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COMMUNICATION

First total synthesis of antrocamphin A and its analogs as anti-inflammatory and anti-platelet aggregation agents†

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Naturally occurring antrocamphin A (1) is a potent anti-inflammatory compound from the edible fungus *Antrodia camphorata* (*Taiwanofungus camphoratus*), whose wild fruiting body is used as a valuable folk medicine in Taiwan. This study is the first total synthesis of antrocamphin A (1) and its analogs. Their inhibition ability on NO release, superoxide anion generation, elastase release and platelet aggregation are reported herein.

Introduction

The endemic fungus *Antrodia camphorata* (*Taiwanofungus camphoratus*), also known as Niu-Chang-Chih (Jang-Jy), is used as a folk medicine and a dietary supplement in Taiwan. Its wild fruiting body is very valuable.[1–3] The chemical composition of this fungus can be classified into three categories: 1). Polysaccharides, 2). Triterpenoids and 3). Enynyl-benzenoids. The polysaccharides are regarded as immunomodulation agents, such as functional foods derived from other fungi, *e.g.*, *Ganoderma lucidum* and *Agaricus blazei*.[1] Triterpenoids are considered to have anti-cancer and anti-inflammatory effects. However, the major chemical component, antrocamphin A (**1**) (Fig. 1), which belongs to the third class, is the key component for anti-inflammatory activity. Evidence is shown below through a complete bioassay-guided fractionation study.[3,4]

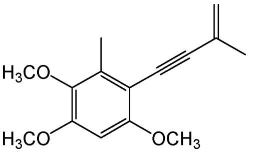

Fig. 1 Antrocamphin A (**1**).

Antrocamphin A (**1**), a potent anti-inflammatory compound naturally found in *A. camphorata* (*T. camphoratus*) and was first isolated by Chen *et al.* in 2007.[4] Wang *et al.* demonstrated its mechanism in suppressing pro-inflammatory molecules (NO and PGE_2), from being released *via* the down-regulation of iNOS and COX-2 expression through the NF-κB pathway.[3] Previous studies indicate that compound **1** may serve as a promising lead drug for the treatment of various diseases that are induced by inflammation. Therefore, antrocamphin A was chosen to be a candidate of further investigation.

Currently, there are only two literature[3,4] reports on the subject of antrocamphin A (**1**). To understand the diverse biological properties and the structure–activity relationship (SAR) of the lead compound **1**, the first total synthesis of compound **1** and its analogs were achieved in the current investigation. All compounds were evaluated for anti-inflammatory and anti-platelet aggregation activities.

Results and discussion

The retrosynthetic analysis of compound **1** is illustrated in Scheme 1. The key step was the Sonogashira reaction. Compound **2** (2-iodo-3,5,6-trimethoxytoluene) was coupled with 2-methyl-1-buten-3-yne (**3**), which led to the target compound. Compound **2** was synthesized from 2,3,5-trimethoxytoluene (**8**), obtained from *o*-vanillin (**5**), in four steps described by Singh and co-workers.[5] We prepared 2-hydroxy-3-methoxytoluene (**4**) by hydrogenating of *o*-vanillin (**5**) over 10% palladium-on-carbon. Compound **4** was then converted to quinone **6** by treatment with the oxidant, potassium nitrosodisulfonate (Fremy's salt). Quinone **6** was reduced to hydroquinone **7** using $TiCl_3$ and then methylated with Me_2SO_4 in the presence of K_2CO_3 resulting in the desired compound **8**. Compound (**2**), 2-iodo-3,5,6-trimethoxytoluene, a key synthon,

