Anticancer and Anti-inflammatory Sulfur-Containing Semisynthetic Derivatives of Sarcophine

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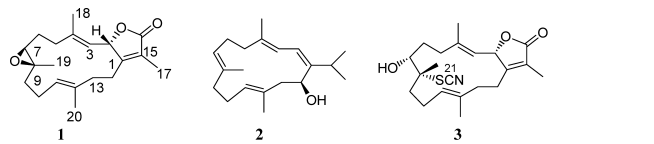
Sarcophine (1), a cembranoid diterpene is known to inhibit the process of tumorigenesis. Sarcophine can be isolated in large amounts from the Red Sea soft coral *Sarcophyton glaucum* and hence is an ideal target for semisynthetic or biocatalytic modifications. Hydroxylated derivatives of 1 were reported to improve its anticancer activity. Despite the promising results and ready availability, there are limited attempts towards further diversifying the library of sarcophine derivatives. Hence, the current study targets the epoxide ring to generate sulfur-containing derivatives of sarcophine by reacting it with ammonium thiocyanate and Lawesson's reagent. Structure elucidation of the products was based on extensive 1D and 2D NMR and high resolution mass spectrometry, in addition to mechanistic considerations. The effect of these derivatives on highly malignant +SA mammary epithialial cell proliferation is reported. Anti-inflammatory potential of sarcophine and its derivatives is also demonstrated.

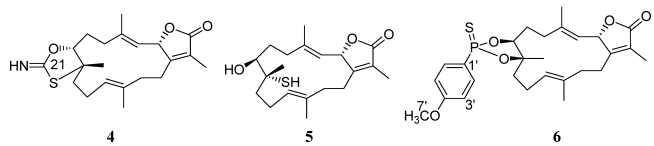
Key words sarcophine; anticancer; anti-inflammatory; semisynthesis; ammonium thiocyanate; Lawesson's reagent

Cembranoids, compounds with 14-membered macrocyclic skeleton, are known to exhibit a wide range of biological activities including anticancer properties.[1)] Since 1998, sarcophine (**1**), a cembranoid diterpene, has been investigated for its potential as a chemopreventive agent.[2)]

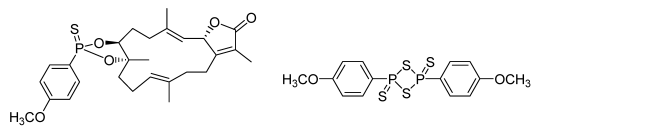
Bioconversion of (**1**) yielded several hydroxylated metabolites.[2)] These metabolites showed improved activity, compared not only to sarcophine, but also to sarcophytol A (**2**), which is a structurally related and well established chemopreventive cembranoid.[2,3)] Despite the promising results, there was one additional attempt toward optimizing the anticancer activity of sarcophine.[4)] This attempt involved semisynthetic transformation of sarcophine and its lactone opened derivative yielding potent hydroxylated derivatives.[4)]



1 **2** **3**



4 **5** **6**



7 Lawesson's reagent

The Red Sea soft coral *Sarcophyton glaucum* is a rich source of sarcophine with yields up to 3% of animal, dry weight.[5)] The high yield along with promising bioactivity makes sarcophine an ideal target for semisynthetic modifications. Hence our goal is to generate a structurally diverse library of sarcophine derivatives and test their biological activities. Previous reports involved generation of oxygenated and nitrogen containing sarcophine derivatives.[2,4,6)] Our first attempt towards semisynthetic modification involved oxymercuration–demercuration and halogenation of sarcophine.[7)] This study targets the generation of sulfur-containing derivatives of sarcophine by targetting the epoxide ring. Sulfur-containing functionalities are known to confer anticancer properties to several natural and synthetic products.[8—10)] For example, the anticancer potential of garlic is attributed to its organic sulfides including allicin.[10)] Sulfur-containing compounds are known to promote detoxification, which helps the liver to breakdown carcinogenic substances.[11)] The anticancer potential of sarcophine and its derivatives was evaluated by studying their effect on the highly malignant +SA mammary epithelial cell proliferation. Finally, the potential of NSAID's in reducing the risk of several malignancies prompted the investigatation of the effect of the chemopreventive sarcophine and its derivatives on the release of inflammatory mediators like thromboxane B_2 and superoxide anion in activated rat neonatal microglia.[12)]

Results and Discussion

Reaction of **1** with ammonium thiocyanate in the presence of antimony trichloride yielded two sulfur and nitrogen-containing derivatives **3** and **4** in reasonable yields.

