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Discovery of novel antitumor dibenzocyclooctatetraene derivatives and related biphenyls as potent inhibitors of NF-κB signaling pathway

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ABSTRACT

Several dibenzocyclooctatetraene derivatives (**5**–**7**) and related biphenyls (**8**–**11**) were designed, synthesized, and evaluated for inhibition of cancer cell growth and the NF-κB signaling pathway. Compound **5a**, a dibenzocyclooctatetraene succinimide, was discovered as a potent inhibitor of the NF-κB signaling pathway with significant antitumor activity against several human tumor cell lines (GI_{50} 1.38–1.45 μM) and was more potent than paclitaxel against the drug-resistant KBvin cell line. Compound **5a** also inhibited LPS-induced NF-κB activation in RAW264.7 cells with an IC_{50} value of 0.52 μM, prevented IκB-α degradation and p65 nuclear translocation, and suppressed LPS-induced NO production in a dose-dependent manner. The antitumor data in cellular assays indicated that relative positions and types of substituents on the dibenzocyclooctatetraene or acyclic biphenyl as well as torsional angles between the two phenyls are of primary importance to antitumor activity.

1. Introduction

Despite many technological advances during the past two decades, natural products remain one of the most important sources of novel leads for new drugs. Dibenzocyclooctadiene lignans are major constituents of the traditional Chinese medicinal plant *Schizandra chinensis*, and show diverse biological activities,[1,2] including antiviral, hepatoprotective, anticancer, and anti-inflammatory effects. Various synthetic studies on natural dibenzocyclooctadiene lignans were reported during the 1970s,[3,4] and recently, interest in this area has revived.[5–7] During our prior attempts to synthesize the natural product gomisin G,[8] we produced and evaluated a series of unsymmetrical biphenyls for cytotoxicity against several human cancer cell lines, resulting in the discovery of a new lead compound **1** (Fig. 1).[9] Compound **1** showed promising cytotoxicity against human A549 (lung), DU145 (prostate), KB (nasopharyngeal), and drug-resistant KBvin cancer cell lines with low GI_{50} values of 0.12, 0.29, 0.41 and 0.51 μM, respectively. Notably, lead **1** displayed similar potencies against KB and paclitaxel-resistant KBvin cell lines, while the anticancer drug paclitaxel exhibited significantly reduced potency against KBvin compared with KB cells (GI_{50} 1800 and 8 nM, respectively). In a continuing study to explore novel antitumor agents, gomisin G analogs (**5**–**7**) with a dibenzocyclooctatetraene skeleton were synthesized from unsymmetrical 2,2′-diformylbiphenyls. Meanwhile, new biphenyls (**8**-**11**) based on lead **1** were also obtained from formylbiphenyl intermediates. Structurally, the biphenyl compounds are analogous to dibenzocyclooctatetraenes lacking a C6–C7 bond to form the cyclooctatetranene ring. Various 2,2′-substituents (R_1 and R_2) with a conjugated double-bond were introduced and the biphenyl A- and B-tings were modified with different patterns of methoxy and methylenedioxy groups, which are commonly found in natural products. Steric compression between the two bulky 2,2′-groups could affect torsion angles between the two phenyl rings of the biphenyl series, which could in turn affect the biological activity. Thus, we were interested in whether correlations would be found between activities of the two series, acyclic biphenyls and dibenzocyclooctatetraenes. To evaluate antitumor activity, newly synthesized compounds were initially evaluated in human tumor cell lines (HTCL) assays, and active compounds were then tested for

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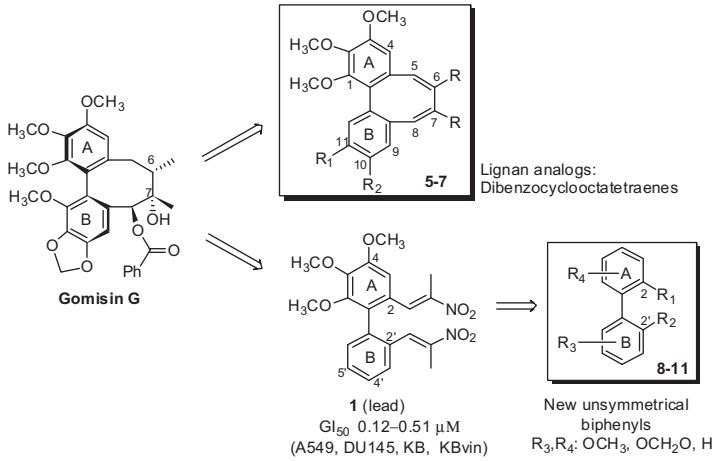
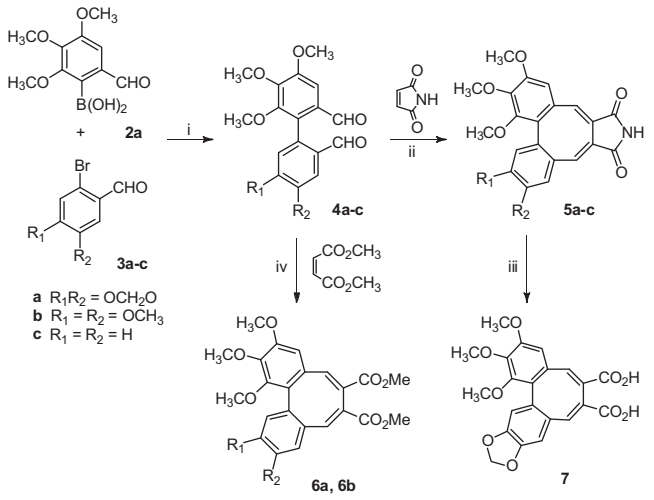


Figure 1. Design strategy and new target compounds (**5**–**11**).

inhibition of the nuclear factor kappa B (NF-κB) signaling pathway, as aberrant NF-κB regulation is observed in various hematological malignancies and solid tumors.[10,11] Continuing activation of NF-κB in tumor cells turns on expression of genes that maintain cell proliferation and protect the cell from death via apoptosis. Thus, blocking NF-κB and its signaling pathway can cause tumor cells to stop proliferating, to die, or to be more sensitive to the action of antitumor agents, providing promising target(s) and approaches for anticancer therapy.[12] Herein, we report the synthesis of dibenzocyclooctatetraene derivatives and related biphenyl compounds, their antitumor activity against a HTCL panel, inhibitory activity against the NF-κB signaling pathway, SAR analysis, and results on mechanism studies using the most active compound **5a** as a probe.

