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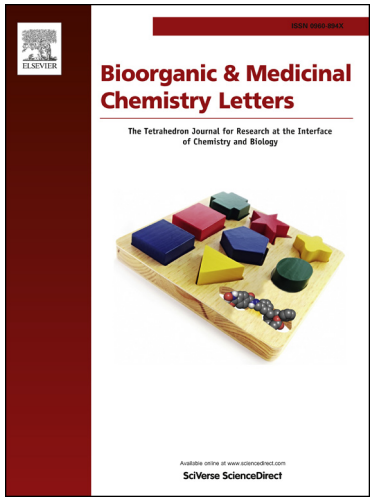

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Syntheses and antitumor activities of N'[1], N'[3]-dialkyl-N'[1], N'[3]-di-(alkylcarbonothioyl) malonohydrazide: the discovery of elesclomol

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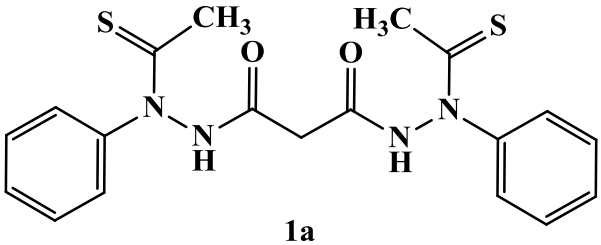
Abstract: A series of N'[1],N'[3]-dialkyl-N'[1],N'[3]-di(alkylcarbonothioyl)malonohydrazides have been designed and synthesized as anticancer agents by targeting oxidative stress and Hsp70 induction. Structure activity relationship (SAR) studies lead to the discovery of STA-4783 (elesclomol), a novel small molecule that has been evaluated in a number of clinical trials as anticancer agent in combination with Taxol.

***Key words*:** STA-4783, elesclomol, discovery, anticancer, Taxol enhancer, Hsp70 induction, SAR, oxidative stress, ROS.

Despite the tremendous progress made in cancer chemotherapy, cancers of various kinds still remain the leading causes of mortality worldwide. Exploiting the unique features of tumor cells and examining the specific molecular pathways or cellular targets that differentiate cancer cells from normal cells are among the key focuses in modern oncology drug discovery.[1] Scientific evidence has shown that elevated levels of reactive oxygen species (ROS) can be detected in almost all cancer cells.[2] It is known that an extremely high level of ROS beyond the threshold in cancer cells can trigger apoptosis.[3] It is also well documented that cancer cells have higher levels of ROS than normal cells and therefore are more sensitive to intruders that induce oxidative stress or ROS production.[4,5] In contrast, the comparably low level of ROS in normal cells can be effectively detoxified by the endogenous antioxidants and makes normal cells much more immune to ROS inducers.[6] In parallel, very high levels of Hsp70, a 70kDa heat shock protein, has also been found in malignant human tumors of various origins[7] and the cellular expression of Hsp70 has been shown to positively correlate with elevated levels of ROS generation.[8] Furthermore, studies also demonstrate that Hsp70 can act as a biomarker of oxidative injury.[9] Therefore, targeting ROS or Hsp70 induction would appear to be a viable approach for new anticancer drug discovery. We started a program by screening compounds in Synta's unique compound library. Since the human Sarcoma cell line MES-SA/Dx5 was a well-established model for multidrug resistance (MDR) modulators screening,[10] we employed this cell-based assay together with Hsp70 induction assay for our initial library screening. Herewith we report the discovery, syntheses, and SAR studies of a series of N'[1], N'[3]-dialkyl-N'[1], N'[3]-di(alkylcarbonothioyl)malonohydrazides as anticancer agents, cumulatively leading to the generation of STA-4783 (elesclomol), a novel small molecule that has been evaluated in a number of clinical trials and showed positive results with melanoma patients displaying low levels of lactate dehydrogenase (LDH).[11]

The first lead molecule **1a**, namely N'[1], N'[3]-diethanethioyl-N'[1], N'[3]-diphenylmalonohydrazide, was discovered from the screening of our compound library (Figure 1).