The HR-MS spectrum **3** displayed a molecular ion peak $[M+H]^+$ at *m/z* 376.1940, suggesting the molecular formula $C_{21}H_{29}NO_3S$, and eight degrees of unsaturation. The IR absorption band at 2159 cm^{-1} indicated the presence of a thio-

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Table 1. ^{13}C- and ^{1}H-NMR Data of Compounds **3**—**5**

Position	**3**[b]		**4**[a]		**5**[b]	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	163.2, s	—	162.4, s	—	163.6, s	—
2	78.7, d	5.71, dq (10.3, 1.5)	79.8, d	5.45, dq (10.3, 1.8)	79.0, d	5.66, dq (10.1, 1.5)
3	122.1, d	4.93, d (10.2)	120.0, d	4.92, d (10.3)	121.6, d	4.92, d (9.9)
4	142.9, s	—	144.4, s	—	143.3, s	—
5	35.3, t	2.49, ddd (13.0, 13.0, 2.9), 2.25, m	35.4, t	2.28, m, 2.22, m	35.7, t	2.25, m, 2.20, m
6	24.7, t	2.00, m, 1.59, m	22.9, t	1.89, m, 1.73, m	25.2, t	1.60, m, 1.55, m
7	71.0, d	3.86, dq (11.4, 1.5)	90.6, d	4.32, dd (10.6, 2.2)	72.0, d	3.67, m
8	65.2, s	—	65.1, s	—	52.4, s	—
9	36.2, t	2.38, m, 2.35, m	32.7, t	2.11, m, 1.58, m	38.1, t	2.09, m, 1.55, m
10	25.8, t	2.40, m, 2.31, m	25.1, t	2.25, m, 1.66, m	25.3, t	2.45, m, 2.20, m
11	124.0, d	5.16, dd (6.6, 6.4)	124.0, d	4.89, d (7.68)	124.7, d	5.26, dd (6.2, 6.2)
12	135.4, s	—	136.4, s	—	134.0, s	—
13	36.6, t	2.27, m, 2.14, m	37.2, t	2.20, m, 1.91, m	36.1, t	2.30, m, 2.13, m
14	28.5, t	2.72, m, 2.20,m	28.9, t	2.38, m, 2.10, m	27.7, t	2.48, m, 2.42, m
15	122.2, s	—	123.9, s	—	121.9, s	—
16	174.0, s	—	174.8, s	—	174.0, s	—
17	8.2, q	1.77, 3H, br s	9.3, q	1.85, 3H, br s	8.3, q	1.77, 3H, br s
18	15.4, q	1.95, 3H, s	17.3, q	1.91, 3H, s	15.2, q	1.88, 3H, s
19	23.5, q	1.53, 3H, s	26.4, q	1.31, 3H, s	27.7, q	1.29, 3H, s
20	14.8, q	1.69, 3H, s	15.3, q	1.61, 3H, s	15.1, q	1.67, 3H, s
21	111.7, q	—	187.7, q	—	—	—
NH	—	—	—	8.00, br s	—	—

a) In $CDCl_3$, 400 MHz for ^{1}H- and ^{13}C-NMR. Coupling constants (*J*) are in Hz. *b*) In CD_3COCD_3, 400 MHz for ^{1}H- and ^{13}C-NMR. Coupling constants (*J*) are in Hz.