2. Chemistry

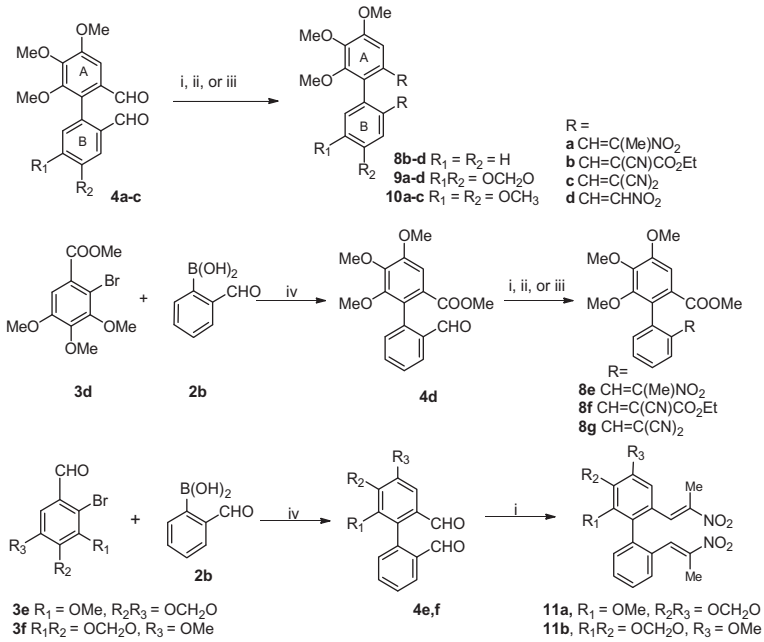
Chemical syntheses of the two series of target compounds are described in Schemes 1 and 2, respectively. The key intermediate unsymmetrical mono- and di-formylbiphenyls (**4**) were synthesized by a Suzuki cross-coupling reaction between a phenylboronic acid (**2**) and a bromobenzene (**3**). As shown in Scheme 1 and 6-formyl-2,3,4-trimethoxyphenylboronic acid (**2a**) was coupled individually with 2-bromopiperonal (**3a**), 2-bromoveratraldehyde (**3b**), or 2-bromobenzaldehyde (**3c**) using $Pd(dppf)Cl_2$ (5% mol) as catalyst in the presence of anhydrous Cs_2CO_3 to produce 2,2′-diformylbiphenyls **4a**–**c**, respectively. Subsequently, **4a**–**c** were reacted with a stable phosphorane Wittig reagent formed by Bu_3P and maleimide,[13] followed by an intramolecular Knoevenagel condensation to afford dibenzocyclooctatetraene succinimides **5a**–**c**, respectively. In a similar manner, dibenzocyclooctatetraene compounds **6a** and **6b** were prepared by Wittig reaction of dimethyl maleate and Bu_3P with diformylbiphenyl **4a** and **4b**, respectively, and a subsequent intramolecular cyclization via Knoevenagel condensation in the presence of piperidine and HOAc in benzene under microwave irradiation. Wittig reagents prepared using Bu_3P are easily purified, because a water-soluble phosphine oxide $Bu_3P{=}O$ is produced. Succinimide compound **5a** was further hydrolyzed under basic conditions to afford 6,7-dicarboxylic dibenzocyclooctatetraene analog **7**. Syntheses of unsymmetrical biphenyls with bulky 2,2′-conjugated substituents (**8**–**11**) are shown in Scheme 2. A Suzuki cross-coupling reaction was used to prepare formylbiphenyl intermediates **4d**–**f** from commercially available 2-formylphenylboronic acid (**2b**) with methyl 2-bromo-3,4,5-trimethoxybenzoate (**3d**), 2-bromo-3-methoxy-4,5-methylenedioxybenzaldehyde (**3e**), or 2-bromo-3,4-methylenedioxy-5-methoxybenzaldehyde (**3f**), respectively. Next, Knoevenagel condensation of formylbiphenyls **4a**–**f** with nitroethane, nitromethane, ethyl cyanoacetate, or malononitrile, respectively, yielded corresponding unsymmetrical biphenyls **8**–**11** with 2- and/or 2′-substituents containing a conjugated double bond. Series **8** compounds have no other substituents on the phenyl B-ring, while series **9** and **10** have a methylenedioxy (**9a**–**d**) or two methoxy groups (**10a**–**c**) at the 4′- and 5′-positions on the B-ring, respectively. In addition, modifications on the A-ring of **1** produced a pair of isomers **11a** and **11b**, in which a methylenedioxy plus a methoxy group replaced the original three methoxy groups on the phenyl A-ring. All new compounds were identified by mass and nuclear magnetic resonance spectra.



Scheme 1. Reagents and conditions: (i) $Pd(dppf)Cl_2$, Cs_2CO_3, 1,2-dimethoxyethane (DME), reflux, 8–12 h; (ii) (a) Bu_3P, THF, reflux, 8–12 h; (b) piperidine, HOAc, 100 °C, 4–6 h; (iii) 10% NaOH aq, THF, 50 °C, 1 h; (iv) (a) Bu_3P, THF, MW, 100 °C, 2 h; (b) piperidine, HOAc, benzene, reflux.

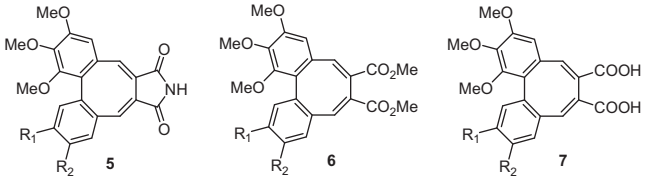
3. Results and discussion

According to procedures described previously,[14,15] the newly synthesized dibenzocyclooctadienes (**5**–**7**) and unsymmetrical biphenyl compounds (**8**–**11**) were tested against a HTCL panel, including A549, DU145, KB, and KBvin cell lines, with paclitaxel as the positive control. In vitro anticancer activity (GI_{50}) was determined using the established SRB (sulforhodamine B) method.[16] To



Scheme 2. Reagents and conditions: (i) $CH_3CH_2NO_2$ or CH_3NO_2, HOAc/*n*-$BuNH_2$, 95 °C, 72 h; (ii) $CH_2(CN)COOEt$, PPh_3/HOAc, 110 °C, 8 h, 63–69%; (iii) $CH_2(CN)_2$, EtOH, rt, 6–24 h, 49–70%; (iv) Pd(dppf)Cl_2, Cs_2CO_3, 1,2-dimethoxyethane (DME), reflux, 8–12 h.

Table 1
Antitumor activity of dibenzocyclooctatetraenes **5**–**7**



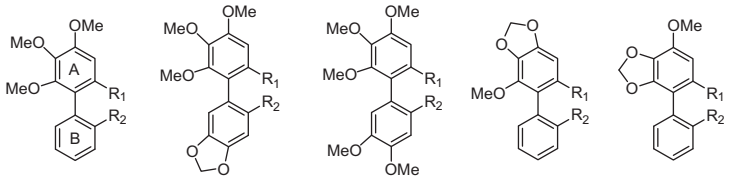
	R_1	R_2	GI_{50}[a] (μM)				IC_{50}[b] (μM)
			A549	DU145	KB	KBvin	Inhibition of NF-κB signaling pathway
5a	$-OCH_2O-$		1.38 ± 0.25	1.43 ± 0.42	1.47 ± 0.16	1.45 ± 0.19	0.52 ± 0.06
5b	OCH_3	OCH_3	10.05 ± 0.50	11.35 ± 1.19	10.09 ± 0.56	10.85 ± 0.47	0.52 ± 0.01
5c	H	H	2.31 ± 0.07	2.34 ± 0.37	2.67 ± 0.26	2.07 ± 0.40	1.02 ± 0.11
6a	$-OCH_2O-$		14.52 ± 3.46	11.74 ± 0.22	14.00 ± 2.24	15.40 ± 2.40	ND[c]
6b	OCH_3	OCH_3	>20	NA	>20	>20	ND
7	$-OCH_2O-$		>20	>20	>20	>20	ND
	Paclitaxel[d]		0.0076	0.006	0.008	1.80	—
	BAY11-7082[d]		—	—	—	—	1.72 ± 0.06

[a] Concentration of compound that inhibits 50% human tumor cell growth, performed at least in triplicate.
[b] NF-κB signaling pathway assays.
[c] Not determined.
[d] Positive control.

explore the possible biological target and mechanism of action, active compounds were screened initially at 10 μM in RAW264.7 cells to evaluate NF-κB inhibitory activity. Compounds that showed greater than 98% inhibition at this concentration were further evaluated at serial concentrations to obtain corresponding IC_{50} values in parallel with positive control BAY 11-7086. Related data of the two compound series are shown in Tables 1 and 2. Interestingly, dibenzocyclooctatetraene succinimide compounds **5a**–**c** showed promising inhibitory activity in the above assays. The most potent compound **5a** exhibited low GI_{50} values (1.38–1.47 μM) against cancer cell proliferation in the tested HTCL panel and a submicromolar IC_{50} value of 0.52 μM in the NF-κB signaling pathway assay. However, when the succinimide ring was opened, dibenzocyclooctatetraene 6,7-dicarboethoxy compounds **6a**, **6b**, or 6,7-dicarboxylate compound **7** were at least 10-fold less potent in comparison with **5a**, suggesting that the succinimide moiety might be important for antitumor activity. In addition, comparison of **5a** with **5b** and **6a** with **6b** indicate that a methylenedioxy on the phenyl ring (B-ring) is more favorable than two methoxy groups at the same positions.