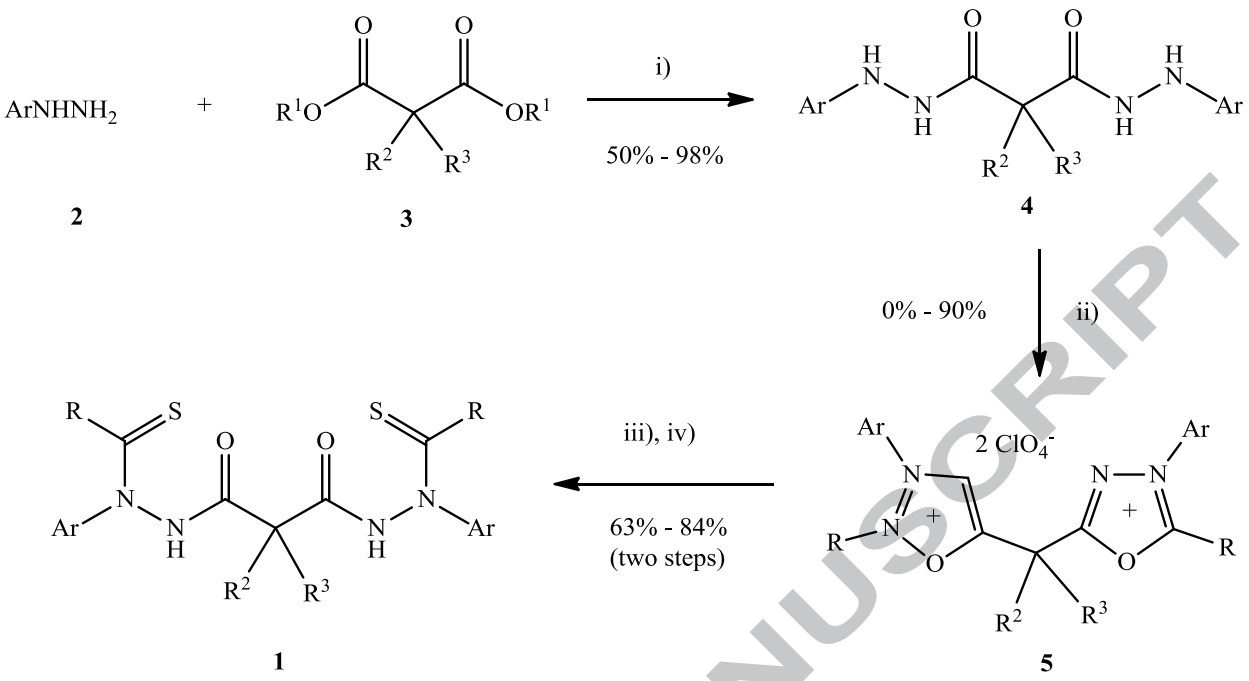
1a

MES-SA/Dx5 IC_{50} = 50 nM

Hsp70 EC_{50} = 0.75 μM

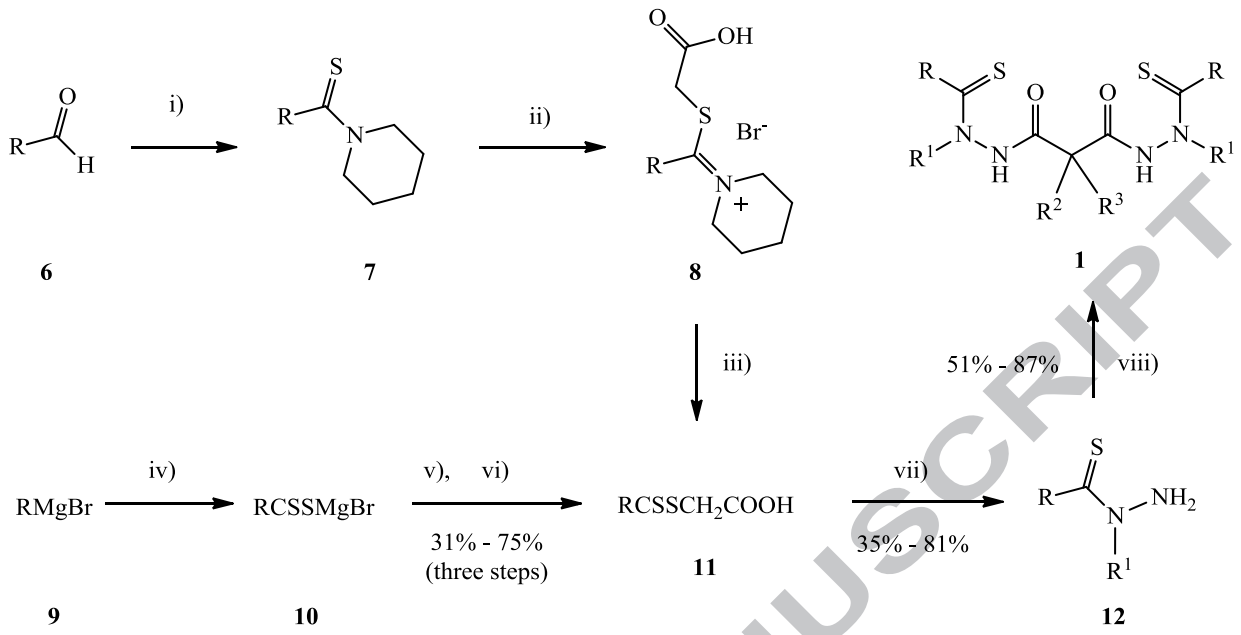
Figure 1. Structure of hit molecule

Compound **1a** showed moderate *in vitro* activities in inducing Hsp70 (EC_{50} = 0.75 μM) and potent anti-proliferative activity against MES-SA/Dx5 cancer cell line (IC_{50} = 50 nM). However, compound **1a** was found to be fairly chemically unstable, especially when it was exposed to air; and metabolically unstable as well (data not shown). The two sulfur atoms in the molecule were easily oxidized to the corresponding oxygen moieties upon direct exposure to air, and this resulted in inactive molecules. Derivatization of this early lead compound was therefore carried out in order to explore the SAR for generating superior inhibitors with improved properties. To our surprise, synthetic methods for these types of malonohydrazides were extremely rare in the literature. This prompted us to seek facile and efficient synthetic methodologies for the construction of such molecules in our lead optimization efforts. As a result, three synthetic routes beginning with different starting materials were eventually developed (Scheme 1, 2, and 3, respectively). As outlined in Scheme 1, reaction of an arylhydrazine **2** and dialkyl malonate **3** at elevated temperature in xylene afforded the corresponding malonyl hydrazide with good yield.[12a] Acylation by an anhydride followed by cyclization with hydroperchloric acid afforded the salt **5**, which, upon thionation with sodium sulfide and acidic hydrolysis, produced the corresponding final product **1** in satisfactory overall yield.