cyanate functionality. The most downfield carbon signals at δ_C 163.2 and 174.0 (Table 1) corresponding to C-1 and C-16 confirmed the presence of an α,β-unsaturated lactone moiety. Analysis of 1D and 2D NMR data of **3** (Table 1) indicated that segments C-1—C-6 and C-9—C-14 are similar to those of sarcophine. The carbon C-7 resonating at δ 71.0 (δ_H 3.86) showed ^{3}J-HMBC correlations with the methyl singlet H_3-19 (δ 1.53) and H-5a (δ 2.49). The proton H-7 showed COSY coupling to H_2-6, which in turn coupled to H_2-5. The carbon signal at δ 65.2 was assigned the quaternary carbon (C-8) based on its ^{2}J-HMBC correlations with H_3-19 and H-7. The location of the thiocyanate group on C-8 rather than C-7 was based on the carbon chemical shifts of the quaternary C-8. The carbon resonating at δ 111.7 was assigned as the thiocyanate carbon C-21. The relative stereochemistry of C-7 and C-8 was established by extensive study of NOESY data. The β-oriented H-2 showed NOESY correlation with H-5a (δ 2.49), indicating similar orientation. The proton H-5a showed a strong NOESY correlation with the methyl singlet H_3-19, which in turn showed NOESY correlation with the proton H-7, suggesting their β-orientation.

The HR-MS, ^{1}H- and ^{13}C-NMR data (Table 1) of **4** suggested the molecular formula $C_{21}H_{29}NO_3S$, eight degrees of unsaturation, and 1,3-oxathiolan-2-imine formation at C-7/C-8. The carbon C-7 (δ_C 90.6) showed ^{3}J-HMBC correlations with H_3-19 methyl singlet (δ 1.31), H-5a (δ 2.28), and H_2-9. The proton H-7 (δ 4.32) also showed ^{2}J-HMBC correlation with the quaternary carbon C-8 (δ_C 65.1). The chemical shift value of C-8 indicated its attachment to the sulfur atom of the 1,3-oxathiolan ring. The methyl singlet H_3-19 showed ^{2}J-HMBC correlation with C-8. The proton H-7 also showed ^{3}J-HMBC correlation with the most downfield carbon signal at δ 187.7, which was assigned as imine C-21 carbon. The exchangeable broad proton singlet at δ 8.00 was assigned as the imine proton, based on its ^{2}J-HMBC correlation with C-21. The *cis* orientation of C-7/C-8 1,3-oxathiolan-2-imine ring was based on NOESY data. The β-orientation of H-7 and H_3-19 was deduced in a similar way discussed in compound **3**.

Reaction of epoxides with ammonium thiocyanate usually afford the β-hydroxy thiocyanates intermediates followed by the end products thiiranes.[13] Although the reaction mechanism was proposed in 1950's, the thiocyanohydrin intermediate was not isolated until recently.[13—15] Cyclic epoxides yield *trans* β-hydroxy thiocyanates under reasonably mild conditions and with proper catalyst.[14—16] These reactions are known to proceed with high regioselectivity, almost exclusively yielding anti-Markovnikov's products.[14—16] However in sarcophine, where the epoxide attached to a 14-membered macrocycle, sulfur attack was exclusively on the more substituted carbon as per Markovnikov's rule, suggesting a carbocation intermediate followed by a thermodynamically stable product. The *syn* addition of the nucleophile resulted in the *cis* β-hydroxy thiocyanate **3**.

In the second step toward conversion of oxiranes to thiiranes, the *trans* thiocyanohydrin intermediate is predicted to undergo a *trans* ring closure to form 1,3-oxathiolan-2-imine, which represents a strained ring system.[13] However, the *cis* thiocyanohydrin formed (**3**) can be expected to undergo a *cis* ring closure to yield a stable cyclic oxathiolan-2-imine (**4**).[13] The thiocyanohydrin intermediates of oxirane conversion with thiocyanate have been particularly difficult to isolate until recently where the regio and stereoselective synthesis and isolation of *trans* intermediate has been reported.[13—16] Additionally, a mixture of *cis* and *trans* thiocyanohydrin and 1,3-oxathiolan-2-imine intermediates was reported earlier from a steroid.[17] Compounds **3** and **4** represent the first regio and stereoselective synthesis and isolation of both intermedi-

ates in *cis* configuration. The selectivity could be attributed to the ring size and conformation.