The new series of unsymmetrical biphenyls extended our prior studies and provided more SAR information. The series **8** compounds were designed to have the same biphenyl scaffold as lead **1**, but different 2,2′-substituents, including nitrovinyl, cyanovinyl, and ester groups. Compound **8d** with 2,2′-dinitrovinyl substitution showed potent antitumor activity with GI_{50} values ranging from 2.09 to 4.60 μM in the HTCL panel, but was less potent than **1**. Compounds **8e** with a 2-carbomethoxy and a 2′-(2-methyl-2-nitro)vinyl

Table 2
Antitumor activity of new unsymmetrical biphenyls **8**–**11**



8 **9** **10** **11a** **11b**

	R_1	R_2	GI_{50}[a] (μM)				IC_{50} (μM)
			A549	DU145	KB	KBvin	Inhibition of NF-κB signaling pathway
8b	CH=C(CN)CO_2Et	CH=C(CN)CO_2Et	7.02	8.08	6.53	8.45	3.02 ± 0. 38
8c	CH=C(CN)$_2$	CH=C(CN)$_2$	>40	>40	>40	>40	ND[c]
8d	CH=CHNO_2	CH=CHNO_2	3.07	2.09	2.58	4.60	1.78 ± 0.15
8e	$COOCH_3$	CH=C(Me)NO_2	6.20	6.56	6.95	6.17	1.61 ± 0. 17
8f	$COOCH_3$	CH=C(CN)CO_2Et	>40	>40	>40	>40	ND
8g	$COOCH_3$	CH=C(CN)$_2$	42.02	21.9	36.2	20.6	ND
9a	CH=C(Me)NO_2	CH=C(Me)NO_2	0.87	1.51	0.72	1.27	0.90 ± 0.10
9b	CH=C(CN)CO_2Et	CH=C(CN)CO_2Et	>22	>22	>22	>22	ND
9c	CH=C(CN)$_2$	CH=C(CN)$_2$	>40	19.7	>40	>40	ND
9d	CH=CHNO_2	CH=CHNO_2	8.95	9.09	5.49	8.60	1.72 ± 0.52
10a	CH=C(Me)NO_2	CH=C(Me)NO_2	5.23	6.75	13.5	7.22	2.16 ± 1.33
10b	CH=C(CN)CO_2Et	CH=C(CN)CO_2Et	13.82	13.1	>36	15.09	3.75 ± 1.16
10c	CH=C(CN)$_2$	CH=C(CN)$_2$	17.98	10.1	23.22	15.92	4.16 ± 1.95
11a	CH=C(Me)NO_2	CH=C(Me)NO_2	4.02	5.02	13.06	12.31	1.61 ± 0.69
11b	CH=C(Me)NO_2	CH=C(Me)NO_2	13.89	19.8	18.02	18.69	0.81 ± 0.08
1	CH=C(Me)NO_2	CH=C(Me)NO_2	0.12	0.29	0.41	0.51	0.75 ± 0.06
Paclitaxel[b]			0.009	0.006	0.008	1.80	
BAY11-7082[b]			ND[c]	ND	ND	ND	1.72 ± 0.06

[a] GI_{50}: concentration that inhibits cell growth by 50%.
[b] Positive references in HTCL and NF-κB signaling pathway assays, respectively.
[c] Not determined.

group and **8b** with 2,2′-di-(2-carboethoxy-2-cyano)vinyl groups exhibited moderate potency with low micromolar GI_{50} values ranging from 6.17 to 6.95 μM and 6.53 to 8.45 μM, respectively, but were less potent than **8d**. In contrast, **8c**, **8f**, and **8g** without a nitrovinyl group at the 2,2′-position were inactive (GI_{50} >20 μM).

The same potency pattern was observed in series **9** and **10** biphenyls, which contain a 4′,5′-methylenedioxy or 4′,5′-dimethoxy substitution, respectively, on the phenyl B-ring. As expected, compound **9a** with 2,2′-di-(2-methyl-2-nitro)vinyl groups showed low micromolar GI_{50} values ranging from 0.72 to 1.51 μM in the cellular assays, which were similar to those of lead **1** and **5a**. Compound **9a** was more potent than 2,2′-dinitrovinyl **9d** (GI_{50} 5.49–9.09 μM), di-2-carboethoxy-2-cyanovinyl **9b** (GI_{50} >22 μM), and 2,2′-dicyanovinyl **9c** (GI_{50} >40 μM). Similarly, **10a** was also more potent than **10b** and **10c**.

Di-(2-methyl-2-nitro)vinyl biphenyls **11a** and **11b** each have with one methylenedioxy (positions 4/5 in **11a** and 5/6 in **11b**) and one methoxy group (position 6 in **11a** and 4 in **11b**) on the A-ring, rather than the three methoxy groups found in lead **1**. Neither compound was more potent than **1** in the antitumor cellular assays. However, compound **11a** displayed selective potency against A549 and DU145 cell proliferation (GI_{50} 4.02 and 5.02 μM respectively) compared with KB and KBvin (GI_{50} 13.6 and 12.31 μM), while **11b** was less potent against all four cell lines (GI_{50} 13.89–19.82 μM).

In summary, the cellular assay results on the biphenyls indicated that (1) 2,2′-substituents significantly affect antitumor potency regardless of other substituents on the biphenyl ring system, (2) conjugated nitrovinyl substituents at the 2,2′-positions are more favorable than other substituents on these positions, which is consistent with prior literature,[17] and (3) trimethoxy substitution on the biphenyl A-ring is preferred compared with both methoxy and methylenedioxy groups at different positions based on a limited data set.

Active dibenzocyclooctatetraene (**5a**–**c**) and nitrovinylbiphenyls (**1**, **8d**, **8e**, **9a**, **9d**, **10a**, **11a** and **11b**) were further assayed for inhibitory effects on the NF-κB signaling pathway in RAW264.7 cells, which are stably transfected with a luciferase reporter gene controlled by NF-κB activation.[18] Interestingly, the compounds exhibited promising inhibitory activity in this assay with IC_{50} values ranging from 0.52 to 2.16 μM. The inhibitory potency was comparable or greater than that of positive control BAY 11-7086 (IC_{50} 1.72 μM), a pharmacological inhibitor of IκB kinase, indicating that these active compounds do interfere with the NF-κB signaling pathway. Consequently, dibenzocyclooctatetraene succinimide **5a** was tested in parallel with positive control BAY 11-7082 in further assays based on the NF-κB signaling pathway. Firstly, we found that **5a** indeed suppressed lipopolysaccharide (LPS)-induced NF-κB activation dose-dependently. As shown in Figure 2, RAW264.7 cells, stably expressing the NF-κB luciferase reporter gene, were stimulated with LPS to increase luciferase activity in the presence or absence of **5a** before measurement of luciferase activity. The cells treated with 10 μM of **5a** completely inhibited LPS-induced NF-κB activation comparable to BAY11-7082 at 20 μM. Next, the underlying mechanisms of action of **5a** were investigated. As a pharmacological inhibitor of the actions of IκB kinase, BAY 11-7082 can prevent the translocation of free NF-κB to the nucleus and, thus, was used as a reference in Western blotting to confirm the action of **5a**. As shown in Figure 3, the p65 subunit of NF-κB, a major component in NF-κB complex activation, was translocated into the nucleus after LPS challenge. However, pretreatment with **5a** substantially inhibited translocation of p65 in a dose-dependent manner. Since IκB protein degradation is an essential step for NF-κB activation induced by LPS, Figure 3 also illustrates that LPS stimulation induced a marked degradation of IκB, while this degradation was suppressed by **5a** in a dose-dependent manner. On the other hand, nitric oxide (NO) is known to function as a pro-inflammatory mediator in the pathogenesis of

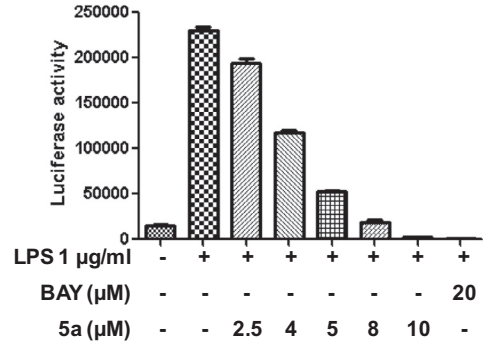


Figure 2. Effect of **5a** on LPS-induced NF-κB activation. RAW264.7 cells were pretreated with **5a** at a dose of 2.5, 4, 5, 8, 10 μM or BAY11-7082 (20 μM) for 30 min before being stimulated with LPS (1 μg/mL) for 6 h, and the luciferase activities were measured by NF-κB luciferase reporter gene assays.