Scheme 1. Reagents and conditions: i) Xylene, reflux, 2-24h; ii) $(RCO)_2O$, 57% $HClO_4$, 0 ^oC to rt; iii) Na_2S, H_2O, acetone, rt, 30 min; iv) HCl, H_2O.

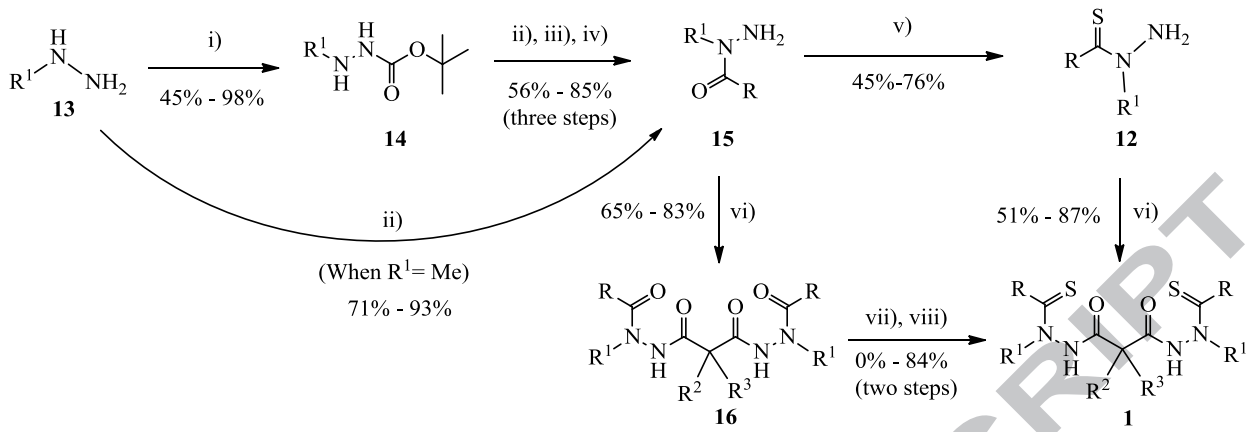
We soon found out that this method had limited success, not only because of the limited commercial sources for the corresponding anhydride, but also because a majority of aliphatic anhydrides, such as trifluoroacetic anhydride and chloroacetic anhydride failed to participate in this type of reaction. Furthermore, in the first step, the scope of the reaction of hydrazine with dimethyl malonate was also very limited. Although much improvement in terms of reaction scope and yield was achieved by the replacement of dimethyl malonate with diphenyl malonate, aliphatic hydrazines, such as methyl hydrazine and butyl hydrazine, failed to give the desired products under various conditions. Therefore, it was necessary to develop more general methods for the synthesis of compounds with more diversified structures for SAR studies. Thus, two new synthetic methods based on a key intermediate, α-thioacylhydrazine **12,** were developed (Schemes 2 and 3). As shown in Scheme 2, the key intermediate **12** was synthesized from an aldehyde **6** or Grignard reagent **9** according to a slightly modified procedure based on Jensen et al.[12b] Coupling of **12** with malonic acid with DCC or BOP reagent provided the final product **1** in very good yield.



Scheme 2. Reagents and conditions: i) Piperidine, S_8; ii) $BrCH_2COOH$; iii) H_2S, EtOH; iv) CS_2, Et_2O; v) $ClCH_2COONa$; vi) H_3O^+; vii) R^1NHNH_2, NaOH; viii) $R^2R^3C(COOH)_2$, DCC or BOP reagent, DMF or DCM, HOBt, 0 ^oC to rt, 1 to 12 hr.

The key intermediate **11** was prepared from two alternative synthetic routes. The first route started from an aldehyde. Treatment of an aldehyde with sulfur in piperidine resulted in the formation of thioamide **7**. Treatment of **7** with bromoacetic acid afforded 1-(1-((carboxymethyl)thio)alkylidene)piperidin-1-ium bromide (**8**) in very good yield. Finally, reaction of **8** with hydrogen sulfide in ethanol provided the key intermediate **11** in satisfactory to very good yield. Alternatively, compound **11** could also be obtained from a Gridnard reagent **9**. Treatment of **9** with carbon disulfide in ether resulted in magnesium bromide alkanedithioate **10**. Reaction of **10** with sodium chloroacetate followed by hydrolyses afforded compound **11** in satisfactory overall yield. The two synthetic routes were complementing each other. For instance, the first route was preferred when R group in **11** contained moiety such as cyano or carboxylic acid or ester that was reactive to Grignard reagent. Alternatively, when the corresponding aldehyde was not readily available or not very stable, the second route would show some advantages.

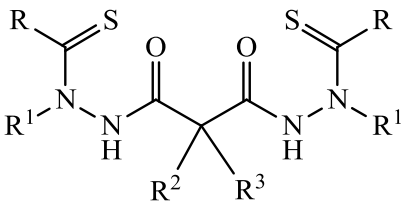
A more general synthetic method from more commercially available carboxylic acids or acyl chlorides was further developed (Scheme 3). Reaction of methylhydrazine with an acylchloride or coupling with a carboxylic acid provided the desired 2-acylhydrazide with high selectivity and good yield. In this case, the more nucleophilic methyl-substituted nitrogen atom in methylhydrazine was selectively acylated.[12c] However, in the case of the more sterically hindered hydrazine, protection of the primary amino group by a Boc group was necessary[12d] in order to achieve the acylation at the 2-position.



