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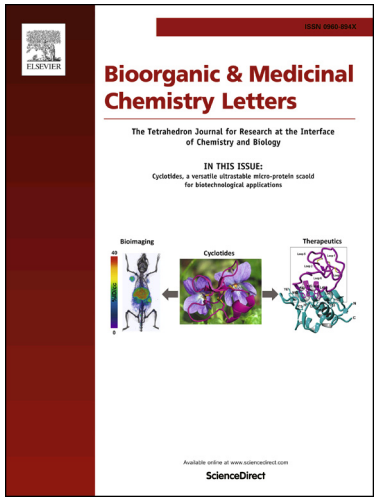

Synthesis, antitumor activity, and cytotoxicity of 4-substituted 1-benzyl-5-diphenylstibano-1*H*-1,2,3-triazoles

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BMCL

Synthesis, antitumor activity, and cytotoxicity of 4-substituted 1-benzyl-5-diphenylstibano-1*H*-1,2,3-triazoles

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ABSTRACT

Trisubstituted 5-organostibano-1*H*-1,2,3-triazoles (**3a-f**) were synthesized by the Cu-catalyzed azide-alkyne cycloaddition of various ethynylstibanes (**1**) with benzylazide (**2**) in the presence of CuBr (5 mol%) under aerobic conditions. The reaction of 5-stibanotriazoles with HCl afforded C5-unsubstituted 1,2,3-triazoles (**4a-f**). The antitumor activity of trisubstituted 5-organostibano-1*H*-1,2,3-triazoles (**3a-f**) and their 5-unsubstituted 1,2,3-triazoles (**4a-f**) were evaluated in several tumor cell lines. All 5-stibanotriazoles (**3a-f**) exerted an excellent antitumor activity. On the contrary, 5-unsubstituted 1,2,3-triazoles (**4a-f**) without a diphenylantimony group in the molecule exhibited very low antitumor activity compared with 5-stibanotriazoles (**3a-f**). In compounds of both the series, the substituted 4-butyl group appeared to decrease antitumor activity. However, results suggested that organometal (antimony) in the molecule was required for greater antitumor activity. In addition, all 5-stibanotriazoles (**3a-f**), but not all 5-unsubstituted 1,2,3-triazoles (**4a-f**), exhibited cytotoxicity in normal vascular endothelial cells derived from bovine aorta. Among the compounds (**3b-e**) that exhibited excellent antitumor activity, those with 4-methylphenyl (**3b**) and 1-cyclohexenyl (**3e**) showed relatively low cytotoxicity to vascular endothelial cells. Together, these results suggest that trisubstituted 5-organostibano-1*H*-1,2,3-triazoles, including compounds **3b** and **3e**, may serve as potential anticancer therapeutic drugs in the future.

Keywords:

Cu-catalyzed azide-alkyne cycloaddition

5-Stibanotriazole

Antimony

Antitumor

Cytotoxicity

1,2,3-Triazoles are an important class of nitrogen-containing heterocyclic ring, and are key in the design and synthesis of novel biologically active agents.[1,2] Among these,

1,4,5-trisubstituted-1,2,3-triazoles have attracted interest as target molecules because of their use as reagents in biological activities. For instance, SST0287CL1 **I**[3] exhibits heat shock protein 90 inhibition, sulfur-containing triazole **II**[4] is a potential herbicide with antifungal activity, triazole **III**[5] behaves as a potent inhibitor of human aromatase, and triazole derivative **IV**[6] having isoxazolyl is a potent non-pseudo-substrate inhibitor of O^6-alkylguanine-DNA-methyltransferase (Fig. 1).

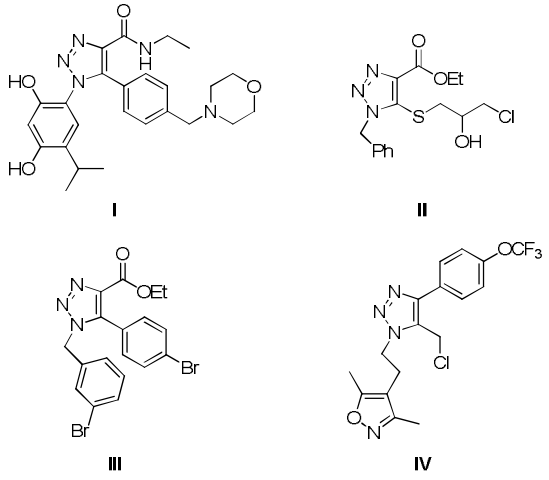
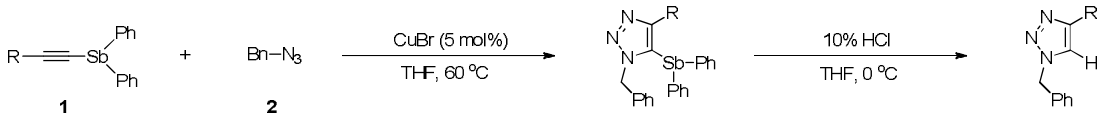


Fig. 1. Biologically active 1,4,5-trisubstituted-1,2,3-triazoles.