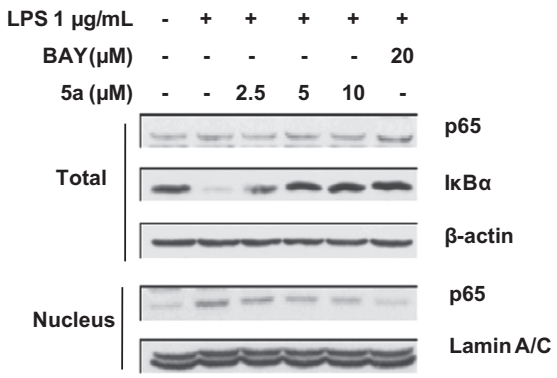


Figure 3. Effect of **5a** on degradation of IκB-α and translation of p65 in LPS-stimulated RAW264.7 cells. RAW264.7 cells were pretreated with **5a** at a dose of 2.5, 5, 10 μM or BAY11-7082 (20 μM) for 30 min, and stimulated by 1 μg/mL LPS for another 15 min. The IκB-α in total cell protein and p65 in nuclear protein were detected by Western blot analysis.

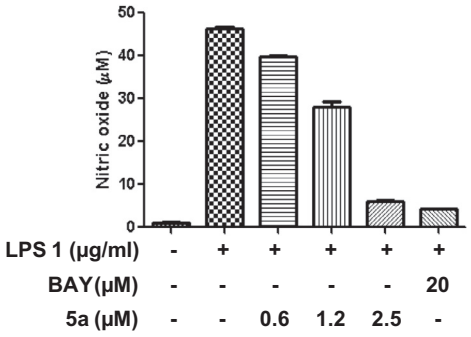


Figure 4. Effect of **5a** on NO production in LPS-induced RAW264.7 cells. After cells were pretreated with either **5a** (0.6, 1.2 and 2.5 μM) or BAY11-7082 (20 μM) for 30 min and then simulated by LPS (1 μg/mL) for 18 h, the production of NO in cell culture medium was analyzed by Griess assay.

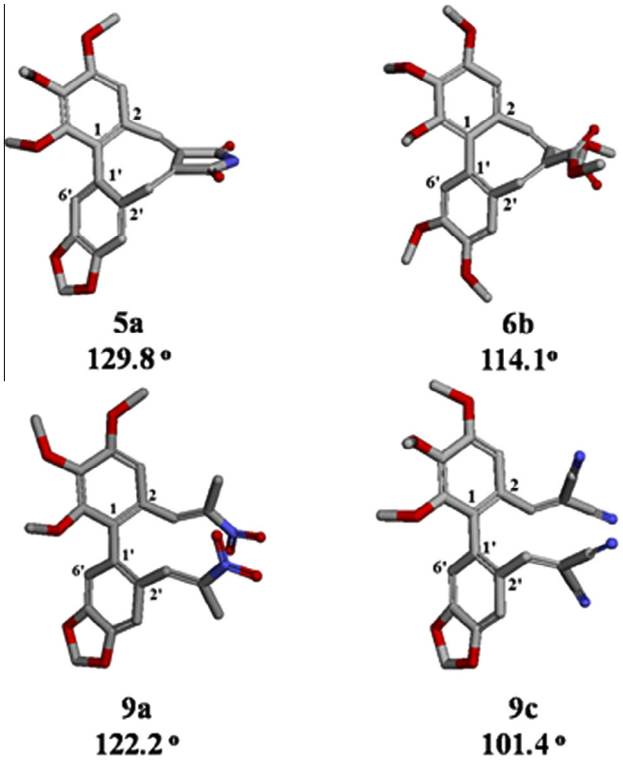


Figure 5. The low energy conformations of **5a**, **6b**, **9a**, and **9c** showing the torsional angles between two phenyl rings. The C2–C1–C1′–C6′ torsional angle is defined as positive if, when viewed along the C1–C1′ bond, atom C2 must be rotated counter-clockwise to eclipse atom C6′.

inflammation. Overproduction of NO may have detrimental consequences and seems to be involved in the pathophysiology of various human diseases. As shown in Figure 4, RAW264.7 cells without LPS stimulation produced minimal NO in the supernatant, while cells released significantly higher levels of NO in response to LPS exposure. When RAW264.7 cells were treated with **5a**, the elevation of NO release was significantly reduced relative to the dose. The reduction in LPS-induced NO production to **5a** at a concentration of 2.5 μM was comparable with the decrease in response to a higher concentration (20 μM) of BAY 11-7082. Based on the results, we suggest that **5a** is a more effective NO inhibitor than BAY 11-7082.

Because molecular binding orientation is related to biological activity, we wanted to observe changes in the biphenyl torsion angle, C2–C1–C1′–C6′ (Fig. 5), as well as determine possible relationships between dibenzocyclooctatetraenes and acyclic biphenyls. Therefore, even though the actual biological target(s) has not been identified, we performed conformational searches on the two compound series using Discovery Studio 3.0. For the minimized conformers of dibenzocyclooctatetraenes, the biphenyl torsion angles of C2–C1–C1′–C6′ ranged from 110° to 130° (defined as in Fig. 5), irrespective to whether the compound was active or inactive. For example, active compound **5a** shows a torsional angle of 129.8° and inactive **6b** has a 114.1° angle. Considering the steric compression of the 2,2′-bulky groups, we postulated that the C2–C1–C1′–C6′ torsional angle in the acyclic biphenyls would deviate from those of related dibenzocyclooctatetraenes. Interestingly, we found that active dinitrovinylbiphenyl **9a** displayed a torsional angle of 122.2° similar to that of active **5a**, while the torsional angle of inactive 2,2′-dicyanovinyl biphenyl **9c** (101.4°) did not fall in the above range. The conformers of representative compounds are shown in Figure 5. We suggest that the torsional angle between the two phenyl rings, either in dibenzocyclooctatetraenes or biphenyls, may impact the molecular antitumor activity because molecular binding orientations can affect interactions between small molecules and their biological target.

4. Conclusion

This study discovered new dibenzocyclooctatetraene succinimides (**5a**–**c**) and related unsymmetric 2- and/or 2′-nitrovinyl

biphenyls (**8b**, **8d–e**, **9a**, **9d**, **10a**, **11a**) with promising cytotoxic (GI_{50} <10 μM in a HTCL panel) and NF-κB inhibitory (IC_{50} values 0.52–3.02 μM) activities. The results suggest that these antitumor agents are potential NF-κB signaling pathway inhibitors. In mechanism of action studies based on NF-κB signaling pathway assays, the most potent compound **5a** suppressed lipopolysaccharide (LPS)-induced NF-κB activation in RAW264.7 cells (IC_{50} 0.52 μM), inhibited LPS-induced degradation of IκBα and nuclear translocation of p65, and significantly blocked LPS-induced nitric oxide (NO) production at low concentrations (0.6–2.5 μM). The results demonstrated that **5a** has an inhibitory profile similar to that of IKK inhibitor BAY 11-7082, implying **5a** may reduce LPS-induced NF-κB activation via inhibition of IKK activities. The antitumor data in cellular assays indicated that the relative positions and types of substituents either on the dibenzocyclooctatetraene or the acyclic biphenyl as well as the torsional angles between the two phenyls are of primary importance to antitumor activity.

