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## Antitumor Agents. 271. Total Synthesis and Evaluation of Brazilein and Analogs as Anti-inflammatory and Cytotoxic Agents

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### Abstract

The first total synthesis of the naturally occurring tetracyclic homoisoflavonoid brazilein (**1**) and 14 new analogs (**1a–n**) is reported. Target compounds and intermediates were assayed for anti-inflammatory effects on superoxide anion generation and elastase release by human neutrophils in response to fMLP/CB, and for cytotoxic activity against nasopharyngeal (KB), vincristine-resistant nasopharyngeal (KBvin), lung (A549) and prostate (DU-145) human cancer cell lines. The most active compound **1b** showed potent effects on superoxide anion generation and elastase release with IC<sub>50</sub> values of 1.2 and 1.9 μM, respectively, and was 65 times more potent than phenylmethylsulfonyl fluoride (PMSF), the positive control, in the latter assay. Additionally, **1b** exhibited broad spectrum in vitro anticancer activity with IC<sub>50</sub> values of 6–11 μM against the four tested cancer cell lines.

### Keywords

homoisoflavonoid; brazilein; anti-inflammatory; cytotoxic

Naturally occurring homoisoflavonoids (3-benzylidene-4-chromanones) are related structurally to flavonoids, and exhibit various biological activities.<sup>1</sup> Synthesis of the 3-benzylidene-4-chromanone skeleton is usually based on the condensation of 4-chromanones with aromatic aldehydes in the presence of an acidic or basic catalyst.<sup>2,3</sup> Although members of this family have been the subjects of several syntheses,<sup>4,5</sup> there are no reports on the total synthesis of brazilein (**1**) nor the preparation of **1**-analogs.

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In our prior study, we isolated the unique tetracyclic homoisoflavonoid brazilein (**1**), whose stereochemistry was obtained as dextrorotatory,<sup>6</sup> from an ethyl acetate extract of *Caesalpinia sappan* L. (Legminosae). Brazilein ((6a*R*)-6a,7-dihydro-3,6a,10-trihydro-benz[*b*]indeno[1,2-*d*]pyran-9-(6*H*)-one) is a natural red pigment used in traditional Chinese medicine. It also exhibits significant cytotoxic activity against HepG2 and Hep3B (liver), MDA-MB-231 and MCF-7 (breast), A549 (lung) and Ca9-22 (gingival) human cancer cell lines (Table 1).<sup>6b</sup> Previous literatures showed that brazilein (**1**) exhibited immunosuppressive activity in mice lymphocytes,<sup>7</sup> cardiotoxic effects in isolated rat hearts,<sup>8</sup> and anti-oxidant properties.<sup>9,10</sup> Brazilin (**2**) is the hydrogenation product of brazilein (**1**) in the proposed biosynthetic route,<sup>11</sup> and can be oxidized to brazilein (**1**) when the extract has been exposed to air and light. Beyond this redox chemistry, our interest in devising a general synthesis for this skeleton was based on literature reports that brazilin (**2**) has cytotoxicity (Table 1)<sup>6</sup> and anti-inflammatory properties,<sup>12,13</sup> acts as a micromolar telomerase inhibitor,<sup>14</sup> and produces DNA nicks.<sup>15</sup> These properties are often associated with useful anticancer agents. Additionally, to the best of our knowledge, brazilein (**1**) and its derivatives have not been studied for anti-inflammatory and cytotoxic effects. Considering the structural similarities between brazilin (**2**) and brazilein (**1**), we wanted to determine whether the latter exhibits a similar spectrum of *in vitro* anti-inflammatory and anticancer effects.

We report herein a strategy that enables the preparation of **1** by construction of the benzylidene moiety *via* acid- or base-catalyzed aldol condensation with aryl aldehydes. All newly synthesized compounds, including structurally related intermediates, were assayed for *in vitro* cytotoxicity against four human cancer cell lines, [KB (nasopharyngeal), KBvin (multidrug-resistant nasopharyngeal over-expressing P-gp), A549 (lung) and DU-145 (prostate)] and for anti-inflammatory action in terms of superoxide anion generation and elastase release by human neutrophils in response to fMLP/CB.

