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A new triphenylethylene derivative, TAT-59; hormone receptors; insulin-like growth factor 1; and growth suppression of hormone-dependent MCF-7 tumors in athymic mice

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Abstract. TAT-59 {(*E*)-4-[1-[4-[2-(dimethylamino)ethoxy]-phenyl]-2-(4-isopropyl)phenyl-1-butenyl]-phenyl-monophosphate} treatment was performed on hormone-dependent MCF-7 tumors in athymic mice. TAT-59 given at 1, 5, and 20 mg/kg inhibited the estrogen-stimulated growth of MCF-7 tumors in athymic mice in a dose-dependent fashion. The most clear decrease in tumor growth was shown in the TAT-59 alone group, although it was not dramatic. Average serum concentrations of DP-TAT-59{(*Z*)-[1-[4-[2-(dimethylamino)-ethoxy]phenyl]-2-(4-isopropyl)phenyl-1-butenyl]-4-hydroxybenzene} and DM-DP-TAT-59(desmethyl-DP-TAT-59), metabolites of TAT-59, increased in a dose-dependent manner. Much higher levels of DP-TAT-59 and DM-DP-TAT-59 were shown in tumors (target tissues of estrogen) as compared with muscles (nontarget tissues of estrogen) or serum. A serum concentration of DP-TAT-59 or DM-DP-TAT-59 corresponding to the physiologic levels of serum estradiol in premenopausal women was sufficient to inhibit the estrogen-stimulated growth of MCF-7 tumors in mice. TAT-59 induced a dose-dependent increase in estrogen receptor levels in the MCF-7 tumors. In contrast, it prevented the estradiol (E_2)-induced increase in progesterone receptor levels in a dose-dependent manner. Insulin-like growth factor 1 levels measured in the MCF-7 tumors significantly decreased in the TAT-59 alone group and in the no treatment group as compared with the E_2 alone group. These results show the pronounced antiestrogenic action of TAT-59 on hormone-dependent MCF-7 tumors in athymic mice.

Key words: TAT-59 – MCF-7 tumors – Athymic mice – Hormone receptors – IGF-1

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Introduction

It is generally accepted that tamoxifen, a nonsteroidal antiestrogen, is the first-line antihormonal therapy for breast cancer. Tamoxifen is recognized to be effective for advanced breast cancer in postmenopausal patients [4, 6, 18, 19] and has proved to be effective in premenopausal patients as well [17, 20]. Recent clinical trials of long-term adjuvant tamoxifen treatment in premenopausal patients [3, 5] have demonstrated an increase in disease-free survival in those women receiving tamoxifen. However, tamoxifen is known to cause an elevation in ovarian steroidogenesis in clinical cases [8, 13, 14, 18, 21, 22]. Therefore, the mechanism of the antitumor action of tamoxifen in premenopausal women is unclear. Tamoxifen inhibits the estrogen-stimulated growth of MCF-7 tumors in athymic mice [7, 11]. However, clinical data published by some investigators [8, 13, 14, 18, 21, 22] as well as data we previously obtained using animal models [11] suggest that at low serum levels, tamoxifen may not act optimally in premenopausal patients because a tamoxifen-induced increase in estradiol may reverse the growth-inhibitory action of tamoxifen.

TAT-59 {(*E*)-4-[1-[4-[2-(dimethylamino)ethoxyl]-phenyl]-2-(4-isopropyl)phenyl-1-butenyl]-phenyl monophosphate} is a new triphenylethylene derivative. The precise mode of action of TAT-59 remains unclear; however, the drug has been more effective at lower doses than tamoxifen both in vivo and in vitro, even against 7,12-dimethylbenz[a]anthracene-induced tumors with low estrogen receptor (ER) content [24].

In the present report the antitumor activity of TAT-59 against MCF-7 tumors, circulating estradiol concentrations, and concentrations of DP-TAT-59 {(*Z*)-[1-[4-[2-(dimethylamino)-ethoxy]phenyl]-2-(4-isopropyl)phenyl-1-butenyl]-4-hydroxybenzene} and DM-DP-TAT-59 (desmethyl-DP-TAT-59) in the serum, tumors, and muscle of athymic mice are presented and the effect of the drug on hormone receptors and on insulin-like growth factor 1 (IGF-1) are examined. The significance of our findings is discussed in a clinical context.