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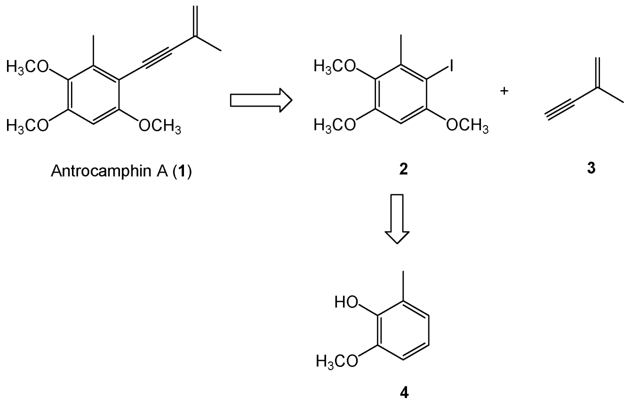
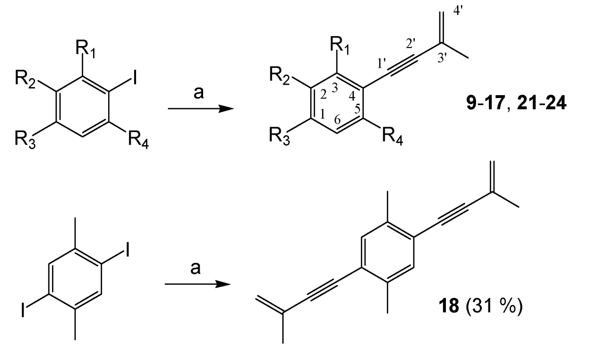


Scheme 1 Retrosynthetic analysis of antrocamphin A.



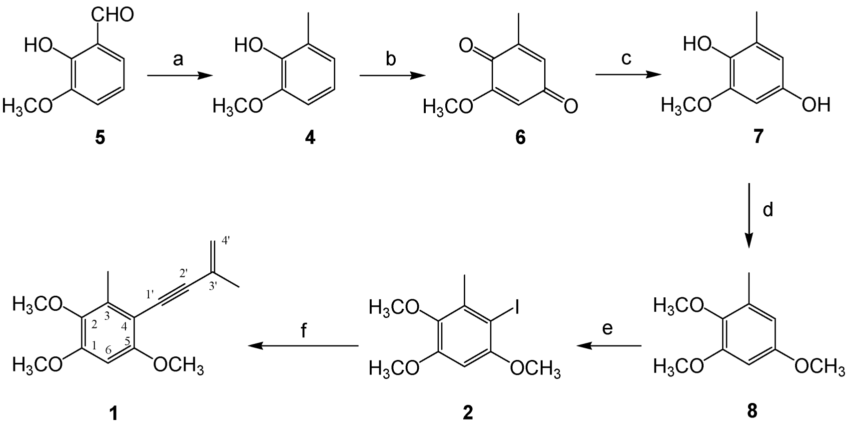
Compound	Substituent group				Yield
	R_1	R_2	R_3	R_4	(%)
9	H	H	H	H	93
10	H	H	OCH_3	H	87
11	OCH_3	H	H	H	73
12	H	OCH_3	H	H	55
13	H	H	CH_3	H	89
14	H	H	CN	H	83
15	H	OCH_3	OCH_3	OCH_3	81
16	H	H	NO_2	H	83
17	CH_3	H	H	H	77
19	CH_3	OCH_3	OCH_3	H	73
20	H	H	OCH_3	OCH_3	63
21	CH_3	H	OCH_3	OCH_3	17
22	CH_3	I	OCH_3	OCH_3	13

Scheme 3 Synthesis of **9–22**. Reagents and conditions: (a) 2-methyl-1-buten-3-yne (**3**), $Pd(PPh_3)_4$, CuI, Et_3N/THF, N_2, rt.

was prepared *via* iodination of **8** using I_2 and CF_3COOAg. The Sonogashira reaction was utilized to couple iodo **2** with 2-methyl-1-buten-3-yne (**3**), using the catalysts $Pd(PPh_3)_4$ and CuI. This key reaction led to the desired compound, antrocamphin A (**1**) (Scheme 2).[6] The Sonogashira coupling reaction was also applied to afford a series of designed analogues (**9–22**) from commercially available iodobenzene products or further iodination benzenoid compounds (Scheme 3).

Synthesized compounds **1** and **9–22** were screened using a nitric oxide (NO) inhibitory assay, where curcumin was used as the positive control. In this study, all analogs were tested on their cytotoxicity toward RAW 264.7 macrophages. Cell viability rates for each test compound showed > 90% at the dosage of 20 μg mL^{-1}. All compounds were evaluated against lipopolysaccharide (LPS)-induced NO production in RAW 264.7 macrophage cell line at the dosage of 20 μg mL^{-1}. As shown in Table 1, compounds **1**, **20**, **21** and **22** showed potent anti-inflammatory activity with NO inhibition rates of 98.1%, 97%, 77%, 84%, respectively.