The general synthesis of brazilein (**1**) was achieved through the route outlined in Scheme 1. 7-Hydroxy-4-chromanone (**5**), a key intermediate, was readily obtained by reaction of resorcinol (**3**) and 3-chloropropionic acid promoted by trifluoromethanesulfonic acid to give 2',4'-dihydroxy-3-chloropropiophenone (**4**), which was further cyclized using 2M NaOH<sup>16</sup> to give **5** in 78% yield. Treatment of **5** with iodomethane in the presence of K<sub>2</sub>CO<sub>3</sub>/acetone gave **6** (60%). Acid catalyzed<sup>17</sup> condensation of **6** with 3,4-dimethoxybenzaldehyde afforded **7** (78%). Epoxidation of **7** with alkaline hydrogen peroxide gave epoxy ketone **8** (90%), which was reduced to the diol **9** (89%) with LiAlH<sub>4</sub>.<sup>3</sup> Cyclization to the desired product was affected by HClO<sub>4</sub><sup>3</sup> to yield trimethylbrazilin **10** in 31% yield. Compound **10** was demethylated using pyridine hydrochloride<sup>18</sup> to give **2** in 36% yield. Brazilin (**2**) was oxidized with a catalytic amount of iodine solution to give brazilein (**1**) in 78% yield.

In addition, the brazilein derivatives **1a**, **1b/c**, and **1d** were obtained by esterification of **1** with acetic anhydride, propionyl chloride, and benzoic chloride, respectively, in pyridine. For **1e** and **1f**, **1** was reacted in strong base (NaH) with dimethylcarbonyl chloride and diethylcarbonyl chloride to produce the desired products **1d** and **1f**, respectively. Compounds **1g–n** were synthesized by the general Williamson method as described in previous reports.<sup>19,20</sup> Specifically, **1g–n** were prepared by alkylation of **1** with iodomethane (**1g** and **h**), iodoethane (**1i** and **j**), benzyl bromide (**1k**), allylbromide (**1l** and **m**) and prenyl bromide (**1n**), in the presence of K<sub>2</sub>CO<sub>3</sub> in acetone. Accordingly, the first total syntheses of **1** and 14 analogs (**1a–n**) (Figure 1) were accomplished.

## Anti-inflammatory activity

Synthesized **1**, **2**, and intermediates **7–10** together with analogs **1a–n**, which are divided into three classes based on the substitution pattern at C-3, C-6a and C-10 and functionalized into

ester, carbamate and ether, were evaluated for anti-inflammatory action based on effects against superoxide anion generation and elastase release by human neutrophils in response to fMLP/CB. The assays were performed using established protocols,<sup>21,22</sup> which are widely used to identify potential anti-inflammatory compounds. Table 2 lists the results for the test compounds, as well as diphenyleneiodonium (DPI) and phenylmethylsulfonyl fluoride (PMSF), included as positive controls for superoxide anion generation and elastase release, respectively. Results are expressed as the mean  $\pm$  S.E.M., and comparisons were made using Student's *t*-test. A probability of 0.05 or less was considered significant.

Among the esterified compounds **1a-d**, analogs **1a** (di-acetate) and **1d** (tri-benzoate) showed selective anti-inflammatory activity (**1a** vs superoxide anion generation, 18.7  $\mu$ M; **1d** vs elastase release, 13.3  $\mu$ M). Analog **1b** (tri-propionate) showed the highest potency against both superoxide anion generation and elastase release with significant IC<sub>50</sub> values of 1.2 and 1.9  $\mu$ M, respectively. Analog **1c** (di-propionate) was slightly less potent with IC<sub>50</sub> values of 1.65 and 3.28  $\mu$ M, but still was more active than the parent compound **1**. Therefore, replacing the hydroxy groups with propionate esters increased potency in both assays, particularly when all three hydroxyls were esterified in **1b**.