Table 1. Concentrations of E_2, DP-TAT-59, and DM-DP-TAT-59 in serum, tumor, and muscle as measured in each experimental group at the 4th week after the start of treatment

Group	Serum			Tumor		Muscle	
	E_2 (pg/ml)	DP-TAT-59 (ng/ml)	DM-DP-TAT-59 (ng/ml)	DP-TAT-59 (ng/g tissue)	DM-DP-TAT-59 (ng/g tissue)	DP-TAT-59 (ng/g tissue)	DM-DP-TAT-59 (ng/g tissue)
E_2[a] alone ($n = 11$)	158.2±77.0	–	–	–	–	–	–
E_2[a]+TAT-59 1 mg/kg ($n = 7$)	101.4±56.0	14.8±6.4	2.3±2.9	236.5±88.1[b]	71.2±24.1[b]	145.9±86.7*13	45.2±30.7*15
E_2[a]+TAT-59 5 mg/kg ($n = 10$)	48.9±48.4*2	247.9±69.9*4	74.9±28.2*6	1,217.0±523.6[b,*8]	357.0±157.9[b,*10]	249.2±87.4*12,*13	86.3±28.4*14,*15
E_2[a]+TAT-59 20 mg/kg ($n = 10$)	38.9±37.2*1	613.4±267.1*4, *5	155.6±59.1*6,*7	14,579,8±9629.1[b,*8,*9]	4,629.0±2712.5[b,*10,*11]	3,076.1±1163.2	1,139.6±369.6
TAT-59 5 mg/kg alone ($n = 11$)	58.2±78.4*2	–	–	–	–	–	–
No treatment ($n = 8$)	33.8±46.2*1,*3	–	–	–	–	–	–

Data represent mean values ± SD
*1 Significantly different from E_2 alone, $P < 0.01$; *2 Significantly different from E_2 alone, $P < 0.05$; *3 Significantly different from E_2+TAT-59 1 mg/kg, $P < 0.05$; *4 Significantly different from E_2+TAT-59 1 mg/kg, $P < 0.01$; *5 Significantly different from E_2+TAT-59 5 mg/kg, $P < 0.05$; *6 Significantly different from E_2+TAT-59 1 mg/kg, $P < 0.01$; *7 Significantly different from E_2+TAT-59 5 mg/kg, $P < 0.05$; *8 Significantly different from E_2+TAT-59 1 mg/kg, $P < 0.01$; *9 Significantly different from E_2+TAT-59 5 mg/kg, $P < 0.01$; *10 Significantly different from E_2+TAT-59 1 mg/kg, $P < 0.01$; *11 Significantly different from E_2+TAT-59 5 mg/kg, $P < 0.01$; *12 Significantly different from E_2+TAT-59 1 mg/kg, $P < 0.05$; *13 Significantly different from E_2+TAT-59 20 mg/kg, $P < 0.01$; *14 Significantly different from E_2+TAT-59 1 mg/kg, $P < 0.05$; *15 Significantly different from E_2+TAT-59 20 mg/kg. $P < 0.01$
[a] 1.0-cm silastic capsule
[b] The DP-TAT-59 or DM-DP-TAT-59 concentration measured in the tumor was significantly higher than that determined in the serum in the TAT-59-treated group ($P < 0.01$), and almost the same relationship was seen between the tumor and the muscle ($P < 0.01$)

Materials and methods

Animals. Athymic mice [BALB/c(nu/nu)] were provided by the Institute of Experimental Animal Research, Gunma University. Sterilized water and diet (MF, Oriental Yeast Co., Ltd, Tokyo) were given ad libitum. Room temperature (24° C), humidity (40%), and 12 h of light were automatically controlled. In all, 2×10^6 cells of ER-positive MCF-7 tumors were inoculated into the bilateral thoracic mammary fat pads (1/side) of 6- to 7-week-old female athymic mice. All animals were also implanted subcutaneously with a 1.0-cm silastic capsule containing 17β-estradiol [11, 12] (described below) because tumors failed to grow in the absence of estrogen.