Compounds **1**, **9**, **15**, **17**, **20** and **21** were synthesized to study the SAR of the CH_3 substitution at C-3 presented in the natural product **1**; however, C3-CH_3 did not influence the ability of NO inhibition. To evaluate the SAR of the OCH_3 functions on the 3′-methyl-but-3′-en-1-ynyl-benzene skeleton, analogs **10–12**, **15** and **20** were designed, which did not have the C3-CH_3 substitution. Compounds **10–12** possess only one OCH_3 group at C-1, C-3 and C-2, respectively. Moreover, **20** and **15** have two and three OCH_3 groups at C-1 and C-5, and C-1, C-2 and C-5, respectively. Among the five compounds, we speculated that the position is more important than the number of OCH_3 substitutions. A similar result was also observed in the test of compounds **19** and **21**, which both have a CH_3 group at C-3 and the same number of OCH_3 substitutions in different positions. Furthermore, a comparison



Scheme 2 Synthetic scheme of antrocamphin A (**1**). Reagents and conditions: (a) 10% Pd/C, H_2, rt, 75%; (b) KH_2PO_4, $(KSO_3)_2NO$, rt, 78%; (c) $TiCl_3$, rt, 90%; (d) $(CH_3O)_2SO_2$, K_2CO_3, acetone, reflux, 88%; (e) I_2, CF_3COOAg, CH_2Cl_2, 0 °C, 74%; (f) 2-methyl-1-buten-3-yne (**3**), $Pd(PPh_3)_4$, CuI, Et_3N/THF, N_2, rt, 10%.

Table 1 Effects of all compounds on NO in LPS-challenged RAW 264.7 cells[a]

Compound[b]	NO inhibition (%)	Cell viability (%)
1	98.10	99.71
9	17.23	101.55
10	33.56	101.98
11	–4.43	90.44
12	27.27	105.80
13	23.11	100.95
14	–0.55	91.77
15	24.98	101.91
16	15.16	94.97
17	6.23	99.73
18	16.61	92.23
19	28.51	99.07
20	97.00	117.08
21	77.00	114.00
22	84.00	102.00
Curcumin[c]	99.00	103.00

[a] All compounds were administrated 1 h before inflammation induction by adding LPS (1 µg mL^{-1}). [b] Dosage of test compound was 20 µg mL^{-1}. [c] Positive control.

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

	Superoxide		Elastase	
Compound	IC_{50}/µg mL^{-1a}	or (Inh %)	IC_{50}/µg mL^{-1a}	or (Inh %)
1	(46.30 ± 7.24)	***	(25.55 ± 3.99)	**
9	4.82 ± 0.41		(37.22 ± 3.12)	***
10	5.63 ± 0.71		4.14 ± 0.35	
11	6.69 ± 0.84		7.94 ± 0.51	
12	4.05 ± 0.41		3.31 ± 0.06	
13	4.66 ± 0.41		44.28 ± 1.67	***
14	(7.95 ± 6.06)		(17.89 ± 1.42)	***
15	3.40 ± 1.49		(26.74 ± 5.88)	*
16	(–10.03 ± 4.84)		22.78 ± 1.01	***
17	(25.55 ± 6.70)	*	(35.55 ± 6.12)	**
18	0.45 ± 0.03		1.32 ± 0.67	
19	1.96 ± 0.28		3.47 ± 0.23	
20	4.69 ± 0.99		8.72 ± 1.15	
21	1.60 ± 0.28		5.33 ± 1.21	
22	(12.13 ± 2.24)	**	(81.36 ± 8.82)	
DPI[b]	0.22 ± 0.13			
PMSF[b]			22.80 ± 5.07	

Per cent of inhibition (Inh%) at a 10-µg mL^{-1} concentration. Results are presented as mean ± S.E.M. (n = 3). *p < 0.05, **p < 0.01, ***p < 0.001 compared with the control value.[a] Concentration necessary for 50% inhibition (IC_{50}). [b] Diphenyleneiodonium (DPI) and phenylmethylsulfonyl fluoride (PMSF) were used as positive controls for superoxide anion generation and elastase release, respectively.

with the structures of **1** and **20–22** concluded that the simultaneous existence of OCH_3 groups at C-1 and C-5 may be necessary for the potential ability of NO inhibition.