*N*-Diethyl carbamate **1f** showed a weak effect (IC<sub>50</sub> 15.5  $\mu$ M) in response to fMLP/CB-induced elastase release, but neither **1f** nor the *N*-dimethyl carbamate **1e** showed a significant effect against superoxide anion generation. Although the ester and carbamate functional group present parallel physical and chemical properties, the data showed that the former did provide better activity against either superoxide anion generation or elastase release.

Among the eight compounds (**1g-n**) containing ether substituents, **1g** (tri-methoxy) and **1j** (di-ethoxy) displayed potent effects in the superoxide anion assay (IC<sub>50</sub> 4.7 and 4.4  $\mu$ M, respectively). Analog **1j** (di-ethoxy) also showed a moderate effect (IC<sub>50</sub> 8.4  $\mu$ M) against elastase release, as did **1k** (di-benzoyloxy) (IC<sub>50</sub> 8.8  $\mu$ M). Compounds **1l-n** (allyl and prenyl ethers) were non-selective, but with only weak inhibitory effects in both assays (IC<sub>50</sub> 10–20  $\mu$ M). These findings suggest that **1g** and **1j** merit further investigation as potential anti-inflammatory compounds.

Furthermore, among the synthetic intermediates **7–10** (Table 1), the epoxy ketone **8** was significantly potent and selective (IC<sub>50</sub> 1.9  $\mu$ M) against superoxide anion generation. The diol **9** was moderately active in both anti-inflammatory assays (IC<sub>50</sub> 8.4 and 10.3  $\mu$ M), while the tetracyclic **10** was potent only against superoxide anion generation (IC<sub>50</sub> 4.5  $\mu$ M).

In conclusion, among all screened compounds, including ester (**1a–d**), carbamate (**1e** and **1f**), and ether (**1g–n**) derivatives, the preliminary structure-activity relationship were addressed in the order: a) ester > ether > carbamate; b) ethyl  $\approx$  methyl > allyl  $\approx$  prenyl > benzyl. In particular, the most potent analog **1b** was 65-fold more active than PMSF, the positive control, in the elastase release assay.

## Anti-cancer activity

The homoisoflavonoids **1a–n** as well as the synthesized **1**, **2** and intermediates **7–10** were examined for *in vitro* cytotoxic activity against four human cancer lines. Table 3 lists the IC<sub>50</sub> values obtained with test compounds compared to the anticancer drug paclitaxel as a positive control. Among the 14 derivatives (**1a–n**), seven compounds (**1a–c**, **1g**, **1i**, **1j** and **1m**) showed moderate potency against the tested human cancer cell lines with IC<sub>50</sub> values of 5–18  $\mu$ M; however, they all were much less active than paclitaxel.

In our previous study, the parent compound **1** showed moderate cytotoxic activity against six cancer cell lines (IC<sub>50</sub> 8–34  $\mu$ M, Table 1).<sup>6b</sup> Comparison of **1** to its synthetic intermediates **2**

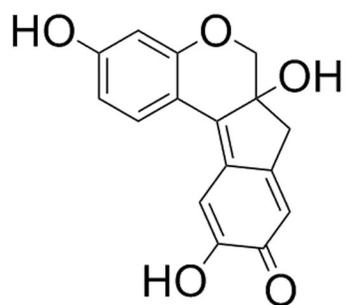
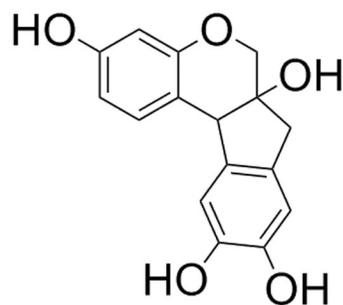
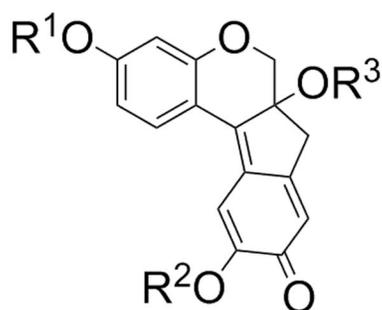
and **7–10** showed that the epoxy ketone **8** had comparable or enhanced activity, while the reduced diol **9** was inactive ( $IC_{50} > 20 \mu M$ ). In addition, trimethylbrazilin **10** was more active than **2** with  $IC_{50}$  values of 10–15  $\mu M$  against all four human cancer cell lines. The enhanced cytotoxicity of epoxide analog **8** could account for its twofold greater potency than **1** against superoxide anion generation.