Tumors. Tumor size were obtained using the formula (length/2 × width/2) × π. After 5 weeks of estrogen treatment, tumors had reached an average size of 0.5 cm^2. Animals were then randomized into six groups and the estradiol capsules were removed. All animals in each group then received one of the following treatments: a 1.0-cm estradiol capsule (11 animals); a 1.0-cm estradiol capsule plus 1 (12 animals, 5 (13 animals), or 20 mg/kg TAT-59 (13 animals); 5 mg/kg TAT-59 (13 animals); or no treatment (12 animals). Each tumor size was recorded weekly as a percentage of the initial tumor size noted on day zero, and the mean values ± SE of percentage changes for each time point were calculated in each group. Tumors were measured for 4 weeks, and then all animals were killed and tissues were taken for determination of TAT-59 metabolites, for ER and progesterone receptor (PgR) assays, and for IGF-1 measurement. ER and PgR levels in the cytosol of MCF-7 tumors were determined by enzymeimmunoassay (EIA) using ER-EIA and PgR-EIA kits (Abbot Laboratories, Chicago, Ill.). IGF-1 levels in the MCF-7 tumors were measured using the supernatant of homogenized MCF-7 tumors and somatomedin C (Ciba Corning) kits.

Drug administration. Estradiol was delivered by subcutaneous implantation of a 1.0-cm silastic capsule [11, 12]. Silastic capsules were prepared by plugging one end of 1.0-cm-long pieces of medical grade silastic tubing (inside diameter, 0.078; outside diameter 0.125; Dow Corning, Midland, Mich.) with silastic silicone type A medical adhesive (Dow Corning) and then filling the tubing with 17-β-estradiol (Sigma Chemical Co., St. Louis, Mo.) mixed 1:3 (w/w) with silastic 382 medical grade elastomer (Dow Corning) without a catalyst [11]. Capsules were completed by filling the open end with adhesive and were then sterilized by irradiaton with ^{60}Co. TAT-59 (supplied by Taiho Pharmaceutical Co., Ltd.) was dissolved in 0.5% carboxymethylcellulose (CMC) containing 0.5% Tween-80 [24]. Each dose of TAT-59 was given orally every day for 4 consecutive weeks. The no treatment group was given 0.5% CMC containing 0.5% Tween-80.

Estradiol and TAT-59 metabolites. Blood samples were obtained by heart puncture using light ether anesthesia at autopsy. After clotting for 2–3 h, samples were centrifuged at 2,000 *g*; the serum was removed and stored at –80° C until analysis. Concentrations of circulating 17β-estradiol and metabolites of TAT-59 were measured by a modified procedure of the original method as previously described [1, 13, 16] in serum samples taken from tumor-bearing mice. Tumors or muscles were homogenized with 5 × cold methanol and samples were centrifuged at 3,000 rpm for 10 min. After centrifugation the supernatant was removed. TAT-59 metabolite (DP-TAT-59 and DM-DP-TAT-59) measurements in tissues were made using the modified procedure of the original high-performance liquid chromatography (HPLC) method described by Langan-Fahey et al. [16] or Brown et al. [1] for tamoxifen. Duplicate assays were performed for each determination.

Statistical analyses. Statistical analyses were performed using Duncan's multiple range test and the Wilcoxon test.

Results

Estradiol and TAT-59 metabolite concentrations in the serum and tissues of animals

Table 1 shows the concentrations of estradiol (E_2), DP-TAT-59, and DM-DP-TAT-59 in the serum, tumors, and muscles of nude mice in each group as determined at the 4th week after the start of treatment. Average circulating E_2 levels