Scheme 3. Reagents and conditions: i) $(Boc)_2O$, DCM; ii) RCOCl, TEA, DCM; iii) TFA, DCM, 0 °C, 30 min; iv) NaOH, 0 °C; v) Lawessen's, toluene or P_2S_5, benzene, reflux, 1- 12 h ; vi) $R^2R^3C(COOH)_2$, EDC or BOP reagent, DMF or DCM, 0 °C to rt, 24h; vii) $HClO_4$; viii) Na_2S.

A handful of compounds with diversified substituent patterns in **1a** were then readily synthesized according to the chemistries described in Scheme 1 through Scheme 3, and were subsequently assessed in Hsp70 induction and MES-SA/Dx5 inhibition assays.[13-14] The corresponding assay results were listed in Table 1.

Table 1. N'[1],N'[3]-dialkyl-N'[1],N'[3]-di(alkylcarbonothioyl)malonohydrazides *in vitro* assay data



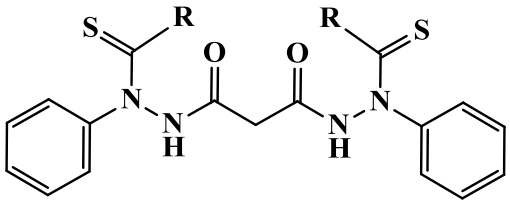
Compound	R	R^1	R^2	R^3	Hsp70 EC_{50} (nM)[a]	MES/Dx5 IC_{50} (nM)[a]
1a	Me	Ph	H	H	750	50
1b	Me	Ph	Me	Me	>1250	1000
1c	4-NO2-Ph	Me	H	H	39	100
1d	3-F-Ph	Me	H	H	42	5
1e	3-CN-Ph	Me	H	H	97	50
1f	4-CN-Ph	Me	Me	H	106	50
1g	2,5-$(MeO)_2$-Ph	Me	H	H	142	5
1h	Ph	Et	H	H	183	50
1i	Ph	n-Bu	H	H	>1250	1000

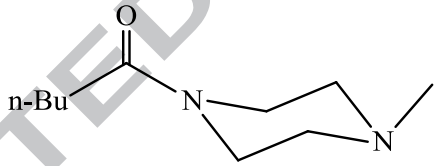
[a] Values are average of 2 or more determination. The assay-to-assay variation was typically within 25% - 35% range.

After observing an obvious correlation between Hsp70 induction activity and MES-SA/Dx5 cell-based inhibition activity (Table 1), we started to use only an Hsp70 induction assay for further SAR exploration.

By employing the synthetic methods depicted in Schemes 1 through 3, derivatives with different R substituents were synthesized and tested in the Hsp70 induction assay. The results were summarized in Table 2. As shown in the table, the Hsp70 induction activity was very sensitive to the R group in **1**. Both the methyl and phenyl groups showed moderate activity, with an IC_{50} value around 0.75 μM. Replacing this substituent with a bulkier alkyl group, either linear or cyclic, resulted in a complete loss of activity (EC_{50} > 1.25μM). In subsequent *in vitro* metabolism studies, we found that when R is a methyl group, the corresponding compound **1a** was very unstable in the media when exposed to the air. The two thio groups in **1a** were oxidized to the corresponding oxocarbonyl groups. This oxidation process was accelerated under basic conditions. However, the phenyl group in compound **1p** demonstrated much better advantages in this regard (data not shown).

Table 2. Effect of R group in **1** on Hsp70 activity

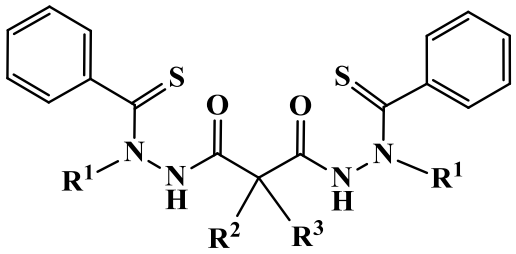


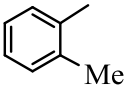
Compound	R	Hsp70 EC_{50} (μM)[a]
1a	Me	0.725
1j	n-Bu	>1.25
1k	n-Pent	>1.25
1l	c-Hex	>1.25
1m	n-Pr-COOH	>1.25
1o		>1.25
1p	Ph	0.725

[a] Values are average of 2 or more determination. The assay-to-assay variation was typically within 25% - 35% range.

Therefore we then investigated the SAR at other positions, while fixing the R as a phenyl group. Eight compounds with different R^1, R^2 or R^3 groups were then synthesized in order to compare their *in vitro* activity. The Hsp70 induction activities of those synthesized new derivatives were listed in Table 3.