5. Experimental section

5.1. Chemistry

Melting points were measured with a RY-1 melting apparatus without correction. The proton nuclear magnetic resonance (^{1}H NMR) spectra were measured on a JNM-ECA-400 (400 MHz) spectrometer using tetramethylsilane (TMS) as internal standard. The solvent used was $CDCl_3$ unless indicated. Mass spectra (MS) were measured on API-150EX mass spectrometer with electrospray ionization connected with an Agilent 1100 system. Thin-layer chromatography (TLC) was performed on silica gel GF_{254} plates. Silica gel GF254 and H (200–300 mesh) from Qingdao Haiyang Chemical Company was used for TLC, preparative TLC, and column chromatography respectively. Medium-pressure column chromatography was performed using a CombiFlash® companion system from ISCO, Inc. to purify target compounds. All chemicals were obtained from Beijing Chemical Works or Sigma–Aldrich, Inc.

5.1.1. General process of Suzuki cross-coupling to prepare mono- or di-formylbiphenyls (4a–f)

A mixture of a substituted bromobenzene (1 equiv) and a phenylboronic acid (1.5 equiv) in the presence of Pd(dppf)Cl_2 (5% mol) and Cs_2CO_3 (2.0 equiv) in 1,2-dimethoxyethane (DME, ca. 10 mL) was heated to 80 °C for 8–12 h under N_2 protection. After cooling to room temperature, the mixture was diluted with EtOAc and filtered through celite. The filtrate was washed with water and brine, successively, and dried over Na_2SO_4. After removal of solvent in vacuo, the residue was purified by a flash column (gradual elution: EtOAc/petroleum ether 0–40%) to afford corresponding formylbiphenyls.

5.1.1.1. 2,2′-Diformyl-4′,5′-methylenedioxy-4,5,6-trimethoxybiphenyl (4a). Starting with 2-bromopiperonal (**3a**, 300 mg, 1.31 mmol) and 6-formyl-2,3,4-trimethoxyphenyl boronic acid (**2a**, 470 mg, 1.96 mmol) to afford 210 mg of **4a**, 47% yield, white solid, 138–140 °C; ^{1}H NMR δ ppm 9.64 and 9.58 (each 1H, s, CHO), 7.51 (1H, s, ArH-3′), 7.38 (1H, s, ArH-3), 6.74 (1H, s, ArH-6′), 6.15 (2H, s, OCH_2O), 4.00 and 3.98 (each 3H, s, OCH_3), 3.63 (3H, s, OCH_3); MS *m*/*z* (%) 367.1 (M+23, 40), 299.3 (M−45, 100).

5.1.1.2. 2,2′-Diformyl-4,4′,5,5′,6-pentamethoxybiphenyl (4b). Starting with 2-bromo veratraldehyde (**3b**, 200 mg, 0.82 mmol) and **2a** (310 mg, 1.29 mmol) to afford 207 mg of **4b**, 70% yield, white solid, mp 142–144 °C; ^{1}H NMR δ ppm 9.65 and 9.52 (each 1H, s, CHO), 7.76 (1H, s, ArH-3′), 7.40 (1H, s, ArH-3), 6.73 (1H, s, ArH-6′), 4.02–3.94 (12H, ms, $OCH_3 \times 4$), 3.61 (3H, s, OCH_3); MS *m*/*z* (%) 383.3 (M+23, 50), 315.2 (M−45, 100).

5.1.1.3. 2,2′-Diformyl-4,5,6-trimethoxybiphenyl (4c). Starting with 2-bromobenzaldehyde (**3c**, 200 mg, 1.08 mmol) and **2a** (390 mg, 1.62 mmol) to afford 300 mg of **4c**, 92% yield, pale-yellow oil; ^{1}H NMR δ ppm 9.85 and 9.60 (each 1H, s, CHO), 8.09 (1H, d, *J* = 8.0 Hz, ArH-3′), 7.67 and 7.60 (each 1H, t, *J* = 8.0 Hz, ArH-4′,5′), 7.42 (1H, s, ArH-3), 7.32 (1H, d, *J* = 8.0 Hz, ArH-6), 4.01 and 3.99 (each 3H, s, OCH_3), 3.55 (3H, s, OCH_3); MS *m*/*z* (%) 323.3 (M+23, 10), 255.3 (M−45, 100).

5.1.1.4. 2-Carbomethoxy-2′-formyl-4,5,6-trimethoxybiphenyl. (4d). Starting with methyl 2-bromo-3,4,5-trimethoxybenzoate (**3d**, 500 mg, 1.64 mmol) and 2-formylphenylboronic acid (**2b**, 295 mg, 1.97 mmol) to afford 326 mg of **4d**, 60% yield, pale-yellow oil. ^{1}H NMR δ ppm 9.84 (1H, s, CHO), 8.23 (1H, d, *J* = 8.0 Hz, ArH-3′), 7.60 and 7. 50 (each 1H, t, *J* = 8.0 Hz, ArH-4′,5′), 7.37 (1H, s, ArH-3), 7.19 (1H, d, *J* = 8.0 Hz, ArH-6′), 3.97, 3.96, 3.58, and 3.54 (12H, ms, $OCH_3 \times 4$); MS *m*/*z* (%) 271.3 (M−59, 40), 256.3 (M−64, 100).

5.1.1.5. 2,2′-Diformyl-4,5-methylenedioxy-6-methoxybiphenyl (4e). Starting with 2- bromo-3-methoxy-4,5-methylenedioxybenzaldehyde (**3e**, 130 mg, 0.5 mmol) and **2b** (150 mg, 1 mmol) to afford 98 mg of **4e**, 68% yield, white solid, mp 125–126 °C; ^{1}H NMR δ ppm 9.86 and 9.47 (each 1H, s, CHO), 8.06 (1H, d, *J* = 7.6 Hz, ArH-3′), 7.67 (1H, t, *J* = 7.6 Hz, ArH-4′), 7.64 (1H, t, *J* = 7.6 Hz, ArH-5′), 7.27 (1H, s, ArH-3), 7.25 (1H, d, *J* = 7.6 Hz, ArH-6′), 6.13 (2H, s, CH_2), 3.83 (3H, s, OMe); MS *m*/*z* (%) 307.3 (M+23, 45), 239.4 (M−45, 100).

5.1.1.6. 2,2′-Diformyl-5,6-methylenedioxy-4-methoxybiphenyl (4f). Starting with 2- bromo-3,4-methylenedioxy-5-methoxybenzaldehyde **3f** (129 mg, 0.5 mmol) and **2b** (150 mg, 1.0 mmol) to afford 100 mg of **4f**, 70% yield, white solid, mp 112–114 °C; ^{1}H NMR δ ppm 9.95 and 9.68 (each 1H, s, CHO), 8.07 (1H, dd, *J* = 1.6 and 7.6 Hz, ArH-3′), 7.70 (1H, t, *J* = 7.6 Hz, ArH-4′), 7.61 (1H, t, *J* = 7.6 Hz, ArH-5′), 7.39 (1H, s, ArH-3), 7.37 (1H, dd, *J* = 1.6 and 7.6 Hz, ArH-6′), 6.13 (2H, s, OCH_2O), 3.83 (3H, s, OMe), MS *m*/*z* (%) 307.5 (M+23, 20).

5.1.2. General procedure for preparing dibenzocyclooctatetraenes (5a–c)

A mixture of 2,2′-diformylbiphenyl (**4a**, **4b**, or **4c**, 1 equiv) and maleimide (1.5 equiv) in anhydrous THF (5–15 mL) in the presence of Bu_3P (1.5 equiv) was heated to reflux for 8–12 h under N_2 protection. After removal of solvent, the residue was dissolved in HOAc (3–15 mL) and 1–3 drops of piperidine were added. The mixture was heated to 100 °C for 4–6 h with TLC monitoring, then poured into ice-water. The solid was filtered and washed with water to neutral, dried, and purified by a flash column (gradual elution: EtOAc/H_2Cl_2 0–20%) to afford corresponding desired pure product.

