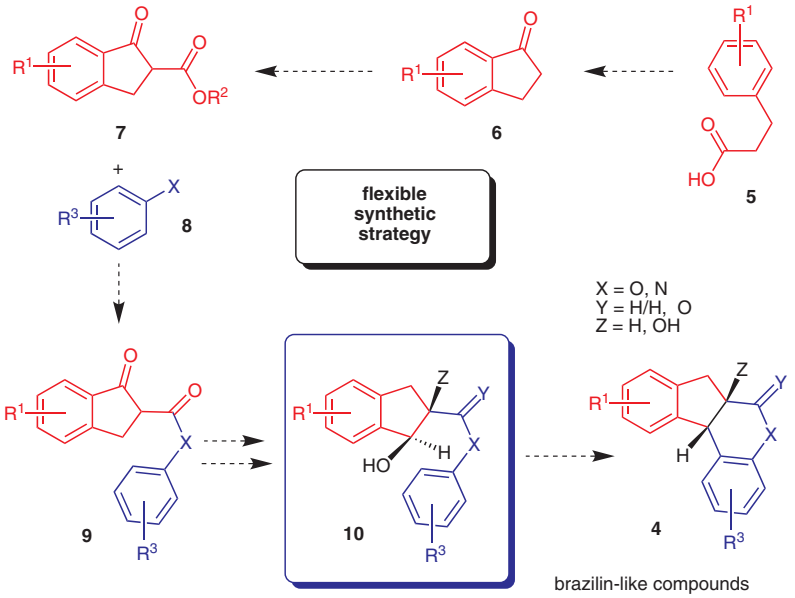
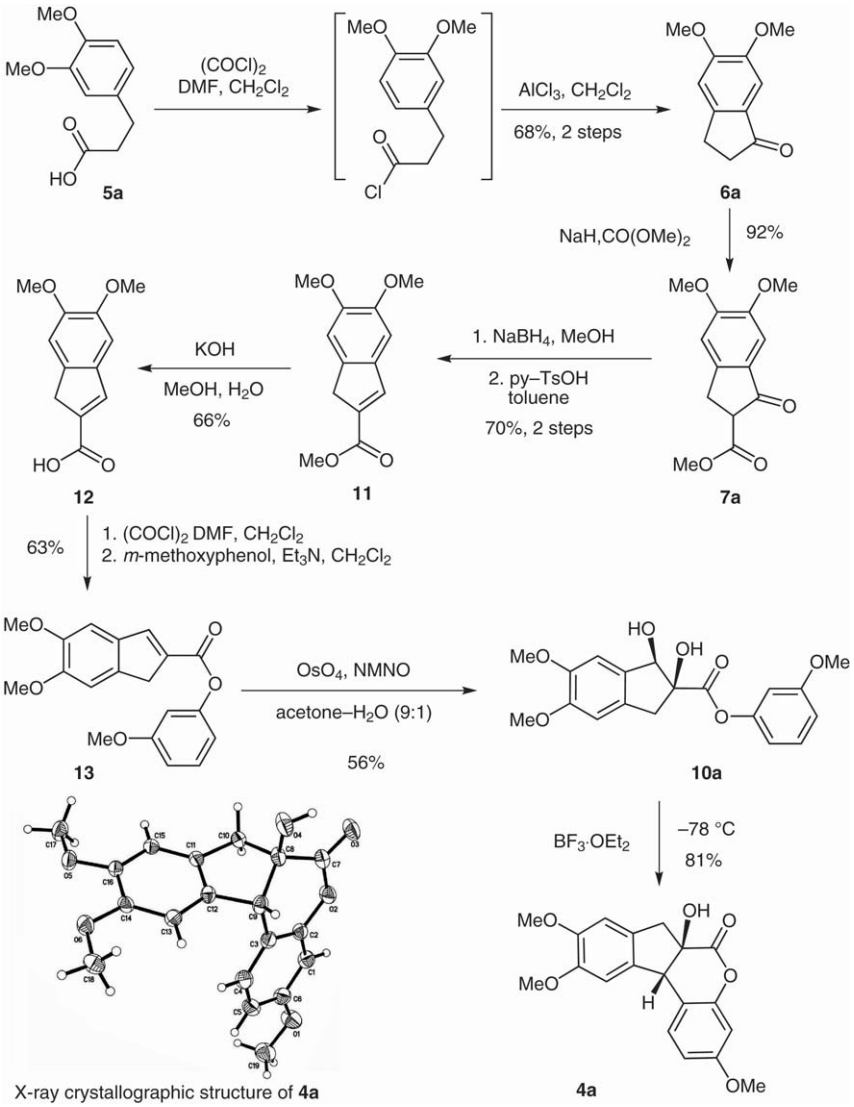