Additionally, antimony compounds have attracted much interest because of their importance as reagents in biological activities.[7-9] Interestingly, sodium stibogluconate, of the pentavalent antimony compound class, has been used for more than half a century in the therapy of the parasitic disease leishmaniasis.[10-12] However, there are few reports on the biological activities of trivalent organoantimony compounds[9] and we have recently reported on the synthesis and biological activity of both trivalent and pentavalent organoantimony compounds.[13-18] Among these, organoantimony (III) compounds such as 1-[(2-di-*p*-tolylstibanophenyl)diazenyl]pyrrolidine[15] and 2-(di-*p*-tolylstibano)-*N*-*p*-tolylbenzamide[16] showed potent antiproliferative activity on human tumor cell lines such as NB4, HeLa, L1210, Mm1, and DLD-1. For instance, 2-(di-*p*-tolylstibano)-*N*-*p*-tolylbenzamide showed moderate antitumor activities [IC_{50} = 4.6–10.8 μM] in several tumor cell lines; however, *N*-*p*-tolylbenzamide without antimony group was inactive [IC_{50}

> 200 μM].[16] Since these results indicate that organoantimony (III) compounds affect antitumor activity, we are interested in the biological activity of fully substituted 1,2,3-triazoles with antimony as a substituent. We present here the synthesis of novel 4-substituted 1-benzyl-5-diphenylstibano-1,2,3-triazoles using the Cu-catalyzed azide-alkyne cycloaddition of ethynyldiphenylstibane with benzyl azide and their antitumor activity and cytotoxicity, including comparisons of the obtained organoantimony compounds and 5-unsubstituted 1,2,3-triazoles.

Recently, we developed the regioselective Cu-catalyzed azide-alkyne cycloaddition of ethynylstibanes with organic azides to form fully substituted 5-organostibano-1,2,3-triazoles.[19,20] However, the ethynylstibanes used required *p*-tolyl group on antimony for determining regioselectivity of the reaction, and their synthesis has been complicated for general synthesis. Therefore, ethynylstibanes (**1a-f**) were selected as the key starting material, as they could be easily prepared according to the general method used previously.[21] The terminal alkynes were treated with *n*-BuLi in dry ether under an argon atmosphere at 0 °C, and followed by trapping with diphenylantimony bromide to afford arylethynyl, vinylethynyl, and alkylethynyl compounds (**1a-f**) in 44–83% yields. The Cu-catalyzed azide-alkyne cycloaddition of ethynylstibanes with aryl (**1a-d**), vinyl (**1e**), and alkyl (**1f**) group with benzylazide (**2**) in the presence of $CuBr_2$ (5 mol%) led to the formation of 5-stibanotriazoles (**3a-f**) in good-to-excellent yields under the optimal conditions, as reported previously (Scheme 1).[19,20,22] The diphenylantimony group in **3** was readily removed by treatment with 10% HCl in THF to obtain the desired C5-unsubstituted 1,2,3-triazoles (**4**) in satisfactory yields. The structures of **4** were confirmed by comparisons of NMR and MS spectra with authentic samples in the literature. Moreover, a nuclear Overhauser effect (NOE) was observed in benzyl protons and C-5 proton on triazole ring in **4**. These results show that Cu-catalyzed azide-alkyne cycloaddition of **1** with **2** proceeds regioselectively to yield 5-stibanotriazole (**3**).



1a: R = 4-Methoxyphenyl
1b: R = 4-Methylphenyl
1c: R = Phenyl
1d: R = 4-Trifluoromethylphenyl
1e: R = 1-Cyclohexenyl
1f: R = *n*-Butyl

3a: 70%
3b: 77%
3c: 75%
3d: 72%
3e: 77%
3f: 74%

4a: 81%
4b: 99%
4c: 98%
4d: 95%
4e: 75%
4f: 75%

Scheme 1. Synthesis of 5-stibanotriazoles **3** and 5-unsubstituted triazoles **4**.