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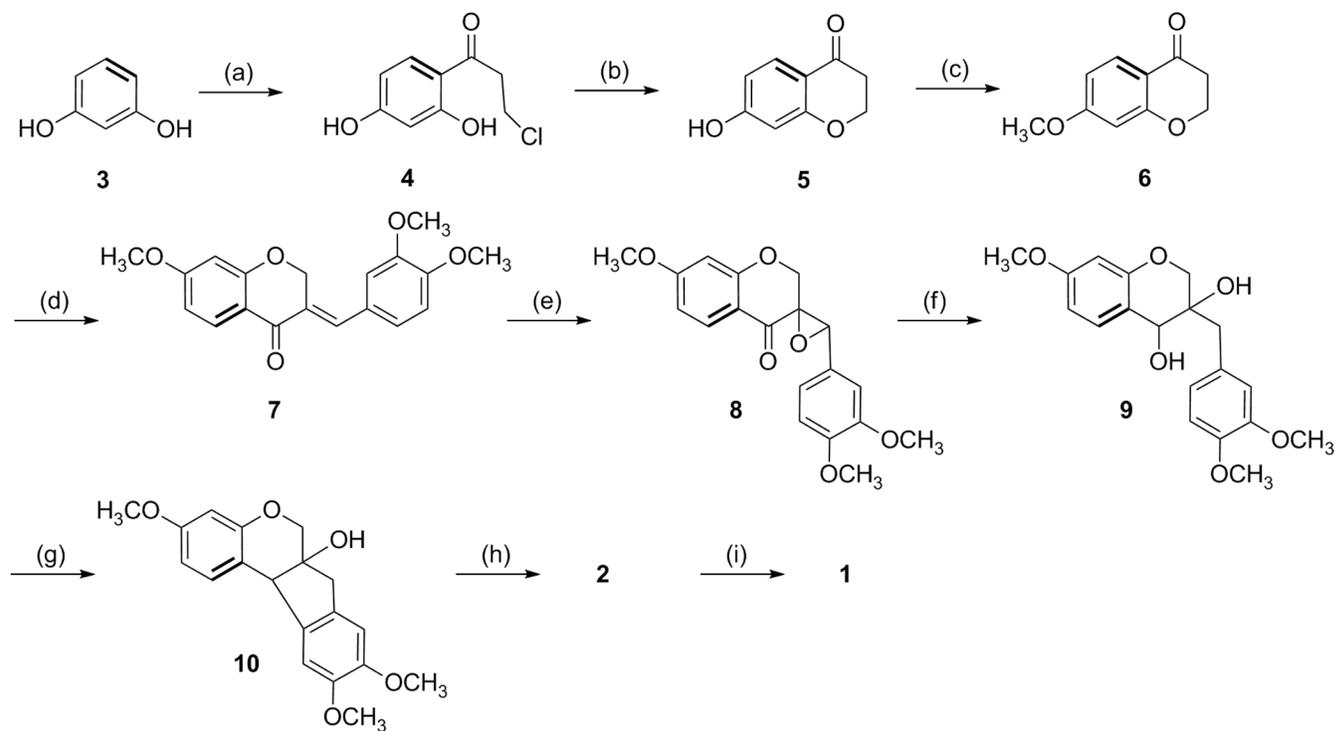
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**1, Brazilein****2, Brazilin**

