Novel multi-kinase inhibitor, T03 inhibits Taxol-resistant breast cancer

YAN LI, CHUNXIA LI, KE TANG, YAN CHEN, KANG TIAN, ZHIQIANG FENG and JINDONG CHEN

1Department of Pharmacology, State Key Laboratory of Bioactive Substances and Functions of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College; 2Department of Synthetic Medicinal Chemistry, Beijing Key Laboratory of Active Substance Discovery and Drugability Evaluation, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050; 3School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang, Liaoning 110016, P.R. China; 4Department of Urology, University of Rochester Medical Center, Rochester, NY 14642, USA

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Abstract. Activation of kinase-associated signaling pathways is one of the leading causes of various malignant phenotypes in breast tumors. Strategies of drug discovery and development have investigated approaches to target the inhibition of protein kinase signaling. In the current study, the anti-tumor activities of a novel multi-kinase inhibitor, T03 were evaluated in breast cancer. T03 inhibited Taxol-resistant breast cancer cell proliferation and induced cell cycle arrest and apoptosis in vitro and in vivo. The current results demonstrated that T03 downregulated c-Raf, platelet-derived growth factor receptor-β and other kinases, thus inhibited Raf/mitogen-activated protein kinase kinase/extracellular signal-regulated kinase and Akt/mechanistic target of rapamycin survival pathways in MCF-7 and MCF-7/Taxol xenograft tumors. At a dose of 100 mg/kg, T03 inhibited tumor growth by 62.90 and 59.98% in tumor weight in MX-1 and MX-1/T xenograft models, respectively and by 62.60 and 60.22% in MCF-7 and MCF-7/T tumors, respectively. These data indicate that the novel multi-kinase inhibitor, T03, may present as a potential compound to develop novel treatments against breast cancer and Taxol-resistant breast tumors.

Introduction

Breast cancer is the most common type of invasive cancer in women. Approximately 1.7 million women are diagnosed with breast cancer annually, and >500,000 succumb to it worldwide (1). While surgery, traditional chemotherapy and radiotherapy are commonly used to treat breast cancer; targeted therapy has drawn the attention of clinicians and researchers in the past decade for its improved therapeutic effect in metastatic cancer, as compared with traditional chemotherapy. Thus, numerous multi-kinase inhibitors have been adopted in the targeted therapy of metastatic breast cancer (2-4). However, the resistance of breast cancer to these inhibitors and drugs remains challenging in chemotherapy and targeted therapy. Therefore, continuing to develop novel anti-cancer drugs is necessary in cancer therapy.

In tumorigenesis of breast and other types of tissue, protein kinases are important in the regulation of proliferation, apoptosis and migration (5). For example, platelet-derived growth factor receptor-β (PDGFRβ), a member of the tyrosine kinase receptors type III family, is associated with the malignancy of breast carcinoma (6-8). In response to survival signals, PDGFRβ activates Akt by upregulating phosphoinositide 3-kinase (PI3K) and phosphoinositide-dependent protein kinase-1 (PDK1) (9). Thus, inhibition of PDGFRβ activity by TKI inhibitor(s) may suppress breast tumor growth (10).

In addition, the Ras/Raf/mitogen-activated protein kinases (MAPK) signaling pathway is critical in breast tumorigenesis (11). Studies have demonstrated that constitutive activation of the MAPK signaling pathway is associated with the progression of breast cancer via the induction of chemoresistance and distant metastases (12-16). Thus, the MAPK signaling pathway may be a potent target for breast cancer chemotherapy (17).

Currently, certain drugs, such as Trastuzumab, Lapatinib, Bevacizumab and Taxol have been identified for breast cancer targeted therapy. Of them, Taxol is the commonly administered drug for the treatment of breast cancer. However, continuous use of Taxol results in acquired drug-resistance of breast cancer (18). Therefore, development of novel drugs is essential to improve targeted therapy of breast cancer and Taxol-resistant breast cancer.