Lawesson's reagent is widely used for the thiolation of carbonyl containing compounds as well as the synthesis of thiols and heterocyclic compounds.[18—20] This reagent is also known to react with epoxides.[21] Lactone carbonyls are known to react with Lawesson's reagent, however at higher temperatures than oxiranes.[18,21] Since sarcophine possesses both oxirane and lactone functions, attempts were made to selctively target the epoxide to form cyclic oxathiophospholane-2-sulfide at room temperature. The resulting products, a thiol (**5**) and two dioxaphospholane-2-sulfide derivatives (**6**, **7**), suggested that the epoxide ring was opened by nucleophilic attack of water to form a diol.

The HR-MS, ^{1}H- and ^{13}C-NMR data of **5** (Table 1) suggested that the C-7/C-8 oxirane group was targeted in a similar fashion to compounds **3** and **4**. The oxygenated proton signal at δ 3.67 was assigned to H-7. This was based on its ^3J-HMBC correlations with methyl singlet H_3-19 (δ 1.29) and H_2-5 (δ 2.25, 2.20). The oxygenated H-7 also showed COSY correlation with H_2-6. The quaternary C-8 (δ_C 52.4) was assigned based on its ^2J-HMBC correlations with H_3-19 and H-7. The upfield location of C-8 confirmed the location of the thiol group. The β-oriented H-2 showed ROESY correlation with H_3-19 methyl singlet, indicating its β orientation. The epoxide ring opening with nucleophilic water is known to yield a diol with two oppositely directed oxygens.[22] Further, the conversion of alcohols to thiols in the presence of Lawesson's reagent is known to proceed with retention of configuration.[20] Thus, H-7 can be predicted to be α-oriented based on these mechanistic considerations and the ROESY data.

The HR-MS spectrum of **6** suggested the molecular formula $C_{27}H_{35}O_5PS$. The IR band at 652 cm^{-1} (P=S) and the aromatic signals in ^{1}H- and ^{13}C-NMR data (Table 2) suggested the formation of cyclic phosphonate diester. The NMR data of **6** confirmed that changes occurred only in C-7/C-8 segment and intact lactone functionality. The proton doublet H-7 (δ 4.96) was assigned based on its ^3J-HMBC correlations with C-19 (δ 24.0) and C-9 (δ 37.1). Proton H-7 showed COSY coupling with H_2-6. Proton H-7 also showed ^2J-HMBC correlation with the quaternary C-8 (δ_C 89.7). Carbon C-8 also showed ^2J-HMBC correlation with H_3-19 and H_2-9. The downfield shift of C-8 suggested that it is bearing oxygen rather than sulfur. The NMR data of **6** also showed 1,4-disubstituted aromatic ring pattern similar to that of Lawesson's reagent. The methoxy singlet at δ 3.83 was assigned as H_3-7′. Methoxy singlet H_3-7′ showed ^3J-HMBC correlation with quaternary aromatic oxygenated carbon C-4′. The two proton doublets at δ 7.85 and δ 7.81 were assigned H-2′ and H-6′, respectively. They showed ^3J-HMBC correlations with C-4′. Protons H-2′ and H-6′ also showed COSY correlations with H-3′ (δ 6.93) and H-5′ (δ 6.92), respectively. Protons H-3′ and H-5′ showed ^3J-HMBC correlations with the quaternary aromatic carbon C-1′ (δ_C 125.3), confirming the assignment of 4-methoxyphenyl moiety of Lawesson's reagent. The relative stereochemistry assignment of C-7 and C-8 was based on NOESY data and molecular modeling. The NOESY correlation between H-7 and H_3-19 suggested the *cis* conformation of the phosphonate diester ring. The NOESY correlation between the β-oriented H-2 and H_3-7′ methoxy singlet and the aromatic H-3′ and H-5′ protons suggested the β orientation of the cyclic phosphonate diester.