The antitumor activities of the novel synthesized 4-substituted 1-benzyl-5-diphenylstibano-1,2,3-triazoles and their 5-unsubstituted 1,2,3-triazoles were evaluated in eight cultured tumor cell lines including those of mouse and human origin (Table 1). All 5-stibanotriazoles (**3a-f**) exerted excellent antitumor activity in all tested tumor cell lines, even human solid tumor cell lines such as breast, colon, and gastric tumors. The range of IC_{50} value was indicated at 0.25–11.9 μM and displayed almost equal IC_{50} values as those of cisplatin (CDDP), a well-known commercial antitumor drug containing a metal in its molecular structure. Among them, the compounds with 4-methylphenyl (**3b**), phenyl (**3c**), 4-trifluoromethylphenyl (**3d**), and 1-cyclohexenyl (**3e**) groups had the lowest IC_{50} values, and those with 4-methoxylphenyl (**3a**) indicated sequential activity. The average IC_{50} values in those cell lines for the compound with 4-*n*-butyl group (**3f**) was the highest recorded value (7.16 μM) but the antitumor activity of it was not disappearance. Conversely, 5-unsubstituted 1,2,3-triazoles (**4a-f**) without diphenylantimony group in the molecule exhibited very low antitumor activity compared with 5-stibanotriazoles (**3a-f**). All IC_{50} values of the compounds without diphenylantimony groups increased compared to that of their corresponding compounds. Among them, 1-benzyl-4-(*p*-trifluoromethylphenyl)-1*H*-1,2,3-triazole (**4d**) exhibited middling antitumor activity with an average IC_{50} value of 82 μM. In compounds of both series, the substituted alkyl chain, such as 4-butyl group, appeared to decrease antitumor activity but the effect of the substituted functional group such as electron-withdrawing and -donating groups at the 4-position was less. However, it appeared that the existence of organometal (antimony) in the molecule was required for greater antitumor activity.

Table 1. Comparison of antitumor activity between 5-stibanotriazoles (**3a-f**) and 5-unsubstituted triazoles (**4a-f**) in tumor cell lines.

	P388	B16-F10	HL-60	KB	HT-1080	MCF-7	DLD-1	NUGC-3
3a	0.54±0.20	0.47±0.04	0.87±0.45	0.86±0.24	0.50±0.12	0.66±0.27	0.80±0.18	1.38±0.59
3b	0.61±0.33	0.32±0.02	0.47±0.07	0.49±0.14	0.35±0.09	0.47±0.10	0.37±0.06	0.84±0.10
3c	0.31±0.04	0.44±0.12	0.93±0.60	0.52±0.09	0.38±0.04	0.59±0.35	0.43±0.12	0.87±0.48
3d	0.25±0.06	0.39±0.05	0.50±0.31	0.50±0.10	0.40±0.11	0.75±0.43	0.38±0.04	0.72±0.28
3e	0.77±0.40	0.34±0.01	0.72±0.28	0.47±0.10	0.41±0.16	0.53±0.07	0.40±0.05	0.71±0.15
3f	6.11±1.73	1.61±0.36	11.9±7.9	6.54±1.06	9.02±3.12	7.73±2.84	4.90±1.39	9.50±3.88
4a	83.8±13.9	88.5±24.5	>100	86.6±7.9	146±106	231±100	139±69	158±29
4b	94.1±31.5	66.4±24.0	>100	79.5±11.0	84.4±23.8	253±190	102±31	129±23
4c	99.1±37.8	96.1±19.1	>100	>100	651±559	490±157	>100	836±429
4d	17.5±7.6	35.4±17.1	171±64.2	60.2±11.6	57.7±10.5	162±97	74.3±52.2	81.0±25.9
4e	49.6±12.6	45.5±18.7	171±47.0	>100	219±145	>100	>100	>100
4f	217±135	421±128	>100	>100	>100	>100	>100	>100

Each value was represented IC_{50} ±SD (μM).