Correspondence to: Dr Yan Li, Department of Pharmacology, State Key Laboratory of Bioactive Substances and Functions of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, 1 Xian Nong Tan Street, Beijing 100050, P.R. China
E-mail: lyhzytt@163.com
Professor Jindong Chen, Department of Urology, University of Rochester Medical Center, 601 Elmwood Avenue Rochester, NY 14642, USA
E-mail: jindong_chen@hotmail.com

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breast cancer. In the current study, a novel multi-kinase inhibitor, T03 is reported. T03 is a novel multi-kinase inhibitor against PDGFRβ and c-Raf, and inhibition of PDGFRβ and c-Raf by T03 may downregulate the Raf/mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) and PDGFR/Akt/mechanistic target of rapamycin (mTOR) survival pathway. In the present study, the anti-tumor activity and underlying mechanism of T03 in regular and Taxol-resistant breast cancer was investigated in vitro and in vivo.

Materials and methods

Cell culture. Breast cancer cell line MCF-7 was obtained from the cell center of Chinese Academy of Medical Sciences (CAMS) and Peking Union Medical College (PUMC; Beijing, China). Cell lines MX-1 and MX-1/T (Taxol-resistant) A549, A549/T (Taxol-resistant) were obtained from the Professor Yongkui Jing (Mount Sinai School of Medicine, New York, NY, USA). The MCF-7/T (Taxol-resistant) cell was established in our laboratory (Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing China) (19). MCF-7/ADM (Adriamycin-resistant) was obtained from the Assistant Professor Hongbo Wang (Yantai University, Shandong, China). The cells were maintained in Dulbecco’s modified Eagle’s medium (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.), 100 IU/ml penicillin and 100 µg/ml streptomycin in a humidified incubator containing 5% CO₂ at 37°C. In the present study, four specific cells (MX-1/T, MCF-7/T, MCF-7/ADM, A549/T) were used.

Drugs. T03, a small molecule compound containing 2-picolinylhydrazide moiety (Chinese patent application no. 201110129115.7) was synthesized by the Department of Pharmacoochemistry at the Institute of Materia Medica, CAMS and PUMC (Purity >97%; high-performance liquid chromatography). For in vitro experiments, T03 and Taxol (Beijing Union Pharmaceutical Factory, Beijing, China) were dissolved in dimethyl sulfoxide (DMSO) and stored at 4°C until use. DMSO served as a vehicle control in all experiments at a final concentration of 0.1%. Cell viability assay. MX-1, MX-1/T, MCF-7 and MCF-7/T cells were trypsinized to single-cell suspensions, and resuspended in DMEM culture medium containing 10% FBS. Approximately 500 cells were plated in 6-well tissue culture plates. After a 24-h incubation at 37°C, the cells were treated with either T03, 0.1% DMSO, or nothing. Cells were incubated in 5% CO₂ at 37°C for 14 days, and the colonies were washed, fixed and stained with 0.005% crystal violet in methanol. The number of colonies was manually counted without a microscope, and experiments were performed in triplicate and repeated three times.

Apoptosis analysis. MX-1, MX-1/T, MCF-7 and MCF-7/T cells were treated with either T03 or 0.1% DMSO for 72 h. Apoptotic cells were measured using Annexin V-fluorescein isothiocyanate (FITC) Apoptosis Detection kit (cat. no. 556570; BD Biosciences, Franklin Lakes, NJ, USA). Briefly, cells were trypsinized and washed with PBS following treatment and stained with Annexin V-FITC according to the manufacturer's protocol, then analyzed by ACCURI C6 flow cytometry with BD Accuri C6 software (BD Biosciences).

Cell-cycle analysis. MX-1, MX-1/T, MCF-7 and MCF-7/T cells were incubated at 37°C with either T03 or 0.1% DMSO for 72 h. Cells were washed in PBS and fixed with 4°C ice-cold 70% ethanol overnight. The cells were then suspended in PBS containing RNase A (100 µg/ml; cat. no. R1030; Beijing Solarbio Science and Technology Co., Ltd., Beijing, China), propidium iodide (50 µg/ml; cat. no. P8080; Beijing Solarbio Science and Technology Co., Ltd.), Triton X-100 (0.1%), and incubated on the ice in the dark for at least 1 h (21). The cell cycle profiles were determined by flow cytometric analysis.