Table 2. ^{13}C- and ^{1}H-NMR Data of Compounds **6** and **7**[a]

Position	**6**		**7**	
	δ_C	δ_H	δ_C	δ_H
1	163.0, s	—	163.9, s	—
2	78.3, d	5.65, dq (10.3, 1.4)	78.4, d	5.64, dq (10.6, 1.8)
3	123.1, d	5.11, d (10.4)	122.7, d	5.09, d (10.6)
4	143.8, s	—	141.7, s	—
5	35.9, t	2.48, ddd (13.0, 12.8, 3.3), 2.30, m	36.4, t	2.45, ddd (10.0, 12.8, 3.3), 2.30, m
6	22.2, t	1.73, 2H, m	22.8, t	1.68, 2H, m
7	79.3, d	4.96, d (11.8)	82.0, d	5.03, d (11.0)
8	89.7, s	—	69.0, s	—
9	37.0, t	2.12, m, 1.63, m	36.4, t	1.87, 2H, m
10	26.0, t	2.75, m, 2.00, m	25.9, t	2.83, m, 2.12, m
11	125.3, d	4.98, d (10.6)	123.4, d	4.94, d (11.7)
12	137.2, s	—	137.2, s	—
13	37.1, t	1.95, m, 1.88, m	37.3, t	2.20, m, 1.92, m
14	27.0, t	2.93, m, 2.24, m	26.1, t	3.00, m, 2.35, m
15	122.6, s	—	123.0, s	—
16	174.9, s	—	175.1, s	—
17	8.9, q	1.83, 3H, br s	9.2, q	1.85, 3H, br s
18	15.8, q	1.98, 3H, s	16.1, q	1.95, 3H, s
19	24.0, q	1.25, 3H, s	28.0, q	1.55, 3H, s
20	16.6, q	1.80, 3H, s	15.9, q	1.86 , 3H, s
1′	125.3, s	—	124.2, s	—
2′	133.0, d	7.85, d (8.8)	134.2, d	7.85, d (8.8)
3′	113.3, d	6.93, d (8.8)	114.1, d	6.97, d (8.8)
4′	163.0, s	—	165.0, s	—
5′	114.0, d	6.92, d (8.8)	114.3, d	6.96, d (8.8)
6′	133.1, d	7.81, d (8.8)	134.4, d	7.79, d (8.8)
7′	55.5, q	3.83, 3H, s	55.5, q	3.83, 3H, s

a) In $CDCl_3$, 400 MHz for ^{1}H- and ^{13}C-NMR. Coupling constants (*J*) are in Hz.

The HR-MS, IR, and NMR data (Table 2) of **7** indicated that compound **7** is the 8-epimeric analog of **6**. This was evident from the huge upfield shifting of C-8 in **7** (−20.7 ppm) compared with that of **6**. Molecular modeling study suggested a folded structure with the aromatic ring stacking over macrocycle in **6**, which justify NOESY correlations of H-2 with aromatic signals. The 8-*epi* analog of **6** (compound **7**) was found to have a flat/unfolded structure. This was also further supported by the huge R_f values differences observed for both compounds (0.41 for **6** *versus* 0.24 for **7**). The folded compound **6** is much less polar due to possible involvement of the aromatic ring and C-4′ methoxy functions in hydrogen bonding with C-16 lactone carbonyl. This is further corroborated by the significant differences in chemical shift values of H_3-19 in both compounds. The upfield shifting of H_3-19 in the folded compound **6** (δ 1.25) compared with that of **7** (δ 1.55) can be attributed to the location of this methyl in the shielding cone of the benzene ring.

Antiproliferative Activity The effects of various concentrations of sarcophine and its semisynthetic derivatives (**3**—**7**) were studied on highly malignant +SA mammary epithelial cell proliferation. Figure 1 shows the antiproliferative effects of sarcophine and its derivatives (**4**, **7**) on the growth of this cell line. As compared to their respective controls, sarcophine, **4**, and **7** inhibited +SA cell growth over 0—100 μM dose range in 4 days. Sarcophine resulted in only minor reduction in +SA mammary epithelial cell prolifera-

tion whereas **4** induced a significant reduction at 100 μM dose. Other tested compounds (results not shown) were equivalent or less potent than sarcophine. Sarcophine and its derivatives were found to be cytostatic, but not cytotoxic, to neoplastic mammary epithelial cells grown in culture.

