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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_{50} 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_{50} values of 6–11 μM against the four tested cancer cell lines.

Naturally occurring homoisoflavonoids (3-benzylidene-4-chromanones) are related structurally to flavonoids, and exhibit various biological activities.[1] 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.[2,3] Although members of this family have been the subjects of several syntheses,[4,5] there are no reports on the total synthesis of brazilein (**1**) nor the preparation of **1**-analogs.

In our prior study, we isolated the unique tetracyclic homoisoflavonoid brazilein (**1**), whose stereochemistry was obtained as dextrorotatory,[6] from an ethyl acetate extract of *Caesalpinia sappan* L. (Legminosae). Brazilein ((6a*R*)-6a,7-dihydro-3,6a,10-trihydrobenz[*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).[6b] Previous literatures showed that brazilein (**1**) exhibited immunosuppressive activity in mice lymphocytes,[7] cardiotonic effects in isolated rat hearts,[8] and anti-oxidant properties.[9,10] Brazilin (**2**) is the hydrogenation product of brazilein (**1**) in the proposed biosynthetic route,[11] 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)[6] and anti-inflammatory properties,[12,13] acts as a micromolar telomerase inhibitor,[14] and produces DNA nicks.[15] 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

Table 1
Cytotoxic effects of brazilein (**1**) and brazilin (**2**)

Compd	Cancer cell lines (IC_{50} value[a], μg/mL)					
	HepG2	Hep3B	MDA-MB-231	MCF7	A549	Ca9-22
Brazilein (**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[b]	0.34	0.56	1.05	0.42	0.32	0.14

[a] IC_{50} represents the 50% inhibitory concentration.
[b] Positive control.

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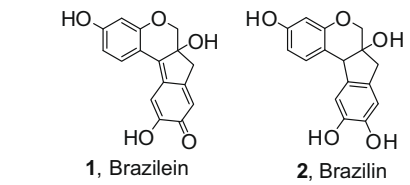
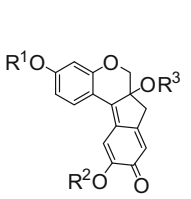
aldol condensation with aryl aldehydes. All newly synthesized compounds, including structurally related intermediates, were assayed for in vitro cytotoxicity against four human cancer cell lines, [KB (nasopharyngeal), KBvin (multidrug-resistant nasopharngeal over-expressing P-gp), A549 (lung), and DU-145 (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 2 M NaOH[16] to give **5** in 78% yield. Treatment of **5** with iodomethane in the presence of K_2CO_3/acetone gave **6** (60%). Acid catalyzed[17] 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 $LiAlH_4$.[3] Cyclization to the desired product was affected by $HClO_4$[3] to yield trimethylbrazilin **10** in 31% yield. Compound **10** was demethylated using pyridine hydrochloride[18] 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 dimethylcarbamyl chloride and diethylcarbamyl chloride respectively. Compounds **1g**–**n** were synthesized by the general Williamson method as described in previous reports.[19,20] 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_2CO_3 in acetone. Accordingly, the first total syntheses of **1** and 14 analogs (**1a**–**n**) (Fig. 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,[21,22] 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





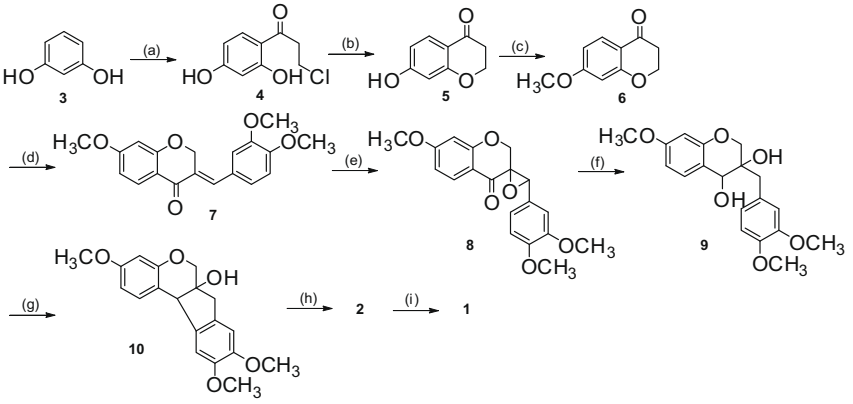
	R^1=R^2	R^3
1a	$COCH_3$	H
1b	$COCH_2CH_3$	$COCH_2CH_3$
1c	$COCH_2CH_3$	H
1d	COC_6H_5	COC_6H_5
1e	$CON(CH_3)_2$	H
1f	$CON(CH_2CH_3)_2$	H
1g	CH_3	CH_3
1h	CH_3	H
1i	CH_2CH_3	CH_2CH_3
1j	CH_2CH_3	H
1k	$CH_2C_6H_5$	H
1l	$CH_2CH{=}CH_2$	$CH_2CH{=}CH_2$
1m	$CH_2CH{=}CH_2$	H
1n	$CH_2CH{=}C(CH_3)_2$	H

