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Synthesis and anti-tumor evaluation of new trisulfide derivatives

Haoyun An,* Jenny Zhu, Xiaobo Wang and Xiao Xu

ACEA Biosciences, Inc., 11585 Sorrento Valley Road, Suite 103, San Diego, CA 92121, USA

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Abstract—New bis-aromatic and heterocyclic trisulfide derivatives **5**, **7**–**10** were synthesized by optimizing lead dibenzyl trisulfide natural product (**4**) to evaluate their anti-tumor activities. Five compounds **5**–**7**, **9**, and **10** exhibited potent anti-tumor activities against eight different tumor cell lines with low cytotoxicity against HepG2. Initial SAR was discussed, and MOA of these anti-microtubule agents was suggested based on cell kinetic response patterns observed on RT-CES system.
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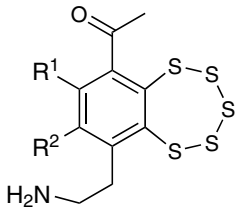
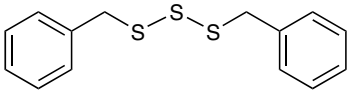
Cancer, including over 200 diseases, is the second biggest cause of death in the developed countries. A variety of cancer chemotherapeutic drugs have been used in clinics and greatly improved survival rates of different human cancers.[1] However, cancer chemotherapy has generally not been curative, and the tumor cells often develop multi-drug resistance (MDR) to various chemotherapeutic agents.[2] The limited efficacy and serious side effects of available cytotoxic agents often resulted in the termination of the chemotherapy for some patients. Therefore, cancer is still the most important unmet medical challenge, and there is an urgent need for new and safe drugs for cancer chemotherapy.

Over 50% of the anti-cancer drugs currently used are either natural products or derived from natural products.[3] The clinical successes of anti-mitotic drugs paclitaxel and epithilone stimulated the search for new natural products. Clinically used anti-microtubule agents of taxanes, alkaloids, and other types of natural products have complicated chemical structures and restricted access to the natural resources.[4] Therefore, derivatization of the biologically active natural compounds and exploration of new small-molecule anti-microtubule agents[5] are efficient strategies to accelerate the discovery and development of novel cancer chemotherapeutic agents.

The naturally-occurring antibiotics varacin (**1**), lessoclinotoxin A (**2**), 5-(methylthio)varacin (**3**)[6] (Fig. 1) Calicheamicin,[7] and esperamicin[8] derivatives have cyclic or acyclic polysulfide moieties, which are critical for biological activities of these natural products. To explore new classes of anti-tumor agents, we discovered anti-tumor activity of natural compound dibenzyl trisulfide (**4**) through cell-based assay screening using RT-CES (real-time cell electronic sensing) cellular screening technology (a label-free and non-invasive cell-based assay technology to quantitatively monitor the dynamic cell response in real time instead of traditional single point cellular assays).[9] Compound **4** is a biologically active trisulfide that was isolated from the sub-tropical shrub *Petiveria alliacea* L.[10] The immunomodulatory activity, molecular mechanism, and some other biological activi-



1, Varacin; $R^1 = COCH_3$, $R^2 = H$
2, Lessoclinotoxin A: $R^1 = OH$, $R^2 = H$
3, 5-(Methylthio)varacin: $R^1 = COCH_3$, $R^2 = SCH_3$



4, Dibenzyl Trisulfide

Figure 1. Organo sulfur natural products.

Keywords: Trisulfide; Anti-tumor activity; Synthesis; SAR.
* Corresponding author. Tel.: +1 858 724 0928; fax: +1 858 724 0927; e-mail: han@aceabio.com

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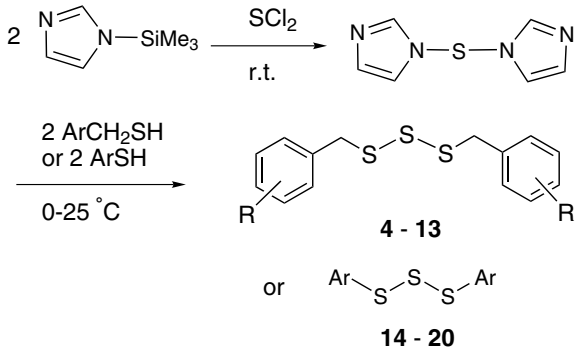
ties of natural compound **4** were reported.[11] However, its anti-tumor, anti-inflammatory, and/or anti-infective activities as well as its potential pharmaceutical application have not been studied. Based on the real-time inhibitory results against various tumor cell lines (see below) and the initial mechanistic studies of compound **4**, we decided to optimize lead **4** and further study in vitro and in vivo anti-tumor activity as well as the mechanistic property of this new class of anti-tumor agents.