Anti-inflammatory Activity The effects of three concentrations (0.1, 1, 10 μM) of sarcophine and the sulfur-containing analogs **3**—**7** on the release of superoxide anion (O_2^-) and thromboxane B_2 (TXB_2) by LPS-activated rat neonatal brain microglia were investigated. Additionally, for each compound, the concomitant release of LDH was measured at the above concentrations as an indicator of cellular toxicity. Although sarcophine weakly inhibited PMA-induced O_2^- and TXB_2 generation ($IC_{50}>10$ μM), better activity was observed with **7** (Fig. 2), which demonstrated both inhibition of O_2^- and TXB_2 generation ($IC_{50}=1$ μM) without significant effect on LDH ($IC_{50}>10$ μM). Other tested compounds **1** and **3**—**6** had $IC_{50}>10$ μM for O_2^- and TXB_2 inhibition.

Conclusion

Five new sulfur-containing derivatives of sarcophine were prepared by reacting sarcophine with ammonium thiocyanate and Lawesson's reagent. Sarcophine and its sulfur-containing derivatives were tested for their antiproliferative and anti-inflammatory activities. The cyclic imine derivative (**4**) showed improved antiproliferative potential over that of sarcophine. The anti-inflammatory activity of sarcophine is demonstrated for the first time, which was further improved by semisynthetic transformation to analog **7** ($IC_{50}=1$ μM). Compound **7**, with postulated flattened structure, showed improved activity in both anticancer and anti-inflammatory assays, unlike its 8-*epi* analog (compound **6**), with folded structure. The loss of activity of **6** could be due to steric hinderence of possible target receptor binding pharmacophores. Targeting the epoxide ring of sarcophine with ammonium thiocyanate and Lawesson's reagent enhanced anticancer and anti-inflammatory activities.

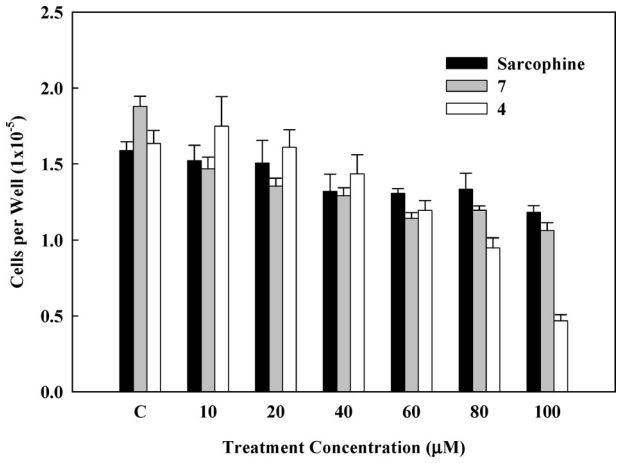

Fig. 1. Effects of Various Doses of Sarcophine and Sarcophine-Derivatives **4** and **7** on Highly Malignant +SA Mammary Epithelial Cell Proliferation *in Vitro*

Data points indicate the mean cell count/well±S.E.M. for 6 replicates in each treatment group on day 5 in culture.

Experimental

General Experimental Procedure The ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ or CD_3COCD_3, on JEOL Ecllipse NMR spectrometer operating at 400 MHz for proton and 100 MHz for carbon. Optical rotations were measured on a Rudolph Research Analytical Autopol III polarimeter. IR spectra were recorded on Nicolet Impact 400D Fourier Transform Infra Red spectrophotometer and the UV spectra were recorded on UV–Visible spectrophotometer. The HR-FAB-MS spectra conducted at the Universities of Kansas and Minnesota. TLC analyses were carried out on precoated silica gel G_{254} 500 μm, using the developing systems hexane : EtOAc (1 : 1) and hexane : isopropanol (8 : 2). For medium pressure liquid column chromatography (MPLC), silica gel <63 μm particle size was used.