5.1.2.1. (5*Z*,7*Z*)-10,11-Methylenedioxy-1,2,3-trimethoxydibenzocyclooctatetraene- 6,7-succinimide (5a). Starting with **4a** (100 mg, 0.29 mmol) to afford **5a**, purple solid, 74 mg, 62% yield, mp 266–268 °C; ^{1}H NMR δ ppm 9.01 (1H, s, =CH), 7.77 (1H, s, =CH), 7.50 (1H, br, NH), 7.23 and 7.18 (each 1H, s, ArH-5 or 12), 6.85 (1H, s, ArH-9), 6.13 (2H, s, OCH_2O), 4.04, 3.99, and 3.96 (each 3H, s, OCH_3); ^{13}C NMR (DMSO-d_6) δ 172.33, 172.20, 151.89, 151.35, 149.00, 146.59, 144.92, 142.50, 130.21, 129.59, 126.83, 126.56, 125.70, 123.18, 118.79, 106.35, 104.10, 103.50, 101.81, 60.94, 60.48, 55.80; MS *m*/*z* (%) 408.5 (M+1, 100).

5.1.2.2. (5*Z*,7*Z*)-1,2,3,10,11-Pentamethoxydibenzocyclooctatetraene-6,7-succinimide (5b). Starting with **4b** (1.0 g, 2.47 mmol) to afford 530 mg of **5b**, 51% yield, orange solid,

231–132 °C, ^{1}H NMR (DMSO-d_6) δ ppm 11.14 (1H, s, NH), 8.97 (1H, s, =CH), 7.82 (1H, s, =CH), 7.47 and 7.32 (each 1H, s, ArH), 7.13 (1H, s, ArH), 4.02–3.92 (15H, ms, $OCH_3 \times 5$); MS *m*/*z* (%) 424.2 (M+1, 100).

5.1.2.3. (5*Z*,7*Z*)-1,2,3-Trimethoxydibenzocyclooctatetraene-6,7-succinimide (5c). Starting with **4c** (300 mg, 1 mmol) to afford **5c**, orange solid, 130 mg, 31% yield, 212–214 °C; ^{1}H NMR δ ppm 9.54 (1H, d, *J* = 8.4 Hz, ArH), 7.89 (2H, m, ArH), 7.70 (1H, t, *J* = 8.4 Hz, ArH-5), 7.60 (2H, m, ArH, NH), 7.20 and 6.88 (each 1H, s, =CH), 4.06, 4.02, and 3.97 (each 3H, s, $OCH_3 \times 3$); ^{13}C NMR (DMSO-d_6) δ 172.66, 172.55, 152.86, 152.53, 145.27, 143.25, 131.20, 130.61, 130.48, 129.95, 129.90, 128.79, 127.57, 126.73, 126.65, 125.45, 118.95, 104.33, 61.33, 60.86, 56.28; MS *m*/*z* (%) 386.2 (M+23, 70), 364.3 (M+1, 100).

5.1.2.4. (5*Z*,7*Z*)-6,7-Dicarbomethoxy-10,11-methylenedioxy-1,2,3-trimethoxydibenzo- cyclooctatetraene (6a). To a solution of **4a** (50 mg, 0.15 mmol) in THF (2–3 mL) was added dimethyl maleate (0.07 mL, 0.56 mmol) and Bu_3P (0.14 mL, 0.56 mmol) in a sealed tube (10 mL). The mixture was heated to 100 °C under microwave irradiation for 2 h, and then cooled to room temperature, diluted with EtOAc, and washed with water several times. After removal of solvent in vacuo, the residue was purified on a flash column (gradual elution: EtOAc/petroleum ether 0–60%). The obtained intermediate was re-dissolved in dried benzene (ca. 10 mL) and 3 drops of piperidine and HOAc were added. The mixture was heated to reflux for 24 h under N_2 protection equipped with a distillation trap to remove water produced during reaction. After removal of solvent in vacuo, the residue was purified on a flash column (gradual elution: EtOAc/petroleum ether 0–50%) and crystallized from MeOH to afford 30 mg of **6a**, 45% yield, pale-yellow needles, 146–148 °C. ^{1}H NMR δ ppm 8.99 (1H, s, =CH), 7.57 (1H, s, =CH), 7.40 and 7.19 (each 1H, s,ArH), 6.27 (1H, s, ArH), 6.11 (2H, s, OCH_2-O), 4.03, 3.99 and 3.98 (each 3H, s, $OCH_3 \times 3$), 3.85 and 3.84 (each 3H, s, $OCH_3 \times 2$); MS *m*/*z* (%) 455.4 (M+1, 50), 423.4 (M−31, 100).

5.1.2.5. (5*Z*,7*Z*)-6,7-Dicarbomethoxy-1,2,3,10,11-pentamethoxydibenzocyclooctatetraene (6b). The procedure was the same as that of **6a**. Starting with **4b** (100 mg, 0.28 mmol) to afford 54 mg of **6b**, 41% yield, pale-yellow needles, 126–128 °C. ^{1}H NMR δ ppm 9.16 (1H, s, =CH), 7.57 (1H, s, =CH), 7.56, 7.09 and 6.29 (each 1H, s, ArH), 4.10 and 4.05 (each 3H, s, OCH_3), 4.03–4.00 (9H, ms, $OCH_3 \times 3$), 3.86 and 3.84 (each 3H, s, $CO_2CH_3 \times 2$); MS *m*/*z* (%) 471.5 (M+1, 40), 439.4 (M−31, 100).

5.1.3. (5*Z*,7*Z*)-6,7-Dicarboxy-10,11-methylenedioxy-1,2,3-trimethoxydibenzocyclooctatetraene (7)

A solution of **5a** (50 mg, 0.12 mmol) in THF (2–3 mL) in the presence of a few drops of aq NaOH (10%) was heated to 50 °C for 1 h. The mixture was then poured into ice-water, acidified pH to 1–2 with aq HCl (5%), extracted with EtOAc three times, and washed with water to neutral. After removal of solvent in vacuo, residue was purified on a flash column (gradual elution: MeOH/CH_2Cl_2 with 3% HOAc 0–20%) to afford **7** as a brown solid, 28 mg, 54% yield, mp >200 °C (des); ^{1}H NMR (DMSO-d_6) δ ppm 8.82 (1H, s, =CH), 7.67 (1H, s, =CH), 7.50, 7.36, 7.36 and 6.27 (each 1H, s, ArH), 6.20 (2H, s, OCH_2O), 3.90 (6H, s, $OCH_3 \times 2$), 3.88 (3H, s, OCH_3); MS *m*/*z* (%) 427.2 (M+1, 100).

5.1.4. General procedure for preparing nitrovinylbiphenyls (8d, 8e, 9a, 9d, 10a, 11a, and 11b)

A mixture of a diformyl or monoformylbiphenyl compound (**4**, 100 mg) and nitroethane or nitromethane (1 mL, excess) in toluene (5–7 mL) in the presence of ice-cold HOAc (0.03 mL) and *n*-butylamine (0.02 mL) was heated at 100–120 °C for 3–4 days. The mixture was poured into ice-water, extracted with EtOAc, and dried over Na_2SO_4. After removal of solvent in vacuo, the residue was purified by thin plate chromatography (TLC) eluted with CH_2Cl_2 or benzene/EtOAc (100:2) to obtain desired products.

5.1.4.1. (*E*)-2,2′-Dinitrovinyl-4,5,6-trimethoxybiphenyl (8d). Starting with **4c** (100 mg) and nitromethane (excess) to afford **8d** in 70% yield, yellow solid, mp 148–150 °C; ^{1}H NMR δ ppm 8.16 (1H, d, *J* = 7.6 Hz, =CH), 7.92 (1H, d, *J* = 7.6 Hz, ArH), 7.50–7.55 (3H, m, ArH), 6.72 (1H, s, ArH), 5.47 (1H, dt, *J* = 2.0 and 8.4 Hz, =CH), 4.06–4.18 (2H, m, $=CHNO_2$), 3.94, 3.92 and 3.47 (each 3H, s, OMe); MS *m*/*z* (%) 326.1 (M+1, 100).

5.1.4.2. (*E*)-2-Carbomethoxy-2′-(2-methylnitrovinyl)-4,5,6-trimethoxybiphenyl (8e). Starting with **4c** (100 mg) and nitroethane (excess) to afford **8e** in 57% yield, yellow oil; ^{1}H NMR δ ppm 7.76 (1H, s, =CH), 7.43 (2H, m, ArH × 2), 7.31 (3H, m, ArH × 2, and =CH), 7.30 (1H, s, ArH-3), 3.96 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 3.55 (3H, s, OCH_3), 3.53 (3H, s, OCH_3), 2.33 (3H, s, CH_3); MS *m*/*z* (%) 388.2 (M+1, 60).