LPS-challenged RAW 264.7 macrophages are an *in vitro* model of murine cell lines for studying anti-inflammatory effects. To further explore the anti-inflammatory activities of these target compounds in human cell systems with different modes of action, all analogs were evaluated against superoxide anion generation and elastase release by human neutrophils in response to *N*-formyl-methionyl-leucyl-phenylalanine (FMLP)/cytochalasin B (CB). Antrocamphin A has been reported as a new anti-inflammatory drug lead against superoxide anion generation in FMLP-activated human neutrophils.[4] Activated neutrophils produced high concentrations of the superoxide anion and elastase known to be involved in airway mucus hypersecretion, which is a neutrophilic inflammatory disease like asthma.[7] Therefore, all analogues were tested for their ability to inhibit superoxide anion generation and elastase release. Diphenyleneiodonium (DPI) and phenylmethylsulfonyl fluoride (PMSF) were used as positive controls in this model assay, respectively.

Interestingly, the analogs showed a broad-spectrum of activity in this model (Table 2). Most of the compounds could inhibit both inflammatory mediators and showed more potent effects than the natural product **1**. Compound **18** with a dienynyl functionality exhibited the best activity, which indicated the enynyl substitution played an important role for this model assay. On the whole, we found dimethoxy (**19–22**) and monomethoxy (**10–12**) derivatives were more favorable than trimethoxy and non-methoxy ones. Some of the compounds showed superior activities to the reference drug, PMSF, against elastase release by neutrophils.

Furthermore, all synthesized derivatives were also subjected to an anti-platelet aggregation assay with collagen and thrombin as inducers; aspirin was used as a positive control. As shown in Table 3, compounds **9**, **10**, **11** and **19** showed potent inhibition against collagen-induced platelet aggregation with IC_{50} values of 3.36, 1.16, 7.55 and 7.22 µg mL^{-1}, respectively; their activities were better than aspirin's activity (IC_{50} 13.58 µg mL^{-1}). Comparing the

Table 3 Anti-platelet aggregation data of all compounds

	IC_{50}/µg mL^{-1}	
Compound	Collagen (10 µg mL^{-1})	Thrombin (0.05 U/mL)
1	>50	>50
9	3.36	40.83
10	1.16	44.06
11	7.55	>50
12	>50	>50
13	>50	>50
14	12.14	36.09
15	15.43	>50
16	>50	>50
17	>50	>50
18	14.50	12.92
19	7.22	>50
20	>50	>50
21	>50	>50
22	>50	>50
Aspirin[a]	13.58	>200

[a] Positive control.

bioassay results, most of the compounds did not possess good inhibition toward the platelet aggregation induced by thrombin. No clear SAR can be concluded from the anti-platelet aggregation effect. Interestingly, compounds **1** and **20–22**, which possess the powerful ability of NO inhibition, did not have anti-platelet aggregation activity.

Conclusions

In summary, we have achieved the first total synthesis of the active natural product antrocamphin A (**1**), in a total yield of 3.7% in six steps. Moreover, 14 analogs, including new compounds, **15**, **16** and **18–22**, were synthesized. Most of these compounds possess OMe groups in the benzene ring. We tried to synthesized derivatives with different substitutions around the ring, for example, 2-iodo-3,6-diacetoxy-5-methoxytoluene or 4-iodo-3,6-dihydroxy-5-methoxytoluene coupled with 2-methyl-1-buten-3-yne using different combinations of catalyst [$Pd(PPh_3)_4$ or $PdCl_2(PPh_3)_2$], solvent (THF or DMF or ether) and base (Et_3N). Unfortunately, this resulted in no reaction or reaction without any target compound. Accordingly, only the reagents with OMe groups, which were easily obtained, were chosen to evaluate the amount and position of OMe groups related to the activity in this investigation.

Comparing anti-inflammatory activity, compounds **1**, **20**, **21** and **22** were identified to be potent NO inhibition agents; the dienynyl compound **18** was a new drug lead against superoxide anion generation and elastase release. Moreover, analog **10** is the most potential compound against platelet aggregation induced by collagen. Overall, our data demonstrate that enynyl-benzenoids may have a chance to be developed as safe and potential anti-inflammatory or a cardiovascular protecting agent in the future.

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