	$R^1=R^2$	$R^3$
<b>1a</b>	COCH <sub>3</sub>	H
<b>1b</b>	COCH <sub>2</sub> CH <sub>3</sub>	COCH <sub>2</sub> CH <sub>3</sub>
<b>1c</b>	COCH <sub>2</sub> CH <sub>3</sub>	H
<b>1d</b>	COC <sub>6</sub> H <sub>5</sub>	COC <sub>6</sub> H <sub>5</sub>
<b>1e</b>	CON(CH <sub>3</sub> ) <sub>2</sub>	H
<b>1f</b>	CON(CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub>	H
<b>1g</b>	CH <sub>3</sub>	CH <sub>3</sub>
<b>1h</b>	CH <sub>3</sub>	H
<b>1i</b>	CH <sub>2</sub> CH <sub>3</sub>	CH <sub>2</sub> CH <sub>3</sub>
<b>1j</b>	CH <sub>2</sub> CH <sub>3</sub>	H
<b>1k</b>	CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	H
<b>1l</b>	CH <sub>2</sub> CH=CH <sub>2</sub>	CH <sub>2</sub> CH=CH <sub>2</sub>
<b>1m</b>	CH <sub>2</sub> CH=CH <sub>2</sub>	H
<b>1n</b>	CH <sub>2</sub> CH=C(CH <sub>3</sub> ) <sub>2</sub>	H

**Figure 1.**  
Molecular structures of brazilein (**1**), brazilin (**2**) and synthetic **1**-analogs.

**Scheme 1.**

Total synthesis of brazilein (**1**).

Reagents: (a) 3-Chloropropionic acid,  $\text{CF}_3\text{SO}_3\text{H}$ , 80 °C, 1h, 92%; (b) 2M NaOH, 0 °C, 2h, 78%; (c) Iodomethane,  $\text{K}_2\text{CO}_3$ , acetone, 60 °C, 5h, 60%; (d) 3,4-Dimethoxybenzaldehyde, EtOH, HCl gas, rt, overnight, 78%; (e)  $\text{H}_2\text{O}_2$ , dioxane, rt, 24h, 90%; (f)  $\text{LiAlH}_4$ , THF, rt, overnight, 89%; (g)  $\text{HClO}_4$ , acetic acid, rt, overnight, 31%; (h) Pyridine-HCl, 190–200 °C, 3h, 36%; (i) 1N  $\text{I}_2(\text{aq})$ , rt, overnight, 78%.

Table 1

Cytotoxic effects of brazillein (1) and brazilin (2).

Compd.	Cancer Cell Lines (IC <sub>50</sub> value <sup>a</sup> , µg/mL)					
	HepG2	Hep3B	MDA-MB-231	MCF7	A549	Ca9-22
Brazillein (1)	3.15	3.42	2.36	3.96	9.68	8.63
Brazilin (2)	3.47	4.54	3.09	6.29	15.56	9.78
Doxorubicin <sup>b</sup>	0.34	0.56	1.05	0.42	0.32	0.14

<sup>a</sup>IC<sub>50</sub> represents the 50% inhibitory concentration.<sup>b</sup>Positive control.

**Table 2**

Inhibitory effects of compounds on superoxide anion generation and elastase release by human neutrophils in response to FMLP/CB.