Table 3. Effect of R^1, R^2, and R^3 groups in **1** on Hsp70 activity

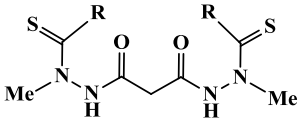


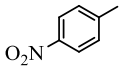
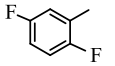
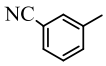
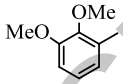
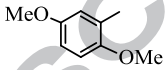
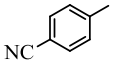
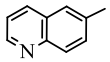
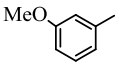
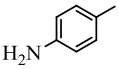
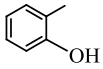
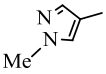
Compound	R^1	R^2	R^3	Hsp70 EC_{50} (μM)[a]
1q		H	H	>1.25
1r	H	H	H	>1.25
1s	Me	H	H	0.02
1h	Et	H	H	0.18
1i	n-Bu	H	H	>1.25
1t	Me	Et	H	>1.25
1u	Me	i-Pr	H	>1.25
1v	Ph	Me	Me	>1.25

[a] Values are average of 2 or more determination. The assay-to-assay variation was typically within a range from 25% - 35%.

As shown in Table 3, when R^2 and R^3 were fixed as H (**1q, 1r, 1s, 1h**, and **1i**), the size of R^1 appeared to be critical for Hsp70 induction activity. When R^1 was a hydrogen group (**1r**), the Hsp70 activity was greatly diminished. Larger groups such as n-butyl (**1i**) or o-methylphenyl (**1q**) resulted in loss of activity as well. An ethyl group (**1h**) was sustainable with quite potent activity (180nM). Best results were achieved when R^1 is a methyl group (**1s**), resulting in potent Hsp70 induction activity (EC_{50} = 20nM). Bulky groups at either or both R^2 and R^3 positions (**1u, 1v**, and **1w**) resulted in loss of activity. Once R^1, R^2, and R^3 were optimized, we retro-tested the SAR at the R position. The results were listed in Table 4.

Table 4. SAR on Hsp70 activity with substituent R in **1**



Compound	R	Hsp70 EC_{50} (μM)[a]	Compound	R	Hsp70 EC_{50} (μM)[a]
1c		0.039	**1z1**		0.116
1e		0.097	**1z2**		0.068
1d		0.042	**1g**		0.142
1s		0.020	**1z3**		0.140
1w		0.029	**1z4**		0.066
1x		>1.25	**1z5**		0.137
1y		>1.25	**1z6**		0.080
1z		>1.25	**1z7**		>1.25

[a] Values are average of 2 or more determination. The assay-to-assay variation was typically within a range from 25% - 35%.

Besides the phenyl group at R^1, mono-substituted phenyl groups were also tolerable for Hsp70 induction activity, especially when the substituents were electron-withdrawing groups such as nitro (**1c**), fluoro (**1d**) and cyano at different positions (**1w** and **1e**). On the other hand, electron-donating groups (**1x**, **1y**, **1z**) appeared to diminish HSP70 induction activity. However, in the case of two substitutions, either electron-withdrawing groups (**1z1**) or electron-donating groups (**1z2** and **1g**) maintained potent Hsp70 induction activity for the parent compounds. In addition to phenyl or substituted phenyl groups, heterocyclic aromatic rings at this position were also tolerable. For examples, potent Hsp70 induction activity was maintained when this position was substituted by a pyridinyl (**1z3**), quinolonyl (**1z4**), thiazolyl (**1z5**), or an oxazolyl group (**1z6**). However, the pyrazinyl group (**1z7)** was an exception, which resulted in loss of activity (**Table 4**).

On the basis of *in vitro* Hsp70 induction activity and preliminary *in vitro* and *in vivo* PK properties, compound **1s** (STA-4783)[15] was selected for further *in vitro* and *in vivo* profiling. The results of cytotoxicity screening against a panel of cancer cell lines by STA-4783 together with Taxol are listed in Table 5. As demonstrated in Table 5, STA-4783 inhibited a wide range of tumor cell lines, but not normal cell lines such as 39 SK or hPBMC (IC_{50} > 10 μM, Table 5).

Table 5. *in vitro* anticancer activity of STA-4783

Compd	IC_{50} (μM)										
	H2	HL60	A549	Clone A	MIP101	MDA435	DU145	MES-SA	MES-SA/Dx5	39 SK	PBMC
STA-4783	0.005	0.4	0.1	0.1	0.05	5	0.02	0.05	0.005	>10	>10
Taxol	0.001	0.005	0.01	0.5	1.1	0.005	0.005	0.005	10	5	5

In addition to the potent activity against a wide range of wild type (WT) tumor cell lines, STA-4783 also demonstrated potent activity with multidrug-resistant (MDR) tumor cell lines that were immune to Taxol (Table 6).

Table 6. IC_{50} (μM) values of STA-4783 and Taxol for Multidrug Resistance (MDR) Cell Lines

Cell Line	Human Tissue	STA-4783		Taxol	
		WT	MDR	WT	MDR
MES-SA	Uterine	0.05	0.005	0.005	5
HL-60	Leukemia	0.4	0.05	0.002	5
Bowes	Melanoma	0.2	0.01	0.005	5

To demonstrate *in vivo* efficacy, compound **1s** (STA-4783 or elesclomol) was administrated alone and in combination with Taxol via intravenous bolus QOD (3x/week; Monday, Wednesday, Friday) for two consecutive weeks in mice with a well-established U937 human lymphoma xenograft model.[16] In this study, no single agent efficacy was observed when STA-4783 was dosed alone at 25 mg/kg via QOD i.v. dosing. Only weak efficacy was seen from the dosing group with Taxol alone at 5mg/kg. However, when STA-4783 was dosing at 25mg/kg together with Taxol at 5mg/kg via similar dose regime, significant efficacy (70% to 100% tumor regression) was observed (Figure 2).