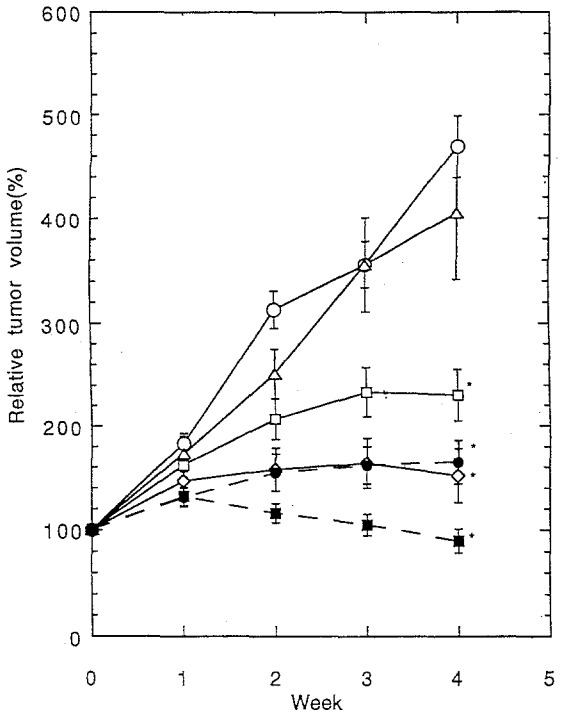

Fig. 1. Antitumor activity of TAT-59 against the E_2-stimulated growth of MCF-7 tumors in athymic mice. The tumor growth observed in the TAT-59 5 mg/kg alone, no treatment, E_2+TAT-59 20 mg/kg, or E_2+TAT-59 5 mg/kg group was significantly blocked as compared with that seen in the E_2 alone group. ○, Control (E_2 alone) group; △, E_2+TAT-59 1 mg/kg group; □, E_2+TAT-59 5 mg/kg group; ◇, E_2+TAT-59 20 mg/kg group; ●, no treatment group; ■, TAT-59 5 mg/kg alone group. E_2: 1.0-cm capsule. Values are mean ±SE. * $P < 0.01$ vs. Control (by Duncan's multiple range test)

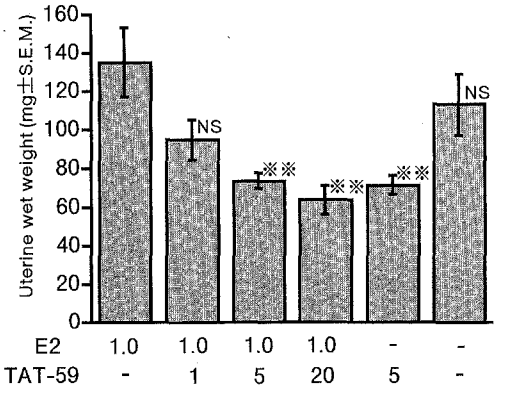

Fig. 2. Effect of TAT-59 on uterine weights of athymic mice in each group. The uterine wet weights determined in the E_2+TAT-59 5 mg/kg, E_2+TAT-59 20 mg/kg, and TAT-59 5 mg/kg alone groups were significantly lighter than those recorded in the E_2 alone group. E_2: 1.0-cm capsule. *NS*, Not significant; ** $P < 0.01$

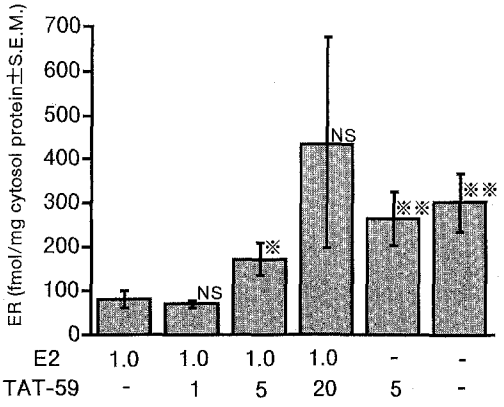

Fig. 3. ER levels measured in the MCF-7 tumors of each group. TAT-59 caused a dose-dependent increase in ER levels in the MCF-7 tumors, and the ER levels significantly increased in the E_2+TAT-59 5 mg/kg and the estrogen withdrawal groups as compared with the E_2 alone group. E_2: 1.0-cm capsule. *NS*, Not significant; * $P < 0.05$; ** $P < 0.01$

measured in the E_2 alone; E_2 plus 1, 5, or 20 mg/kg TAT-59; TAT-59 5 mg/kg alone; and no treatment groups were 158.2, 101.4, 48.9, 38.9, 58.2, and 33.8 pg/ml, respectively. The E_2 levels decreased in a TAT-59-dose-dependent manner. Average DP-TAT-59 and DM-DP-TAT-59 concentrations detected in the serum increased in a TAT-59-dose-dependent fashion, and those measured in the tissues also showed almost the same trend. DP-TAT-59 and DM-DP-TAT-59 concentrations determined in the tumors (target tissue) were significantly (more than 5 times) higher than those measured in the muscles (nontarget tissue) or serum of each treated group. DP-TAT-59 concentrations were about 3 times higher than DM-DP-TAT-59 concentrations in the serum, tumors, and muscles of each group.