Compound	Superoxide anion	Elastase release
	IC <sub>50</sub> (μM) <sup>a</sup> or (Inh %)	IC <sub>50</sub> (μM) <sup>a</sup> or (Inh %)
Brazilein (1)	4.0 ± 0.42	14.2 ± 1.32
Brazilin (2)	4.6 ± 0.63	16.7 ± 0.41
<b>1a</b>	18.71 ± 2.82	(40.87 ± 3.49) <sup>***</sup>
<b>1b</b>	1.2 ± 0.02	1.9 ± 0.31
<b>1c</b>	1.65 ± 0.09	3.28 ± 0.51
<b>1d</b>	(14.14 ± 5.70) <sup>*</sup>	13.25 ± 1.14
<b>1e</b>	(16.37 ± 3.71)	(11.39 ± 4.76)
<b>1f</b>	(-7.00 ± 4.90)	15.50 ± 2.40
<b>1g</b>	4.65 ± 1.31	13.6 ± 1.09
<b>1h</b>	(18.61 ± 6.29) <sup>*</sup>	(26.76 ± 6.60) <sup>*</sup>
<b>1i</b>	23.94 ± 5.48	(35.54 ± 1.71) <sup>***</sup>
<b>1j</b>	4.35 ± 0.91	8.37 ± 0.41
<b>1k</b>	(33.38 ± 7.75) <sup>**</sup>	8.76 ± 1.51
<b>1l</b>	16.16 ± 0.40	11.83 ± 2.00
<b>1m</b>	19.72 ± 4.44	16.54 ± 1.70
<b>1n</b>	19.69 ± 5.82	10.06 ± 0.60
<b>7</b>	NT <sup>b</sup>	NT <sup>b</sup>
<b>8</b>	1.90 ± 0.32	(43.71 ± 2.00) <sup>***</sup>
<b>9</b>	8.38 ± 0.02	10.25 ± 0.14
<b>10</b>	4.52 ± 1.21	(35.76 ± 2.60) <sup>*</sup>
<b>DPI<sup>c</sup></b>	0.7 ± 0.4	
<b>PMSF<sup>c</sup></b>		130.9 ± 2.91

<sup>a</sup> IC<sub>50</sub> represents the 50% inhibitory concentration of the compound. If 50% inhibition was not reached at any test dose, the percentage of inhibition obtained at a test dose of 10 μg/mL is given in parentheses (Inh %). Results are presented as mean ± S.E.M. (n = 3–5).

\*  $p < 0.05$ ,

\*\*  $p < 0.01$ , and

\*\*\*  $p < 0.001$  compared with the control value.

<sup>b</sup> Abnormal absorption at OD405 and 550 nm.

<sup>c</sup> DPI and PMSF were used as positive controls.

**Table 3**Cytotoxic effects of **1**-analogs.

Compound	Cancer Cell line (IC <sub>50</sub> value <sup>a</sup> , $\mu$ M)			
	KB	KBvin	A549	DU-145
<b>1a</b>	10.0 $\pm$ 1.3	ND <sup>b</sup>	8.1 $\pm$ 1.2	8.2 $\pm$ 0.72
<b>1b</b>	6.6 $\pm$ 2.21	11.3 $\pm$ 1.53	13.5 $\pm$ 1.12	11.0 $\pm$ 0.47
<b>1c</b>	7.7 $\pm$ 3.5	ND <sup>b</sup>	7.8 $\pm$ 0.65	7.1 $\pm$ 0.44
<b>1g</b>	15.2 $\pm$ 1.41	16.9 $\pm$ 0.42	> 20	17.5 $\pm$ 0.67
<b>1i</b>	18.9 $\pm$ 1.11	17.9 $\pm$ 0.9	> 20	18.1 $\pm$ 1.54
<b>1j</b>	11.3 $\pm$ 0.78	16.5 $\pm$ 1.56	15.3 $\pm$ 1.04	11.0 $\pm$ 0.67
<b>1m</b>	5.4 $\pm$ 0.29	ND <sup>b</sup>	9.4 $\pm$ 0.49	9.1 $\pm$ 0.75
<b>7</b>	> 20	18.3 $\pm$ 0.26	> 20	> 20
<b>8</b>	9.8 $\pm$ 1.28	6.4 $\pm$ 0.67	> 20	10.6 $\pm$ 0.42
<b>9</b>	> 20	> 20	> 20	> 20
<b>10</b>	10.5 $\pm$ 0.48	7.6 $\pm$ 0.73	> 20	13.8 $\pm$ 1.26
Paclitaxel <sup>c</sup>	1.55 $\times$ 10 <sup>-3</sup>	1.09 $\times$ 10 <sup>-3</sup>	1.93 $\times$ 10 <sup>-3</sup>	2.35 $\times$ 10 <sup>-3</sup>

<sup>a</sup>Data are expressed as mean  $\pm$  SD (n = 3).<sup>b</sup>ND: Not determined.<sup>c</sup>Positive control.