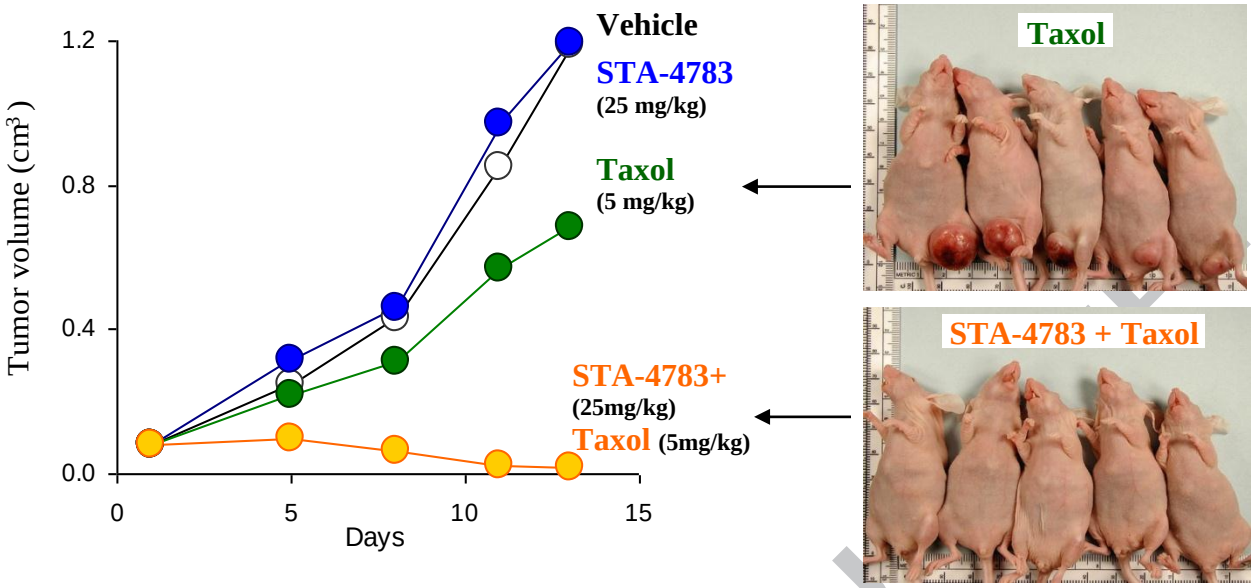


Figure 2. Taxol-Enhancing Effects of **STA-4783** in U937 human lymphoma xenograft model. Mice used: CD-1 nu/nu Female (n=5);Tumor type: U937 (Human lymphoma); Subcutaneous implantation: 5x106 cells; Formulation: 10% DMSO, 18% Cremophor RH40; Administration route: intravenous bolus; Dose schedule: 3x/week; Tumor measurement: 2x/week.

In addition to the U937 human lymphoma xenograft model, Taxol-enhancing effects were also observed for **STA-4783** in an MDA-MB-435 human melanoma xenograft model in nude mice.[16] Significant tumor regression was observed for the dosing group with **STA-4783** (25 mg/kg) and Taxol (5mg/kg). (Figure 3).