Growth-inhibitory action of TAT-59 on breast tumors and uterine tissue

Figure 1 shows the growth-inhibitory action of TAT-59 on breast tumors in each group. MCF-7 tumors in the 1.0-cm E_2 capsule group grew continuously. There was no significant difference between the E_2 alone group and the E_2 + TAT-59 1 mg/kg group in terms of the percentage changes in tumor size; however, the E_2 + 5 or 20 mg/kg TAT-59 groups showed a significantly pronounced antitumor effect as compared with the E_2 alone group. TAT-59 showed an augmented antitumor effect dose-dependently on the E_2-stimulated growth of MCF-7 tumors. No significant difference was seen among the E_2 + TAT-59 20 mg/kg group, the no treatment group, and the TAT-59 alone group in terms of the percentage changes in tumor size. Uterine wet weights were also measured in all animals at the end of the experiment. TAT-59 reversibly controlled the uterine growth induced by estrogen (Fig. 2).

Effect of an increase in the dose of TAT-59 on the steroid receptor content and levels of insulin-like growth factor 1 in MCF-7 tumors

TAT-59 induced a dose-dependent increase in ER levels in the MCF-7 tumors (Fig. 3), whereas it blocked the E_2-induced increase in PgR levels in the tumors in a dose-dependent fashion (Fig. 4). IGF-1 levels measured in the tumors were lower in the TAT-59 alone ($P < 0.05$) and no treatment groups ($P < 0.01$) as compared with the E_2 alone group (Fig. 5).

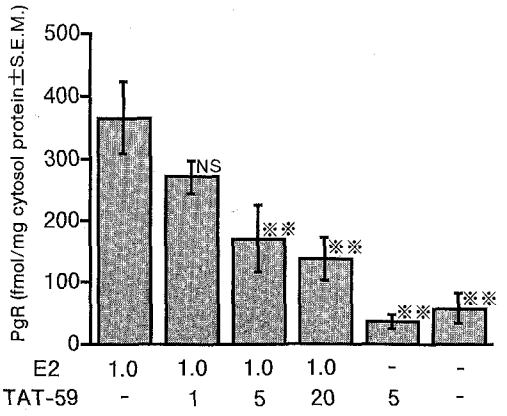

Fig. 4. PgR levels measured in the MCF-7 tumors of each group. TAT-59 blocked an estrogen-induced increase in PgR levels in a dose-dependent manner. Significant differences in PgR levels were seen in the E_2+TAT-59 5 mg/kg, E_2+TAT-59 20 mg/kg, and E_2 withdrawal groups as compared with the E_2 alone group. E_2: 1.0-cm capsule. *NS*, Not significant; ** $P < 0.01$

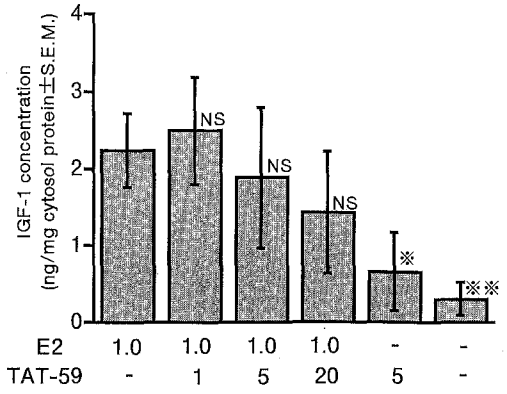

Fig. 5. IGF-1 concentrations measured in the MCF-7 tumors of each group. The IGF-1 concentrations determined in the E_2 withdrawal groups were significantly lower than those recorded in the E_2 alone group. E_2: 1.0-cm capsule. *NS*, Not significant; * $P < 0.05$; ** $P < 0.01$

Discussion

Phase I and phase II trials in Japan have shown the safety and efficacy of TAT-59, and a dose of 20 mg/day was demonstrated to be moderate among the doses tested (10, 20, or 40 mg/day) [23]. Toko et al. [24] reported that in a comparison of the growth-inhibitory effects of TAT-59 and tamoxifen against DMBA-induced rat mammary carcinoma or MCF-7 human mammary carcinoma transplanted into nude mice, TAT-59 was capable of suppressing tumor growth at a low dose, whereas a correspondingly low dose of tamoxifen had no inhibitory effect. In the present study, MCF-7 tumors in nude mice did not grow markedly in the no treatment group, and the tumors tended to regress in relation to the initial size measured on day zero in the TAT-59 alone group. In the above mentioned two groups, the average circulating E_2 levels were 34 and 58 pg/ml, respectively. These results demonstrated that TAT-59 was more effective against MCF-7 tumors under low E_2 conditions. Furthermore, TAT-59 inhibited the estrogen-stimulated growth of MCF-7 tumors in a dose-dependent manner. Average circulating E_2 levels were decreased by elevating doses of TAT-59. These results suggest that TAT-59 may control circulating E_2 levels in a dose-dependent fashion and, subsequently, the antitumor activity of TAT-59 against MCF-7 tumors may be operative under low E_2 conditions. Furthermore, much higher levels of DP-TAT-59 and DM-DP-TAT-59 were found in tumors (target tissues of estrogen) as compared with muscles (nontarget tissues of estrogen) or serum.