Dibenzyl trisulfide (**4**) was synthesized from benzyl sulfenylthiocarbonate in low yield and from labile benzyl hydrodisulfide intermediate.[12] It was also synthesized by the reaction of labile benzyl sulfur chloride with bis(tributyltin)sulfide.[13] The direct coupling of halides catalyzed by copper (II) and tin (II) reagents resulted in a mixture of di-/tri-/tetra-sulfides.[14] Banerji and Kalena[15] synthesized compound **4** in 66% yield through diimidazolylsulfide derivative. This would be an efficient method for the synthesis of new trisulfide compounds if the distillation of the toxic and easily decomposed sulfur dichloride reagent can be avoided. Therefore, we simplified the synthetic procedures by directly utilizing commercially available sulfur dichloride solution. The new substituted benzyl trisulfide derivatives **5**–**13** were then synthesized in good to excellent isolated yields using the modified procedure (Table 1 and Scheme 1). In order to explore different heterocyclic and flexibility effect of trisulfide derivatives on their anti-tumor activity, we also utilized the modified protocol and synthesized new heterocyclic or flexible trisulfide derivatives **14**–**20** (Table 2). The bis-aryl methyl type of trisulfides **5**–**12** and **14** as well as bis(phenyl-ethylene)trisulfide (**16**) were synthesized in higher yields, while the sterically hindered bis(2,4,6-trimethyl-benzyl)trisulfide (**13**) was obtained in 68% yield. The bis-heterocyclic trisulfides **18**–**20** were also obtained in relatively lower yields because of the low nucleophilicity of the corresponding heterocyclic mercaptans. Bis(4-fluorobenzyl)trisulfide (**5**) was fully characterized by ^{1}H NMR, ^{13}C NMR, ^{19}F NMR, MS, FT-IR, and UV–vis spectroscopic as well as elemental analyses.[16] Other derivatives were also characterized by ^{1}H NMR spectroscopic analysis.[17]

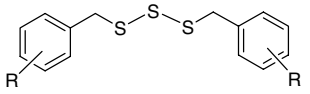


Scheme 1. Synthesis of trisulfide anti-tumor agents.

Typical procedure for the synthesis of bis-aryl trisulfides is as follows: To a stirred solution of *N*-trimethylsilylimidazole (1.42 mmol) in 1.5 mL of anhydrous hexanes was added slowly sulfur dichloride solution in dichloromethane (0.7 mL, 1.0 M, 0.7 mmol) at room temperature under a nitrogen atmosphere. The reaction mixture having white precipitate was stirred for 40 min and then cooled to 0 °C. A solution of a mercaptan (1.41 mmol) in 2 mL of anhydrous hexanes was added dropwise under stirring and nitrogen atmosphere. The resulting reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 3 h. The white solid was filtered off through a pad of Celite and washed with small amount of hexanes. The filtrate was washed with water and then brine. The organic phase was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by flash chromatography on a silica gel column using hexanes–ethyl acetate (40–60:1) as an eluent. The fractions were monitored with silica gel TLC using hexanes–ethyl acetate (40:1) as a developing solvent. The resulting white solid product (except for oily products **10**, **14**, and **16**–**18**) was re-crystallized from hexanes to give desired white crystalline products.

Compounds **4**–**20** were screened against Ovarian (A2780 and OVCAR4), fibrosarcoma (HT1080), non-small cell lung (H460), breast (MCF7 and M231), and adenocarcinoma (HeLa) tumor cell lines as well as toxicity-indicating cell line HepG2 using RT-CES cell-based assay.[9]

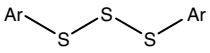
Table 1. Anti-tumor cellular activity of trisulfides (IC_{50}, μM)

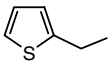
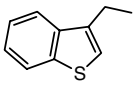
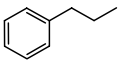
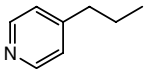
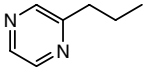
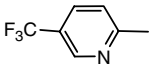
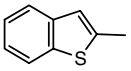