In order to test the cytotoxicity of the novel synthesized 4-substituted 1-benzyl-5-diphenylstibano-1,2,3-triazoles and their 5-unsubstituted 1,2,3-triazoles in normal cells, the non-tumor vascular endothelial cells derived from bovine aorta were used. All 5-stibanotriazoles (**3a-f**) exhibited higher antitumor activity (Table 1) and cytotoxicity (Table 2) than the 5-unsubstituted 1,2,3-triazoles (**4a-f**). The highest cytotoxic effect to vascular endothelial cells was observed for compounds with 4-methoxylphenyl (**3a**, CC_{50} = 1.9 ± 0.45 μM), phenyl (**3c**, CC_{50} = 2.1 ± 0.22 μM), and 4-trifluoromethylphenyl (**3d**, CC_{50} = 1.5 ± 0.07 μM) group. Although the compounds with 4-methylphenyl (**3b**) and 1-cyclohexenyl (**3e**) group, together with the compounds with phenyl (**3c**) and 4-trifluoromethylphenyl (**3d**) group, had the lowest IC_{50} values on the tumor cell lines (Table 1), they did not have the lowest CC_{50} values on the vascular endothelial cells (**3b**, CC_{50}= 5.4 ± 1.61 μM; **3e**, CC_{50}= 7.4 ± 0.71 μM). The compound with 4-*n*-butyl (**3f**) showed a lower toxicity on the vascular endothelial cells as well as lower antitumor activity among all 5-stibanotriazoles (**3a-f**). Moreover, the treatment of compounds with 4-methylphenyl (**3b**) and 1-cyclohexenyl (**3e**) at a concentration of 0.5 μM supported antitumor activity in tumor cell lines, but did not exhibit a cytotoxic effect in non-tumor vascular endothelial cells (data not shown). These results indicate that tumor cell lines are more sensitive to compounds with 4-methylphenyl (**3b**) and 1-cyclohexenyl (**3e**) than non-tumor vascular endothelial cells. Moreover, since compounds **3b** and **3e** exhibited excellent

antitumor activity in all tested tumor cell lines and relatively low cytotoxicity in non-tumor vascular endothelial cells, it is possible that these compounds can effectively suppress tumor growth without damage to normal cells.

Table 2. Comparison of cytotoxicity between **3** and **4** in non-tumor vascular endothelial cells.

Compounds	CC_{50} (mean ± SD, μM) [a]
3a	1.9 ± 0.45
3b	5.4 ± 1.61
3c	2.1 ± 0.22
3d	1.5 ± 0.07
3e	7.4 ± 0.71
3f	22.9 ± 6.87
4a	> 100
4b	> 100
4c	> 100
4d	> 100
4e	> 100
4f	> 100

[a] CC_{50}: 50% cytotoxic concentration.

In conclusion, the Cu-catalyzed azide-alkyne cycloaddition of various ethynylstibanes with benzylazide, under mild reaction conditions, afforded 4-substituted 1-benzyl-5-diphenylstibano-1*H*-1,2,3-triazoles. The 5-stibanotriazoles exerted greater antitumor activity in eight cultured tumor cell lines, including the human solid tumor cell line, than the corresponding 5-unsubstituted triazoles. Among the compounds **3b-e** that exhibited excellent antitumor activity, compounds with 4-methylphenyl (**3b**) and 1-cyclohexenyl (**3e**) showed relatively low cytotoxicity to normal cells. These results suggest that compounds **3b** and **3e** may serve as potential anticancer therapeutic drugs in future.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://

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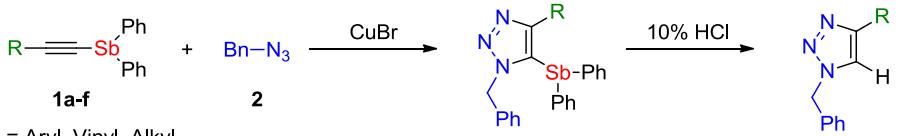
Highlights:

Trisubstituted 5-stibanotriazoles were prepared by the Cu-catalyzed cycloaddition.

5-Substituted triazoles exerted great antitumor activity in several tumor cell lines.

Two 5-stibanotriazoles showed low cytotoxicity to normal vascular endothelial cells.

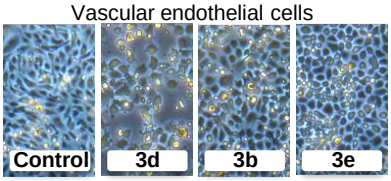


1a-f **2** **3a-f** **4a-f**

R = Aryl, Vinyl, Alkyl

1) A novel **3a-f** with excellent antitumor activity were designed and synthesized
2) Antitumor activity of **3a-f** against DLD-1 with IC_{50} = 0.37 – 4.9 μM
3) **3b** (4-tolyl) and **3e** (4-cyclohexenyl) showed low cytotoxicity to non-tumor cells

Vascular endothelial cells



	(μM)	
	IC_{50}	CC_{50}
3d	0.38	1.5
3b	0.37	5.4
3e	0.40	7.4