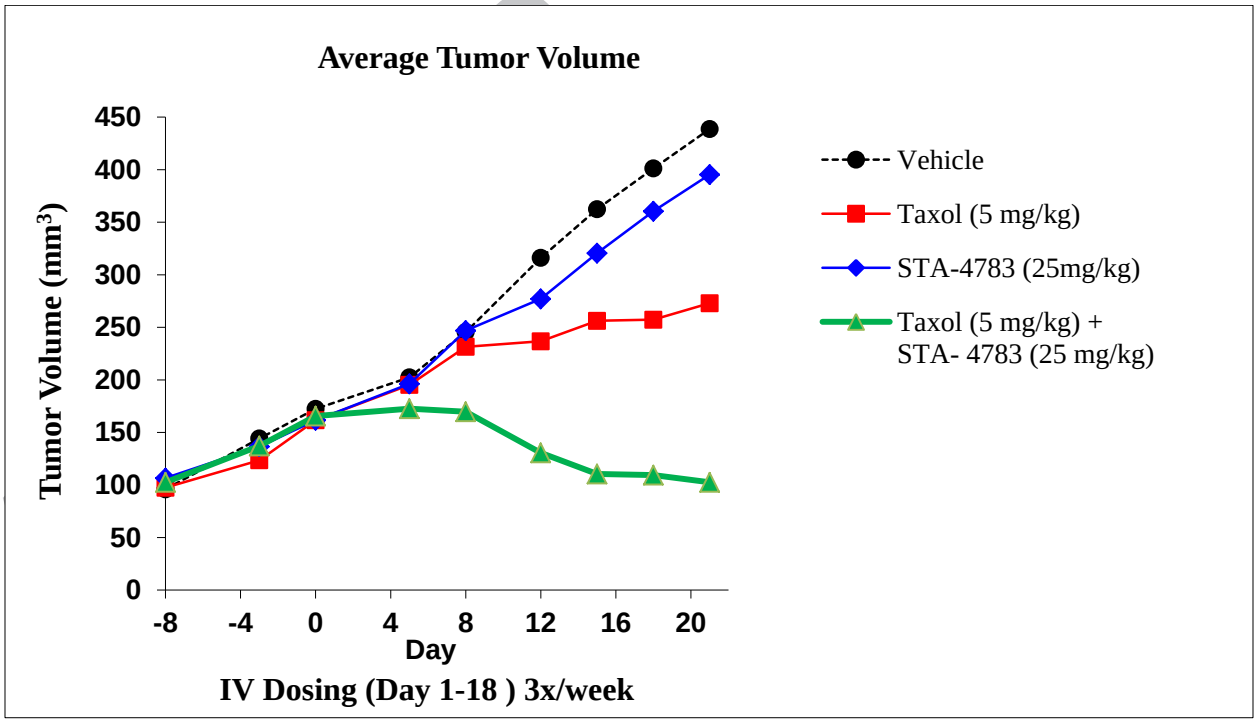


Figure 3. Taxol-Enhancing Effects of STA-4783 (25 mg/kg) in the MDA-MB-435 human melanoma xenograft model. Mice: CD-1 nu/nu female (n=5); Tumor cells: MDA-MB-435 (human melanoma); Mammary fat pad implantation: 5x106 cells; Formulation: 10%DMSO, 18% Cremophor RH40; Administration route: intravenous bolus; dose schedule: 3x/week; tumor measurement: 2x/week.

To determine the corresponding pharmacokinetics (PK) of STA-4783, the compound was administered intravenously to nude/nude mice or Sprague-Dawley male rats. Results showed high systemic clearance, and short apparent terminal half-life (Table 7).

Table 7. PK results of STA-4783 in rats and mice

Species	Dose (mg/kg)	Cmax (μM)	$T_{1/2}$ (hr)	AUC (μM·hr)	Cl (L/hr/kg)	Vss (L/Kg)	Formulation[c]
Rat[a]	25	56.3	1.94	32.7	0.79	0.1	DRD
Mouse[b]	25	85.2	1.90	35.9	1.74	0.51	DRD

[a] Male SD rat data; [b] Female nu/nu mouse, Cmax: the first time point for PK blood draw, approximately 5 min after the injection; [c] DRD: 10% DMSO, 18% CrRH40.

Combination PK studies for STA-4783 and Taxol in male rats and nude/nude mice were also conducted, and the results did not show apparent difference in Taxol PK parameters (C_{max}, $T_{1/2}$ and AUC) between single (Taxol 5mg/kg) and combination (Taxol 5mg/kg + STA-4783 25mg/kg.) No pharmacokinetic drug-drug interaction was observed. The corresponding pharmacokinetic parameters ($T_{1/2}$, AUC, C_{max}) are listed in Table 8. Co-administration of STA-4783 with Taxol resulted in minimum pharmacokinetic interaction, causing no toxicity impact in nonclinical species.

Table 8. DMPK of STA-4783 and Taxol in rats and mice

Species	Taxol (mg/kg)	STA-4783 (mg/kg)	Taxol			STA-4783			Formulation[c]
			Cmax (μM)	$T_{1/2}$ (hr)	AUC (μM*hr)	Cmax (μM)	$T_{1/2}$ (hr)	AUC (μM*hr)	
Rat[a]	5	0	21.7	3.1	10.3	-	-	-	DRD
	5	25	20.4	3.4	11.9	63.3	1.7	36.8	DRD
Mouse[b]	5	25	10.6	2.26	2.95	37.8	1.2	11.9	EED

[a] Male rats: Approximately 5 min after injection; [b] Nu/nu mouse slow bolus injection; [c] DRD: 10% DMSO, 18% CrRH40; EED: 5% Ethanol, 5% CrEL, 4.5% Dextrose.

Furthermore, STA-4783 was well tolerated when given alone or in combination with taxol in mice, rats, and dogs evaluated in acute non-GLP or GLP toxicology studies. Throughout most of the doses tested, the dose limiting toxicity of the STA-4783/Taxol drug combination was similar to that of Taxol administered alone or attributed to Taxol based on clinical observations and pathological assessments consistent with Taxol toxicity. Additional studies also suggested that there was no significant toxicity as a result of STA-4783 treatment in combination with Taxol after repeat-dose toxicity assessment in rats or dogs.

In summary, a hit molecule, N'[1], N'[3]-diethanethioyl-N'[1], N'[3]-diphenylmalonohydrazide **(1a)** was discovered from the screening of Synta unique compound library by Hsp70 induction assays, together with a cytotoxicity assay using a MDR cancer cell line (MES-SA/Dx5). Medichem optimization of the hit molecule **1a** through SAR studies led to the generation of a novel anticancer agent, N'[1], N'[3]-dimethyl-N'[1], N'[3]-di(phenylcarbonothioyl)malonohydrazide (**1s**, STA-4783 or elesclomol). STA-4783 demonstrated potent anti-cancer activity against a wide range of tumor cell lines including a number of MDR tumor cell lines that was immune to Taxol. In addition, STA-4783 demonstrated remarkable enhancement effects for the anticancer activity of Taxol in multiple human tumor xenograft models conducted in mice. As a first-in-class investigational drug, elesclomol administered in combination with Taxol was shown to prolong progression-free survival compared with taxol alone in a phase II clinical trial in patients with metastatic melanoma.[11a] However, in a more comprehensive phase III clinical trial, it showed a statistically significant improvement in median PFS for the combination only in a prospectively defined subgroup patients with normal baseline LDH.[17] The mechanism of action is still not yet fully defined and is currently under further investigation.