5.1.4.3. (*E*)-2,2′-Di(2-methylnitrovinnyl)-4′,5′-methylenedioxy-4,5,6-trimethoxybiphenyl (9a). Starting with **4a** (100 mg) and nitroethane (excess) to afford **9a** in 37% yield, yellow solid, mp 98–99 °C; ^{1}H NMR δ ppm 8.05 (1H, s, CH=), 7.47 (1H, s, CH=), 6.87 (1H, s, ArH), 6.75 (1H, s, ArH), 6.66 (1H, s, ArH), 6.13 (2H, s, OCH_2O), 3.95–3.90 (9H, ms, OCH_3), 2.50 (3H, s, CH_3), 2.29 (3H, s, CH_3); MS *m*/*z* (%) 459.2 (M+1, 95), 384.2 (100).

5.1.4.4. (*E*)-2,2′-Dinitrovinyl-4′,5′-methylenedioxy-4,5,6-trimethoxybiphenyl (9d). Starting with **4a** (100 mg) and nitromethane (excess) to afford **9d** in 72% yield, yellow solid, mp 114–115 °C; ^{1}H NMR δ ppm 8.04 (1H, s, ArH), 7.24 (2H, d, *J* = 12.8 Hz, =CH), 6.83 (2H, d, *J* = 12.8 Hz, =CH), 6.05 and 6.00 (each 1H, s, ArH), 4.28 (2H, m, OCH_2O), 4.05, 3.96, and 3.51 (each 3H, s, OMe); MS *m*/*z* (%) 431.1 (M+1, 10), 453.2 (M+Na, 50), 370.0 (100).

5.1.4.5. (*E*)-2,2′-Di(2-methylnitrovinyl)-4,4′,5,5′,6-pentamethoxybiphenyl (10a). Starting with **4b** (100 mg) and nitroethane (excess) to afford **10a** in 27% yield, yellow oil; ^{1}H NMR δ ppm 7.53 (1H, s, =CH), 7.36 (1H, s, =CH), 6.75–6.87 (2H, m, ArH), 6.59 (1H, s, ArH), 3.87–3.97 (18H, ms, $OCH_3 \times 5$, $=CCH_3$), 3.60 (3H, s, CH_3); MS *m*/*z* (%) 497.3 (M+Na, 100).

5.1.4.6. (*E*)-2,2′-Di(2-methylnitrovinyl)-6-methoxy-4,5-methylenedioxybiphenyl (11a). Starting with **4e** (95 mg, 0.33 mmol) and nitroethane (10 mL, excess) to give 54 mg of **11a**, 41% yield, yellow oil; ^{1}H NMR δ ppm 7.74 (1H, s, =CH), 7.46–7.21 (4H, m, ArH), 6.69 (1H, s, =CH), 6.02 (1H, s, ArH), 4.62 (2H, s, OCH_2O), 3.79 (3H, s, OCH_3), 2.36 (3H, s, CH_3), 1.97 (3H, s, CH_3); MS *m*/*z* (%) 399.2 (M+1, 20).

5.1.4.7. (*E*)-2,2′-Di(2-methylnitrovinyl)-4-methoxy-5,6-methylenedioxybiphenyl (11b). Starting with **4f** (50 mg, 0.18 mmol) and nitroethane (10 mL) to give 40 mg of **11b**, 57% yield, yellow oil, ^{1}H NMR δ ppm 7.68 (1H, s, =CH), 7.51–7.36 (4H, m, ArH), 6.61 (1H, s, =CH), 6.07 (1H, s, ArH), 5.98 (2H, s, OCH_2O), 3.98 (3H, s, OCH_3), 2.30 (3H, s, CH_3), 2.26 (3H, s, CH_3); MS *m*/*z* (%) 399.2 (M+1, 30).

5.1.5. General procedure of preparing (2-carboethoxy)cyanovinylbiphenyls (8b, 8f, 9b, 10b)

A solution of a formylbiphenyl **4** (50 mg) and ethyl cyanoacetate (0.1 mL) in HOAc (3 mL) in the presence of 3 drops of piperidine was heated to 100 °C with stirring under N_2 protection for

6–8 h. The mixture was poured into ice-water, extracted with EtOAc, and washed with water to neutral. After removal of solvent in vacuo, the residue was simply washed with MeOH or purified on a flash column (gradual elution: EtOAc/petroleum ether 0–40%) or thin plate chromatography (elution: cyclohexane/EtOAc 5:2) to afford pure product.

5.1.5.1. (*E*)-2,2′-Di(2-carbomethoxy)cyanovinyl-4,5,6-trimethoxybiphenyl (8b). Starting with **4c** (50 mg) and ethyl cyanoacetate (0.1 mL) to give **8b** in 50% yield, pale yellow oil; ^{1}H NMR δ ppm 8.40 (1H, m, ArH-3′), 7.93 (1H, s, ArH-3), 7.84 and 7.73 (each 1H, s, =CH), 7.60 (2H, m, ArH-4′,5′), 7.23 (1H, m, ArH-6′), 4.28 (4H, m, $OCH_2 \times 2$), 4.01, 4.00 and 3.54 (each 3H, s, $OCH_3 \times 3$), 1.32 (6H, m, $CH_3 \times 2$); MS *m*/*z* (%) 513.5 (M+23, 50), 445.2 (M−45, 100).

5.1.5.2. 2-Carbomethoxy-2′-(2-carbomethoxy)cyanovinyl-4,5,6-trimethoxybiphenyl (8f). Starting with **4d** (50 mg) and ethyl cyanoacetate (0.1 mL) to give **8f** in 58% yield, colorless oil; ^{1}H NMR δ ppm 8.40 (1H, d, J = 8.0 Hz, ArH-3′), 8.06 (1H, s, =CH), 7.52 (2H, m, ArH-4′,5′), 7.35 (1H, s, ArH-3), 7.21 (1H, d, J = 8.0 Hz, ArH-6′), 4.28 (2H, q, J = 8.0 Hz, CH_2), 3.97 and 3.95 (each 3H, s, $OCH_3 \times 2$), 3.53 (6H, s, $OCH_3 \times 2$), 1.32 (3H, t, J = 8.0 Hz, CH_3); MS *m*/*z* (%) 448.4 (M+23, 20), 348.2 (M−77, 100).

5.1.5.3. (*E*)-2,2′-Di(2-carbomethoxy)cyanovinyl-4′,5′-methylenedioxy-4,5,6-trimethoxybiphenyl (9b). Starting with **4a** (50 mg) and ethyl cyanoacetate (0.1 mL) to give **9b** in 50% yield, white needles, mp 66–68 °C; ^{1}H NMR δ ppm 7.90 (1H, s,ArH), 6.81 (1H, s, ArH), 6.63 (1H, s, ArH), 6.03 and 6.01 (each 1H, s, =CH), 4.52 and 4.44 (each 2H, m, $OCH_2 \times 2$), 3.92 (6H, s, $OCH_3 \times 2$), 3.80–3.70 (5H, m, OCH_2O, OCH_3), 1.44 (6H, t, $CH_2CH_3 \times 2$); MS *m*/*z* (%) 557.3 (M+23, 95), 552.5 (M+18, 100), 535.4 (M+1, 80).

5.1.5.4. (*E*)-2,2′-Di(2-carbomethoxy)cyanovinyl-4,4′,5,5′,6-pentamethoxybiphenyl (10b). Starting with **4b** (50 mg) and ethyl cyanoacetate (0.1 mL) to give **10b** in 52% yield, white solid, mp 96–98 °C; ^{1}H NMR δ ppm 8.16 (1H, s, ArH-3′), 7.84 (1H, s, ArH-3), 7.81 and 7.74 (each 1H, s, $=CH \times 2$), 6.68 (1H, s, ArH-6′), 4.30–4.26 (4H, m, $OCH_2 \times 2$), 4.04 (3H, s, OCH_3), 4.01 (6H, s, $OCH_3 \times 2$), 3.91 and 3.58 (each 3H, s, $OCH_3 \times 2$), 1.32 (6H, t, J = 8.0 Hz, $CH_3 \times 2$); ^{13}C NMR ($CDCl_3$) δ 162.72, 161.12, 153.73, 152.62, 152.49, 151.48, 149.17, 146.26, 132.80, 129.23, 126.32, 124.22, 116.28, 115.76, 114.38, 110.28, 107.59, 103.24, 100.64, 67.78, 62.55, 61.36, 61.09, 56.37, 56.30, 14.17, 14.13; MS *m*/*z* (%) 551.4 (M+1, 20), 573.5 (M+23, 50).