Scheme 1 Synthetic route to brazilin-like compounds



Scheme 2 Synthesis of lactone analogue of brazilin

We began our research with commercially available 3-(3,4-dimethoxyphenyl)propanoic acid (**5a**). Treatment of the acid with oxalyl chloride followed by aluminum chloride provided indanone **6a**. The indanone **6a** was then reacted with dimethylcarbonate in the presence of sodium hydride and resulted in β-keto ester **7a** (92%). Reduction of keto ester **7a** with sodium borohydride followed by dehydration with pyridinium *p*-toluenesulfonate gave an unsaturated ester **11** (70%). Hydrolysis of the methyl ester in the presence of potassium hydroxide in methanol afforded acid **12** (66%). The ester **13** was obtained by a two-step procedure as indicated in Scheme 2 (63%), treatment of acid **12** with oxalyl chloride in the presence of DMF in dichloromethane followed by reaction of the acyl chloride with 3-methoxyphenol. After dihydroxylation with osmium tetroxide, the resultant was then treated with boron trifluoride diethyl etherate in dichloromethane to yield a lactone analogue of brazilin (**4a**, Scheme 2). The structure was unambiguously established by an X-ray crystallography.[10]

Next, we initiated the synthesis of lactam analogue of brazilin by utilization of acid **12** as the starting material. We successfully synthesized the lactam analogue **4c**, and the synthetic procedure is outlined in Scheme 3.

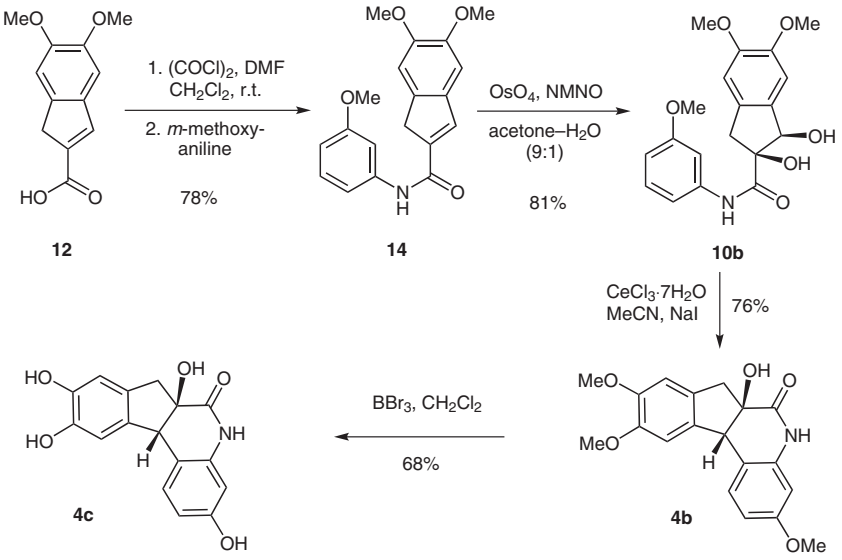
Having successfully established the synthetic route to lactone and lactam analogues of brazilin, we then focused our attention on aza-brazilins, which were expected to be better analogues from the viewpoint of solubility. The keto esters **7a** and **7b** obtained by similar procedure indicated in Scheme 2 were converted to β-ketoanilides **9a–h** by treatment with aniline derivatives in refluxing xylene. β-Ketoanilides were then converted to compound **15a–h** by an oxidative hydroxylation with cerium(III) chloride in the presence of oxygen.[11] Reduction of compound **15a–h** led to a mixture of diols **10c–j**, which upon treatment with ethereal boron trifluoride in dichloromethane at 0 °C provided the desired aza-brazilins **4d–k**. Brazilane (**3**)-like compounds **4l** and **4m** (Scheme 4) were also obtained by reduction of **9a** and **9h** followed by cyclization with a Friedel–Crafts reaction.