Compound	R	Yield[a] (%)	Jurkat[b]	A2780	OVCAR4	HT1080	H460	MCF7	M231	HeLa	HepG2
4	H[15]		0.35	0.40	1.4	1.9	5.1	6.6	2.4	2.5	>100
5	*p*-F	87	0.15	0.5	0.85	1.0	2.1	2.1	3.1	0.29	>100
6	*p*-Cl[18]	90	0.47	0.7	2.25	1.2	9.6	4.4	2.3	8.5	>100
7	*o*-Cl	77	0.51	0.75	0.50	2.2	23.2	7.8	1.06	2.0	>100
8	*p*-Br	84	—	1.50	1.31	1.39	4.8	16	1.33	1.1	>100
9	*p*-Me	97	—	1.43	0.9	0.82	0.6	2.3	0.78	0.42	>100
10	*m*-Me	99	0.34	0.75	0.60	1.9	12.5	2.75	1.06	6.1	>100
11	*m*-CF_3	100	8.0	11.8	33.5	27.5	50	49	41	2.7	>100
12	*p*-^{t}Bu	96	—	>50	>50	>50	>50	>50	>50	>50	>100
13	2,4,6-tri-Me	68	—	>50	>50	>50	>50	>50	>50	>50	>100

[a] Isolated yields.
[b] Result from MTT assay.

Table 2. Anti-tumor cellular activity of bis-aromatic/heterocyclic trisulfides (IC_{50}, μM)



Compound	Ar	Yields[a] (%)	Jurkat[b]	A2780	OVCAR4	HT1080	H460	MCF7	M231	HeLa	HepG2
14		100	2.6	1.3	12.2	3.6	33.5	6.25	17.5	7.0	>100
15		61	1.2	2.6	5.1	5.3	20	5.6	4.8	2.2	>100
16		96	4.68	6.25	10.5	9.4	42.2	8.8	19.1	4.3	>100
17		79	7.27	4.4	11.5	9.0	18.2	11.9	16.0	—	>100
18		47	>25	17.4	34.8	36	31.5	31.6	>50	—	>100
19		53	>50	>50	>50	>50	>50	>50	>50	—	>100
20		51	>50	>50	>50	>50	>50	>50	>50	—	>100

[a] Isolated yields.
[b] Result from MTT assay.

The inhibitory activities, expressed as IC_{50} (Tables 1 and 2), were obtained at the 48 h time point-although the real time inhibitory effect for any given time point was recorded. The inhibitory activity against leukemia (Jurkat) tumor cell line was obtained by MTT assay.[19] Bis-aryl trisulfide derivatives **4**–**10** substituted with small functional groups exhibited potent cellular anti-tumor activity. Bis(4-fluoro-benzyl)trisulfide (**5**) and bis(4-methylbenzyl)trisulfide (**9**) showed the most potent activity against most of the tested tumor cell lines. Compounds **5** and **9** have slightly different inhibitory preferences against different cell lines. However, compounds **12** and **13** with bulky or more alkyl substituents are inactive. Bis(thiophen-2-yl-methyl)trisulfide (**14**) and bis-(benzo[*B*]thiophen-2-yl-methyl)trisulfide (**15**) showed similar anti-tumor activities. Inserting a CH_2 group on both sides of the trisulfide **4** gave more flexible compound **16**, which was less active than **4**. Compound **17** having pyridinyl moiety has equivalent activity with that of compound **16**. However, pyrizinyl derivative **18** having two nitrogen atoms in aromatic rings was much less active. The less flexible compounds **19** and **20** completely lost activity. The tested trisulfide derivatives have more potent inhibitory effect against Jurkat, A2780, OVCAR4, HT1080, and HeLa tumor cells than against H460, MCF7, and M231 tumor cells. The non-inhibitory effect of these compounds against HepG2 cell line indicated low cytotoxicity of these compounds, which also shows the potential for this type of compound to be used as anti-tumor agents.

The real-time cell electronic sensing (RT-CES™) system is a label-free and cell-based screening assay.[9] The key advantage of this assay is the real-time measurement of the kinetic responses. It provides important insights in to various cellular processes and useful mechanistic information for the action of drugs. Therefore, high-quality lead compounds and the mechanism of action can be easily discovered from the screening results. Some of the potent trisulfide derivatives such as **4**, **5**, **7**, and **9** showed the same kinetic responding pattern as that of paclitaxel. Therefore, these compounds were predicted and then verified to have the same mode of action as anti-cancer drug paclitaxel.[20] These compounds also inhibit tumor cells by disturbing tubulin-microtubule dynamic equilibrium, therefore, arrest the cells to M phase to block cell division, eventually causing cell apoptosis.[20] This new class of anti-microtubule agents has potential to become useful cancer chemotherapeutic agents. The detail mechanism of action, drug target, and cell cycle specific inhibition as well as in vivo anti-tumor activities of these agents will be discussed in due course.