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

controls for superoxide anion generation and elastase release, respectively. Results are expressed as the mean ± 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 μM; **1d** vs elastase release, 13.3 μM). Analog **1b** (tri-propionate) showed the highest potency against both superoxide anion generation and elastase release with significant IC_{50} values of 1.2 and 1.9 μM, respectively. Analog **1c** (di-propionate) was slightly less potent with IC_{50} values of 1.65 and 3.28 μM, 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_{50} 15.5 μM) 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,



Scheme 1. Total synthesis of brazilein (**1**). Reagents and conditions: (a) 3-Chloropropionic acid, CF_3SO_3H, 80 °C, 1 h, 92%; (b) 2 M NaOH, 0 °C, 2 h, 78%; (c) iodomethane, K_2CO_3, acetone, 60 °C, 5 h, 60%; (d) 3,4-dimethoxylbenzaldehyde, EtOH, HCl gas, rt, overnight, 78%; (e) H_2O_2, dioxane, rt, 24 h, 90%; (f) $LiAlH_4$, THF, rt, overnight, 89%; (g) $HClO_4$, acetic acid, rt, overnight, 31%; (h) pyridine–HCl, 190–200 °C, 3 h, 36%; (i) 1 N $I_{2(aq)}$, rt, overnight, 78%.

Table 2
Inhibitory effects of compounds on superoxide anion generation and elastase release by human neutrophils in response to FMLP/CB

Compd	Superoxide anion IC_{50} (μM)[a] or (Inh%)	Elastase release IC_{50} (μM)[a] or (Inh%)
Brazilein (**1**)	4.0 ± 0.42	14. 2 ± 1.32
Brazilin (**2**)	4.6 ± 0.63	16.7 ± 0.41
1a	18.71 ± 2.82	(40.87 ± 3.49)***
1b	1.2 ± 0.02	1.9 ± 0.31
1c	1.65 ± 0.09	3.28 ± 0.51
1d	(14.14 ± 5.70)*	13.25 ± 1.14
1e	(16.37 ± 3.71)	(11.39 ± 4.76)
1f	(−7.00 ± 4.90)	15.50 ± 2.40
1g	4.65 ± 1.31	13.6 ± 1.09
1h	(18.61 ± 6.29)*	(26.76 ± 6.60)*
1i	23.94 ± 5.48	(35.54 ± 1.71)***
1j	4.35 ± 0.91	8.37 ± 0.41
1k	(33.38 ± 7.75)**	8.76 ± 1.51
1l	16.16 ± 0.40	11.83 ± 2.00
1m	19.72 ± 4.44	16.54 ± 1.70
1n	19.69 ± 5.82	10.06 ± 0.60
7	NT[b]	NT[b]
8	1.90 ± 0.32	(43.71 ± 2.00)***
9	8.38 ± 0.02	10.25 ± 0.14
10	4.52 ± 1.21	(35.76 ± 2.60)*
DPI[c]	0.7 ± 0.4	
PMSF[c]		130.9 ± 2.91

[a] IC_{50} 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.
[b] Abnormal absorption at OD 405 and 550 nm.
[c] DPI and PMSF were used as positive controls.

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_{50} 4.7 and 4.4 μM, respectively). Analog **1j** (di-ethoxy) also showed a moderate effect (IC_{50} 8.4 μM) against elastase release, as did **1k** (di-benzoxy) (IC_{50} 8.8 μM). Compounds **1l**–**n** (allyl and prenyl ethers) were nonselective, but with only weak inhibitory effects in both assays (IC_{50} 10–20 μM). 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_{50} 1.9 μM) against superoxide anion generation. The diol **9** was moderately active in both anti-inflammatory assays (IC_{50} 8.4 and 10.3 μM), while the tetracyclic **10** was potent only against superoxide anion generation (IC_{50} 4.5 μM).

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 = methyl > allyl = 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_{50} 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_{50} values of 5–18 μM; 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_{50} 8–34 μM,

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

Compd	Cancer cell line (IC_{50} value[a], μM)			
	KB	KBvin	A549	DU-145
1a	10.0 ± 1.3	ND[b]	8.1 ± 1.2	8.2 ± 0.72
1b	6.6 ± 2.21	11.3 ± 1.53	13.5 ± 1.12	11.0 ± 0.47
1c	7.7 ± 3.5	ND[b]	7.8 ± 0.65	7.1 ± 0.44
1g	15.2 ± 1.41	16.9 ± 0.42	>20	17.5 ± 0.67
1i	18.9 ± 1.11	17.9 ± 0.9	>20	18.1 ± 1.54
1j	11.3 ± 0.78	16.5 ± 1.56	15.3 ± 1.04	11.0 ± 0.67
1m	5.4 ± 0.29	ND[b]	9.4 ± 0.49	9.1 ± 0.75
7	>20	18.3 ± 0.26	>20	>20
8	9.8 ± 1.28	6.4 ± 0.67	>20	10.6 ± 0.42
9	>20	>20	>20	>20
10	10.5 ± 0.48	7.6 ± 0.73	>20	13.8 ± 1.26
Paclitaxel[c]	1.55×10^{-3}	1.09×10^{-3}	1.93×10^{-3}	2.35×10^{-3}

[a] Data are expressed as mean ± SD (n = 3).
[b] ND: not determined.
[c] Positive control.

Table 1).[6b] 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 μM). In addition, trimethylbrazilin **10** was more active than **2** with IC_{50} values of 10–15 μM 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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