With the brazilin-like compounds in hand,[12] we then initiated studies towards the biological activities of these natural product mimics. Considering the antitumor activities of brazilin reported in the literature,[7c] we decided to focus our initial biological study on the anticancer bioassay. The

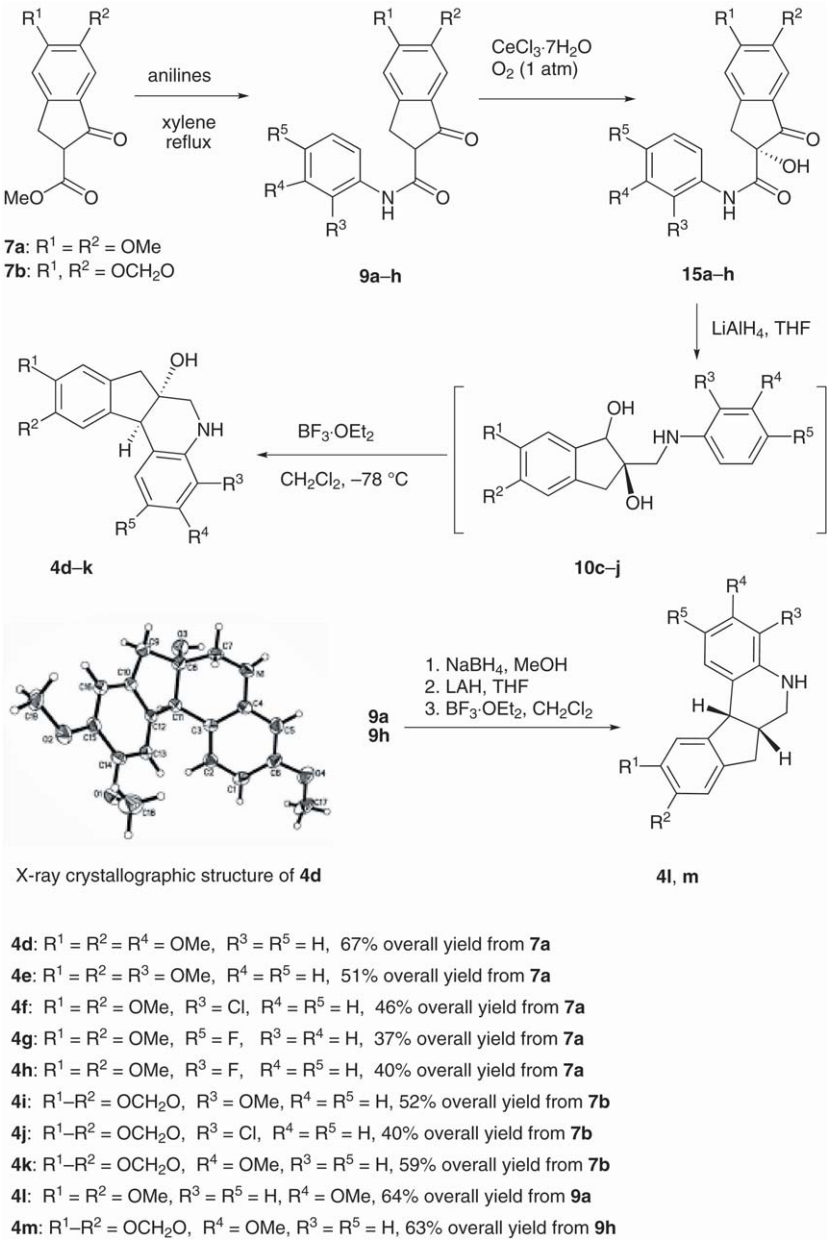
Table 1 Anticancer Activity of Brazilin-Like Compounds (IC_{50}, μM/mL)

Compd	K562 (μM)	A549 (μM)	HT-29 (μM)	HL60 (μM)
4a	>40	>40	>40	>40
4b	15.4 ± 2.9	>40	>40	>40
4c	13.2 ± 3.4	>40	>40	19.9 ± 9.6
4d	9.5 ± 3.9	>40	>40	>40
4e	2.4 ± 0.8	3.7 ± 1.7	2.0 ± 0.8	0.4 ± 0.6
4f	3.6 ± 1.2	>40	7.1 ± 3.3	4.7 ± 1.3
4g	11.5 ± 2.8	26.3 ± 8.	26.7 ± 6.0	30.0 ± 6.5
4h	30.0 ± 6.5	>40	>40	18.0 ± 5.6
4i	2.8 ± 1.1	5.2 ± 2.3	5.4 ± 2.9	2.2 ± 1.2
4j	6.2 ± 2.2	14.2 ± 7.2	6.1 ± 2.3	18.6 ± 4.7
4k	2.8 ± 1.3	>40	>40	12.0 ± 4.4
4l	3.9 ± 1.1	> 40 μM	20.0 ± 9.8	9.9 ± 2.8
4m	6.2 ± 1.9	>40	>40	>40
DDP	3.1 ± 0.5	5.8 ± 0.6	2.3 ± 0.7	8.2 ± 1.1