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16. Characterization of **5**: mp 61.0–62.0 °C; UV–vis (EtOH) $\lambda = 205$ nm (ω, 23,339), $\lambda = 219$ nm (ω, 22,324), $\lambda = 273$ nm (ω, 2,382); FT-IR (KBr, cm^{-1}): 3047, 3010 (w, ν_{CH}), 2950, 2917 (w, ν_{CH_2}), 1603 (m, $\nu_{C=C}$), 1511 (s, $\nu_{C=C}$), 1415 (m, $ArCH_2$), 1233 (s, ν_{C-F}), 834 (s, δ_{CH}, 2ortho-H), 652 (m, ν_{CH_2-S}), 527 (s, Φ_{C-C}, para-substitution), 462 (m, ν_{S-S}); ^{1}H NMR (499.1 MHz, $CDCl_3$) δ 4.00 (s, 4H), 7.01 (t, 4H, $J = 8.8$ Hz), 7.27 (dd, 4H, $J = 8.8$, 5.4 Hz); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 42.4, 115.6, 115.8, 131.2, 131,3, 132.4, 162.5 (C–F, $J = 250$ Hz); ^{19}F NMR (376.5 MHz, $CDCl_3$) δ −114.2; MS (EI) *m/z* 314 $(M)^+$. Anal. Calcd for $C_{14}H_{12}F_2S_3$: C, 53.48; H, 3.85; F, 12.08; S, 30.59. Found: C, 53.54; H, 3.84; F, 12.35; S, 30.73.
17. ^{1}H NMR (499.1 MHz, DMSO-d_6 for **21** and $CDCl_3$ for other compounds) spectra of compounds: **6**: δ 3.98 (s, 4H), 7.22 (d, 4H, $J = 8.4$ Hz), 7.29 (d, 4H, $J = 8.4$ Hz); **7**: δ 4.17 (s, 4H), 7.23–7.28 (m, 4H), 7.35–7.43 (m, 4H); **8**: δ 3.96 (s, 4H), 7.17 (d, 4H, $J = 8.3$ Hz), 7.45 (d, 4H, $J = 8.3$ Hz); **9**: δ 2.33 (s, 6H), 4.01 (s, 4H), 7.14 (d, 4H, $J = 8.0$ Hz), 7.21 (d, 4H, $J = 8.0$ Hz); **10**: δ 2.39 (s, 6H), 4.05 (s, 4H), 7.08–7.20 (m, 6H), 7.23–7.29 (m, 2H); **11**: δ 4.04 (s, 4H), 7.41–7.49 (m, 4H), 7.51–7.58 (m, 4H); **12**: δ 1.30 (s, 18H), 4.02 (s, 4H), 7.25 (d, 4H, $J = 8.3$ Hz), 7.35 (d, 4H, $J = 8.3$ Hz); **13**: δ 2.27 (s, 6H), 2.42 (s, 12 H), 4.23 (s, 4H), 6.87 (s, 4H); **14**: δ 4.24 (s, 4H), 6.92–6.96 (m, 2H), 6.99–7.04 (m, 2H), 7.24–7.28 (m, 2H); **15**: δ 3.74 (s, 4H), 7.01 (s, 2H), 7.34–7.45 (m, 4H), 7.75 (d, 2H, $J = 7.4$ Hz), 7.85 (dd, 2H, $J = 7.8$, 1.1 Hz); **16**: δ 3.05–3.11 (m, 4H), 3.13–3.18 (m, 4H), 7.19–7.27 (m, 6H), 7.30–7.35 (m, 4H); **19**: δ 7.70 (d, 4H, $J = 8.4$ Hz), 7.84 (dd, 4H, $J = 8.4$, 2.4 Hz), 8.73 (s, 2H); **20**: δ 7.50–7.62 (m, 4H), 8.34 (d, 2H, $J = 6.6$ Hz), 8.58 (d, 4H, $J = 4.4$ Hz).
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