5.1.6. General procedure for preparing 2,2′-dicyanovinyl)biphenyls (8c, 8g, 9c, and 10c)

A solution of di- or mono-formylbiphenyl **4** (1 equiv) and malononitrile (4 equiv) in CH_3CN (5 mL) in the presence of NH_4OAc (100 mg) was heated to 60 °C with stirring under N_2 protection for 6–8 h. The mixture was poured into ice-water, extracted with EtOAc, washed with water and brine successively. After removal of solvent in vacuo, the residue was purified on a flash column (gradual elution: EtOAc/petroleum ether 0–40%) to afford desired pure product.

5.1.6.1. 2,2′-Di(2,2′-dicyanovinyl)-4,5,6-trimethoxybiphenyl (8c). Starting with **4b** (50 mg) and malononitrile to provide **8c** in 47% yield, white solid, mp 178–180 °C; ^{1}H NMR δ ppm 8.34 (1H, d, J = 8.0 Hz, ArH-3′), 7.81 (1H, s, ArH-3), 7.71–7.67 (2H, m, ArH-4′,5′), 7.47 (1H, s, =CH), 7.25 (1H, s, =CH), 7.22 (1H, d, J = 8.0 Hz, ArH-6′), 4.06, 4.02, and 3.53 (each 3H, s, $OCH_3 \times 3$); MS *m*/*z* (%) 419.3 (M+23, 100), 397.2 (M+1, 10).

5.1.6.2. 2-Carbomethoxy-2′-(2,2-dicyanovinyl)-4,5,6-trimethoxybiphenyl (8g). Starting with **4d** (50 mg) and malononitrile to provide **8g** in 70% yield, white solid, mp 102–104 °C; ^{1}H NMR δ ppm 8.30 (1H, d, J = 8.0 Hz, ArH-3′), 7.61–7.51 (3H, m, ArH), 7.38 (1H, s, =CH), 7.23 (1H, d, J = 8.0 Hz, ArH-6′), 3.99, 3.98, 3.58 and 3.54 (each 3H, s, $OCH_3 \times 4$); MS *m*/*z* (%) 401.4 (M+23, 50), 288.2 (M−90, 100).

5.1.6.3. 2,2′-Di(2,2′-dicyanovinyl)-4′,5′-methylenedioxy-4,5,6-trimethoxybiphenyl (9c). Starting with **4a** (50 mg) and malononitrileto provide **9c** in 58% yield, light yellow solid, mp 172–174 °C; ^{1}H NMR δ ppm 7.94 (1H, s, ArH-3′), 7.80 (1H, s, =CH), 7.24 (1H, s, =CH), 7.18 and 6.65 (each 1H, s, ArH), 6.25 (2H, d, J = 8.0 Hz, OCH_2O), 4.05 and 4.02 (each 3H, s, $OCH_3 \times 2$), 3.63 (3H, s, OCH_3); MS *m*/*z* (%) 463.3 (M+23, 90), 458.4 (M+18, 100).

5.1.6.4. 2,2′-Di(2,2′-dicyanovinyl)-4′,5′,4,5,6-pentamethoxybiphenyl (10c). Starting with **4b** (50 mg) and malononitrile to provide **10c** in 40% yield, light yellow solid, mp 103–104 °C; ^{1}H NMR δ ppm 8.07 (1H, s, ArH-3′), 7.81 (1H, s, ArH-3), 7.23 (1H, s, =CH), 7.21 (1H, s, =CH), 6.62 (1H, s, ArH-6′), 4.07–4.02 (9H, ms, OCH_3 - $\times 3$), 3.96 (3H, s, OCH_3), 3.61 (3H, s, OCH_3-6); MS *m*/*z* (%) 479.2 (M+23, 95).

5.2. Bioassays

5.2.1. Anti-tumor activity against tumor cell lines in HTCL assay

Target compounds were assayed for in vitro anticancer activity by using the SRB method according to procedures described previously.[14,15,19] The panel of cell lines included human lung carcinoma (A-549), epidermoid carcinoma of the nasopharynx (KB), P-gp-expressing epidermoid carcinoma of the nasopharynx (KBvin), and prostate cancer (DU145). The cytotoxic effects of each compound were expressed as GI_{50} values, which represent the molar drug concentrations required to cause 50% tumor cell growth inhibition.

5.2.2. NF-κB luciferase reporter gene assays

The effect of the tested compounds on LPS-induced NF-κB activity was measured using RAW264.7 cells stably transfected with an NF-κB luciferase reporter construct. Cells were seeded at 10^4 cells per well in a 96-well plate overnight for attachment, then cells were pretreated with **5a** or BAY11-7082 for 30 min before being stimulated with LPS for 6 h. The treated cells were harvested and lysed, and the firefly luciferase activities in lysates were measured using the Promega Luciferase Assay System according to manufacturer's instructions (Promega).

5.2.3. Protein isolation and Western blot analysis

RAW264.7 cells were seeded in petri-dishes and incubated overnight for attachment. Cells were incubated with LPS (1 μg/mL) for 15 min. In order to study the effects of the inhibitor, cells were pretreated with either **5a** (2.5, 5, and 10 μM) or BAY11-7082 (20 μM) for 30 min prior to treatment with LPS. The total protein was isolated from cells in radio-immunoprecipitation assay buffer containing protease and phosphatase inhibitors. The nuclear and cytosolic extracts were isolated by using the Nuclear Extraction Kit (Cayman) according to the manufacturer's recommendations. The proteins were then subjected to Western blot analysis. Briefly, equal amounts of protein were resolved with SDS–PAGE and transferred to PVDF membranes (Roche). The membranes were probed with primary antibodies overnight at 4 °C and incubated for 1 h with secondary peroxidase-conjugated antibody at room temperature. The signals on the membrane were visualized by Lumiglo reagent (Cell Signaling) and exposed to X-ray film (Fuji Photo Film).

5.2.4. Nitric oxide determination

The measurement of NO_2^- in the cell culture medium was carried out by using Griess reagents I and II (Beyotime), as a measure of NO production. Briefly, RAW264.7 cells were seeded in petridishes and incubated overnight for attachment. Cells were stimulated by LPS (1 μg/mL) for 18 h. In order to study the effects of the inhibitor, cells were pretreated with either **5a** (0.6, 1.25 and 2.5 μM) or BAY 11-7082 (20 μM) for 30 min prior to treatment with LPS. After treatment, the cells culture medium was collected and centrifuged (69g/5 min). Griess reagents I and II were equilibrated to room temperature before use. Fifty-microliter volumes of cell culture supernatants were reacted with Griess reagent I (50 μL) and Griess reagent II (50 μL). Finally, absorbance was measured at 540 nm.

5.3. Conformation search method

Conformation searching was performed by Discovery Studio 3.0 software (Accelrys, Inc.). The default atom types and parameters supplied with Discovery Studio 3.0 were used throughout the conformation searching. Before search, all target compounds were assigned to the same configuration where the phenyl-A ring rotates clockwise and the phenyl-B ring rotates counter-clockwise along the biphenyl axis. Small molecules were prepared using the ligand preparation tool available in the software and then systematic search conformation method was deployed by generate conformations tool with default CHARMm force-field. The indicated torsion angle and absolute energy of the global minimum were measured.

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Supplementary data

Supplementary data (HPLC purity and conditions for active compounds **5a**–**5c**, **8b**–**8g**, **9a**, **9d**, **10a**–**10c**, **11a**, **11b**.) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.11.018.

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