Materials The soft coral *Sarcophyton glaucum* was collected from the Red Sea, Hurghada, Egypt. A voucher specimen (03RS24) is deposited in the Department of Basic Pharmaceutical Sciences, College of Pharmacy, University of Louisiana at Monroe, LA. The details of extraction and isolation of sarcophine were previously reported.[7] The identification of **1** was accomplished by comparing its physical and spectral data with literature.[5]

Antiproliferative Assay The antiproliferative effects of sarcophine and its semisynthetic derivatives were studied on highly malignant +SA mouse mammary epithelial cell line maintained in defined media containing 10 ng/ml EGF and 10 μg/ml insulin as co-mitogens, as described previously.[23] Cells were plated in 24-well culture plates at a density of 5×10^4/well (6 wells/group) and fed media having different concentrations of each compound (0—100 μM) on day 1. On day 5, viable cell count was determined by the 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay as described previously.[23]

Anti-inflammatory Assay Rat neonatal microglia (2×10^5 cells/24-well cell culture clusters) were stimulated with *Escherichia coli* lipopolysaccharide (LPS) (0.3 ng/ml) in 1 ml Dulbecco's modified Eagle medium +10% fetal bovine serum+penicillin+streptomycin for 17 h in a humidified 5% CO_2 incubator at 37 °C.[24] Media was then removed and the microglia the cells were washed with warm (37 °C) Hanks' balanced salt solution (HBSS) and then incubated with compounds **1**, **3**—**7** (0.1—10 μM) or vehicle (DMSO) for 15 min prior to stimulation with phorbol 12-myristate 13-acetate (PMA) (1 μM). All experimental treatments were run in triplicate and in final volume of 1 ml. The cells were stimulated for 70 min with PMA, after which, HBSS was aspirated and O_2^-, TXB_2 and LDH release were determined as described elsewhere.[24]

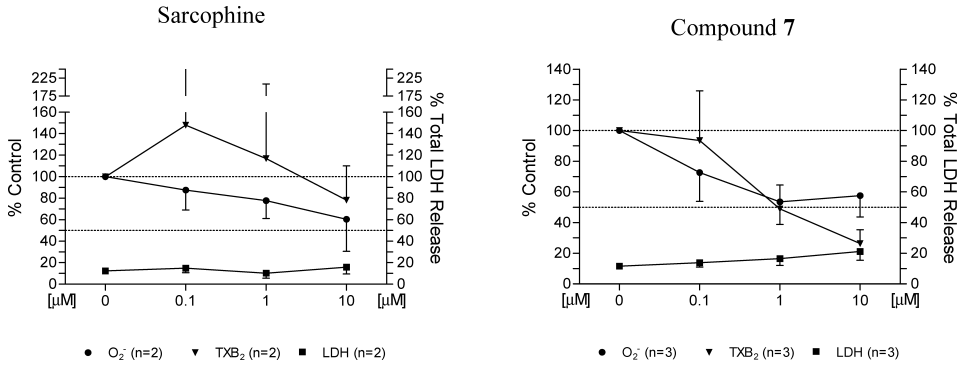

Fig. 2. Effects of Different Concentrations of Sarcophine and Compound **7** on PMA Stimulated O_2^-, TXB_2 and LDH Release by LPS-Activated Rat Neonatal Microglia

Reaction Procedures. Reaction of Ammonium Thiocyanate and Antimony Trichloride with 1 Sarcophine (100 mg, 0.32 mM) was dissolved in 20 ml of anhydrous acetonitrile. To this solution, 49 mg NH_4SCN (0.64 mM) and 23 mg of $SbCl_3$ (0.1 mM) was added and stirred under reflux conditions. The reaction was monitered by TLC using hexane : isopropanol (8 : 2) as the mobile phase. After 1 h the reaction was stopped, acetonitrile was evaporated and EtOAc was added. The precipitate was filtered and the filtrate was extracted with EtOAc (2×10 ml) and 10% methanol in EtOAc (1×10 ml). EtOAc layer was then evaporated under vacuum to give a crude product mixture (120 mg) . The residue was fractionated by MPLC on silica gel 60 (10 g) using gradient elution with hexane–EtOAc to yield compounds **3** (22 mg, R_f 0.51, hexane–isopropanol 8 : 2) and **4** (42 mg, R_f 0.31, hexane–isopropanol 8 : 2).