TAT-59 is rapidly metabolized to its active metabolites DP-TAT-59 and DM-DP-TAT-59 [23]. The antiestrogenic activity of DP-TAT-59 or DM-DP-TAT-59 is almost the same as or greater than that of TAT-59 [24]. The present findings suggest that the metabolites of TAT-59 may stay in the target cancer cells at higher concentrations and that they may act effectively against cancer cells through the ER, their direct actions, or by unclarified mechanisms. The mechanism of action of TAT-59 is not clearly understood. If TAT-59 can decrease circulating E_2 levels in premenopausal women, it may be an effective treatment for premenopausal patients with breast cancer as well as for postmenopausal patients. We do not know the precise reason why circulating E_2 levels decrease in a TAT-59-dose-dependent manner. In our previous studies, circulating E_2 levels in nude mice decreased in a dose-dependent fashion during tamoxifen [11] or toremifene treatment [12].

Our previous and present studies suggest that elevating the antiestrogen dose can control circulating E_2 levels. It is possible that our results may have been due to drug effects on E_2 metabolism or clearance. The radioimmunoassay was checked and it was found that neither TAT-59 nor its metabolites interfered with the measurement of E_2. It is generally accepted that long-term tamoxifen treatment induces ovarian steroidogenesis in premenopausal women. The data we previously obtained in nude mice showed that tamoxifen or toremifene administration decreased circulating E_2 levels dose-dependently. The above-mentioned observations suggest that elevating the dose of tamoxifen or toremifene is more effective for premenopausal women with breast cancer, even if the drugs induce ovarian steroidogenesis.

TAT-59 acted as a potent antagonist on the mouse uterus in the present experiments. The uterine growth induced by estrogen continued in the no treatment group after E_2 capsule removal because lower levels of circulating E_2 remained.

ER and PgR levels in tumors were determined using ER-EIA and PR (PgR)-EIA kits. Tamoxifen caused a dose-dependent increase in ER levels in MCF-7 tumors, and it also blocked the E_2-induced increase in PgR levels in a dose-dependent fashion in our previous study [11]. Although tamoxifen is known to increase levels of PgR in some target tissues in vivo and in tumor cells in vitro [2, 9, 10, 15], it prevented the estrogen-induced increase in PgR levels in MCF-7 tumors [11] in vivo. Welshons and Jordan [25] reported that estrogen withdrawal caused an increase in ER levels and a decrease in PgR levels in MCF-7 cells in vitro. In the present study TAT-59 showed almost the same trends noted in our previous study and in the report of Welshons and Jordan [25] in terms of ER and PgR levels.

The IGF system has been reviewed by Yee et al. [26]. Breast cancer cells produce only IGF-2, whereas both IGF-1 and IGF-2 may be produced by fibroblasts. Breast cancer cells express both type 1 and type 2 IGF receptors, and IGF-binding proteins produced by the breast cancer cell may influence the interaction between the ligand and the receptor. Serum IGF-1 levels were not determined in the present study because all sera were used for the assays of circulating E_2 and metabolites of TAT-59. TAT-59 significantly decreased the IGF-1 levels measured in the MCF-7 tumors in the absence of estrogen in this experiment. A possible mechanism of action of TAT-59 is through the IGF system. TAT-59 may decrease both tissue and serum IGF-1 levels that are stimulated by estrogen, thereby inhibiting the growth of MCF-7 cells. There is a high possibility that TAT-59 can act effectively against breast cancer cells irrespective of the patient's menopausal status. We hope that the data obtained in the present study will be helpful in clarifying the exact mechanism of action of TAT-59.

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