[a] Cytotoxicity as IC_{50} values for each cell line, the concentration of compound that caused 50% reduction in absorbance at λ = 570 nm relative to untreated cells using the MTT assay.
[b] Human chronic myelogenous leukemia (K562), human lung adenocarcinoma (A-549), human colon carcinoma (HT29), human promyelocytic leukemia (HL-60).



Scheme 3 Synthesis of lactam analogue of brazilin



Scheme 4 Synthesis of aza-brazilin analogues

cytotoxic properties of the synthesized brazilin-like compounds were evaluated in vitro against four human tumor cell lines (including K562, A-549, HT-29, HL-60) through MTT assay, and IC_{50} values (concentration required to produce 50% cell-growth inhibition) were calculated. Cisplatin (DDP) was used as the reference drug.[13] The results are summarized in Table 1.

Lactone **4a** showed no activity (>40 μM for four cancer cell lines), lactam **4c** displayed mild activity against K562 and HL-60 cancer cell lines. To our delight, compound **4e** and **4i** displayed potent cytotoxic activity in vitro with IC_{50} values at the range of 0.4–5.4 μM towards all cancer cell lines tested. Compound **4e** (IC_{50} = 0.4 μM), **4f** (IC_{50} = 4.7 μM), **4i** (IC_{50} = 2.2 μM), **4j** (IC_{50} = 18.6 μM) and **4l** (IC_{50} = 9.9 μM) showed potential activities against myeloid leukaemia (HL-60); compounds **4d**,**e**,**f**,**i**,**j**,**l** also exhibited potent activities against K562 cancer cell. Among the 13 derivatives **4a**–**m**, compound **4e** and **4i** showed promising antiproliferative activities against a broad spectrum of human tumor cell lines, including leukemia and solid tumors (lung, colon cancer cell lines).

In conclusion, we have developed a flexible strategy for the synthesis of brazilin-like compounds. A series of new brazilin-like compounds have been synthesized. Preliminary screening of a number of representative derivatives has already resulted in some significant cytotoxic properties. Interpretation of natural lead structure as a combination of easily accessible common building block, in particular as indane and dihydrobenzopyranyl unit in this research, provided an alternative way for design and synthesis of biologically active natural product-like com-

pounds. Further analogues are now being prepared for bioassay, and the results will be reported in due course.

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett.

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(10) CCDC804781 (for compound **4a**) and CCDC804782 (for compound **4d**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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(12) **Representative Brazilin-Like Compounds**
Lactone analogue **4e**: pale red syrup. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.02 (1 H, d, *J* = 7.5 Hz), 6.70–6.86 (4 H, m), 4.15 (1 H, s), 3.84 (3 H, s), 3.81 (3 H, s), 3.77 (3 H, s), 3.34 (1 H, d, *J* = 15.3 Hz), 3.04–3.13 (2 H, m), 2.84 (1 H, d, *J* = 15.6 Hz) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 148.41, 148.12, 146.88, 137.37, 133.69, 130.87, 122.96, 121.67, 117.82, 108.72, 77.83, 56.10, 55.48, 52.51, 48.07, 42.13 ppm. MS (EI): *m/z* (%) = 328 (25) [M^+ + 1], 327 (100) [M^+], 309 (23), 308 (86), 294 (32), 278 (9), 250 (4), 239 (4), 220 (4), 208 (4), 191 (4), 176 (58), 151 (65), 133 (13), 107 (12). HRMS: *m/z* calcd for $C_{19}H_{21}NO_4$ [$M]^+$: 327.1471; found: 327.1479.

(13) The cytotoxicity assay was carried out on four cell lines (K562, A549, HT-29, and HL60). Cells were cultured at 37 °C under a humidified atmosphere of 5% CO_2 in RPMI 1640 medium supplemented with 10% fetal serum and dispersed in replicate 96-well plates. Compounds were then added. After 48 h exposure to the compounds, cells viability were determined by the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) cytotoxicity assay by measuring the absorbance at λ = 570 nm with a microplate spectrophotometer. Each test was performed in triplicate. Cisplatin (DDP) was used as the reference drug.