Reaction of Lawesson's Reagent with 1 A solution of 75 mg (0.24 mM) of **1** in 5 ml of toluene was prepared. To this solution, 48 mg (0.12 mM) of Lawesson's reagent was added and the reaction mixture was stirred for 7 h at room temperature. The reaction was monitored by TLC using hexane : EtOAc (1 : 1) as the mobile phase. The reaction was stopped by adding cold water and partitioned with $CHCl_3$ (2×10 ml). $CHCl_3$ layer was the evaporated under vacuum to give a crude product mixture (90 mg). The residue was then fractionated by MPLC on Si gel 60 (<63 μm particle size, 30 g) using gradient elution with hexane–EtOAc to yield compounds **5** (8 mg, R_f 0.48, hexane–EtOAc 1 : 1), **6** (7.7 mg, R_f 0.41, hexane–EtOAc 1 : 1), and **7** (8.3 mg, R_f 0.24, hexane–EtOAc 1 : 1).

Analytical Data Compound (**3**): White powder, $[\alpha]_D^{25}$ +63.7° (c=0.90, MeOH); UV λ_{max} (MeOH) nm (log ε) 256 (3.83), 250 (3.82), 221 (4.09); IR (neat) ν_{max}: 3688, 3015—2860, 2403, 2159, 1742, 1431, 1225, 1052, 935 cm^{-1}; ^{1}H- and ^{13}C-NMR: Table 1; HR-FAB-MS: $(M+H)^+$ m/z 376.1940 (Calcd for $C_{21}H_{30}NO_3S$: 376.1946).

Compound (**4**): Colorless oil, $[\alpha]_D^{25}$ −7.2° (c=1.50, MeOH); UV λ_{max} (MeOH) nm (log ε) 243 (4.3), 221 (4.2); IR (neat) ν_{max}: 3019, 2395, 1746, 1477, 1215, 1156, 775 cm^{-1}; ^{1}H- and ^{13}C-NMR: Table 1; HR-FAB-MS: $(M+H)^+$ m/z 376.1949 (Calcd for $C_{21}H_{30}NO_3S$: 376.1946).

Compound (**5**): Colorless oil, $[\alpha]_D^{25}$ +45.5° (c=0.44, MeOH); UV λ_{max} (MeOH) nm (log ε) 256 (3.9), 250 (3.9), 224 (4.1); IR (neat) ν_{max}: 3489, 2297, 2143, 1721, 1419, 1213, 1115, 948 cm^{-1}; ^{1}H- and ^{13}C-NMR: Table 1; HR-FAB-MS: $(M+H)^+$ m/z 351.2000 (Calcd for $C_{20}H_{31}O_3S$: 351.1994).

Compound (**6**): Colorless oil, $[\alpha]_D^{25}$ −17.0° (c=0.90, MeOH); UV λ_{max} (MeOH) nm (log ε) 250 (4.6), 224 (4.7) nm; IR (neat) ν_{max}: 3500, 3158, 2127, 1735, 1640, 1410, 1334, 1288, 1240, 956, 652 cm^{-1}; ^{1}H- and ^{13}C-NMR: Table 2; HR-FAB-MS: $(M+H)^+$ m/z 503.2023 (Calcd for $C_{27}H_{36}O_5PS$: 503.2021).

Compound (**7**): Colorless oil, $[\alpha]_D^{25}$ +69.0° (c=0.58, MeOH); UV λ_{max} (MeOH) nm (log ε) 248 (4.5), 224 (4.5) nm; IR (neat) ν_{max}: 3627, 2928, 2380, 1731, 1598, 1445, 1378, 1240, 958 cm^{-1}; ^{1}H- and ^{13}C-NMR: Table 2; HR-FAB-MS: $(M+H)^+$ m/z 503.2007 (Calcd for $C_{27}H_{36}O_5PS$: 503.2021)

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