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13. Hsp70 induction assay was conducted as follows: Hsp70 induction is measured in Ramos B lymphoma cells. Cells are seeded at 80-tl/well in 96-plate and treated with compounds at 10, 50, 250 and 1250 nM for 5h. The cell density is determined by titration where testing compound shows Hsp70 induction in linear manner with concentration. Cells are lysed in culture medium with 14-vol of alkaline-detergent buffer composed of 200 mM bicarbonate, pH 9.4, and 20% Triton X100 to include Hsp70 that would be released into culture supernatant. The whole culture lysis significantly contributes to throughput as omitting experimental labor of cell separation from culture supernatant may cause experimental error. Fifty microliter of the whole culture lysate is transferred to Hsp70 ELISA assay well (Assay Design) including 150uL of immunoenhancer solution (Toyobo, Japan). Then the assay steps are carried out according to the protocol of the manufacturer.
14. Cell culture and assays were carried out in a similar manner as described by our lab previously: James, D; Koya, K.; Li, H.; Chen, S.; Xia, Z.; Ying, W.; Wu, Y.; Sun, L. *Bioorg. Med. Chem. Lett.* **2006**, 16, 5164-5168.
15. a) In the i*n vitro* PK studies, numerous metabolites were observed in plasma. Amide hydrolysis, sulfur replacement to oxygen, and hydroxylation were the main metabolic pathways. This multiple metabolic pathways may account for the short half-life observed

for these derivatives. STA-4783 was selected based on its relative higher metabolic stability and more potent *in vitro* activities.

b) Typical procedures for the synthesis of STA-4783: to a stirred solution of N-methyl-N-thiobenzoylhydrazine[12b] (**12**, 1.66 g, 10 mmol), HOBt.H_2O (0.15 g, 11 mmol) and malonic acid (0.052 g, 5 mmol) in DMF (2 mL) was added DCC (0.22 g, 10.7 mmol) at 0 °C. The resultant suspension was stirred at 0 °C for 3 h and at rt for 18h. Precipitated material was filtered off and washed with EtOAc (3 x 15 mL). Combined filtrate and washings was washed successively with H_2O (2 x 20 mL), 5% citric acid (20 mL), H_2O (20 mL), Saturated $NaHCO_3$ (20 mL) and brine (20 mL). After being dried over Na_2SO_4, the solvent and volatile components were removed under reduced pressure to afford the crude product as a yellow solid, which was recrystallized from THF/Hexane. 0.16 g (yield 80%) of pure product was obtained as a fine powder. **STA-4783:** R_f 0.3 (Hexane/EtOAc 1:1 v/v); mp 191-193 °C; ^{1}H NMR ($CDCl_3$) δ 3.1 – 3.8 (m, 6H), 3.4 (s, 2H), 7.1 – 7.45 (m, 10 H), 9.5 – 10.5 (m, 1H) ppm; ^{13}C NMR (DMSO-d_6) δ 40.5, 44.8, 127.6, 129.2, 130.7, 143.4, 165.0, 204.2 ppm; FTIR (KBr): 3205, 1688, 1299, 1247, 1091 cm^{-1}; MS (ES^-) m/z 399 (M – 1); Anal. Calcd for $C_{19}H_{20}N_4O_2S_2$ (400.10): C 56.98, H 5.03, N 13.99, O 7.99, S 16.01; found: C 57.12, H 5.12, N 13.90, O 7.99, S 16.00.

16. General procedure for *in vivo* efficacy testing: The *in vivo* anticancer enhancing effects of STA-4783 were assessed in tumor bearing mice using the tumor growth inhibition assay. U-937 human lymphoma cells were purchased from American Type Culture Collection (ATCC Cat. No. CRL-1593.2). The cells were implanted by injection of a tumor cell suspension subcutaneously into the mammary fat pad of nude mice. MDA-MB-435 human melanoma tumor cells were also purchased from ATCC (Cat No. HTB-129) and implanted subcutaneously into nude mice. These cells were previously characterized as mammary gland pleural effusion. Treatment of the tumor with STA-4783 and Taxol was initiated after the tumor had been established (tumor volume ~ 100 mm^3). The animals were then started on a multiple injection schedule where STA-4783 and Taxol were administered by IV route of administration. Tumor volumes were measured 2x/week. During the course of this assay, animals were monitored daily for signs of toxicity including body weight loss.

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Graphical Abstract

Syntheses and antitumor activities of N'[1], N'[3]-dialkyl-N'[1], N'[3]-di-(alkylcarbonothioyl) malonohydrazide: the discovery of elesclomol

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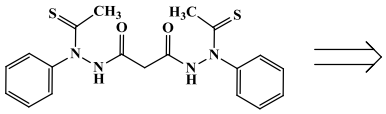
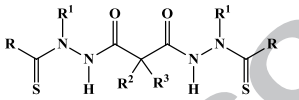
Shoujun Chen*, Lijun Sun, Keizo Koya, Noriaki Tatsuta, Zhiqiang Xia,
Timothy Korbut, Zhenjian Du, Jim Wu, Guiqing Liang, Jun Jiang, Mitsunori Ono, Dan Zhou, and Andrew Sonderfan



1a

(Hit molecule)

Hsp 70 EC_{50} = 750 nM
MES-SA/Dx5 IC_{50} = 50 nM



1s

(STA-4783, Elesclomol)
(R = Ph; R^1 = Me; R^2, R^3 = H)

Hsp 70 EC_{50} = 20 nM
MES-SA/Dx5 IC_{50} = 5 nM