Tumor implantation and growth in MX-1, MX-1/T, MCF-7 and MCF-7/T xenografts. All animal studies were performed in compliance with the policies of the Institute of Materia Medica Animal Care and Use Committee. Six-week-old, female BALB/c/cu nude mice were used in the present study (all had 20 mice/experiment). The body weight was 15-16 g for MX-1 and MX-1/T xenograft model, and 16-22 g for MCF-7 and MCF-7/T xenograft model. They were purchased from Vital River Laboratory Animal Technology Co., Ltd., (Beijing, China) and housed in the controlled environment at 25°C on a 12-h light/dark cycle (5 mice per group). When tumors grew to an average volume of 100-250 mm³, tumor-bearing mice were randomly separated into four groups of five animals. A total of one group received per os Cremophor EL/ethanol/water and served as a vehicle control; the other groups received injections of 5 mg/kg Taxol (twice per week), or received an oral dose of 50 or 100 mg/kg T03 six times per week for 13 days (MX-1 and MX-1/T) and 33 days (MCF-7, MCF-7/T). Mice were euthanized at the end of the treatment period. Tumors were removed and weighed, and samples of all of the sections were stored at -80°C for western blot analysis.

Kinase inhibition assay. Inhibition of kinase activity against target kinases was measured using Caliper and Glo-ATP assays (ADP-Glo assay buffer: 25 mM HEPES, 10 mM MgCl₂, 0.01% Triton X-100, 100 µg/ml BSA, 2.5 mM DTT, (pH 7.4); Caliper assay buffer: 100 mM HEPES, 10 mM MgCl₂, 100 µl/l Brij35
(30%), 1 mM DTT, (pH 7.4); Other reagents: ATP (cat. no. A7699 Sigma-Aldrich; Merck KGaA, Darmstadt, Germany); ADP Gloreagent (cat. no. V9102; Promega Corporation, Madison, WI, USA). The Biochemical assay was performed according to the manufacturer's protocol. The assay was performed by HD Biosciences (China) Co., Ltd. (Shanghai, China).

Western blot analysis. Lysates (portions of two or three randomly selected tumors from MCF-7 and MCF-7/T xenograft mice) were prepared as previously described. The protein extraction buffer was a radioimmunoprecipitation buffer (1 mM phenylmethylsulfonyl fluoride) (21). Protein concentration was determined using the bicinchoninic acid method. Total proteins (50 µg) were separated by 10.0% SDS-PAGE and transferred to a nitrocellulose membrane by semi-wet electrophoresis were incubated with primary antibodies overnight at 4˚C following blocking with TBS containing 1% Tween-20 and 5% skimmed dry milk for 1 h at room temperature. The antibodies were as follows: Rabbit anti-phosphorylated (p)-c-Raf (Ser259; cat. no. 9421), c-Raf (cat. no. 9422), p-MEK (cat. no. 9127), MEK (cat. no. 9903), p-ERK (cat. no. 4370), ERK (cat. no. 9101), p-PDK (cat. no. 3061), PDK (cat. no. 3062), p-Akt (Thr 308; cat. no. 9275), Akt (cat. no. 4691), p-mTOR (cat. no. 2971), mTOR (cat. no. 2983), p-AuroraA (cat. no. 2914), AuroraA (cat. no. 14475) (all from Cell Signaling Technology, Inc., Danvers, MA, USA) and mouse anti-actin (cat. no. 3700; CST Biological Reagents Co., Ltd., Shanghai China). All the primary antibodies were used at 1:1,000. The samples were detected with peroxidase-conjugated anti-rabbit or anti-mouse IgG (1:5,000, cat. no. sc-2004, Santa Cruz Biotechnology, Inc., Dallas, TX, USA) for 1 h at room temperature and developed using an enhanced chemiluminescence system western blot detection and analysis system (Applygen Technologies, Inc., Beijing, China). The membranes were assessed for equal loading by probing for β-actin.

Statistical analysis. Data were expressed as means ± standard deviation. Statistical analysis of the results was performed
Results

**T03 inhibits the proliferation of breast cancer cells.** To evaluate the suppressive efficacy of T03 on breast cancer cells in vitro, the regular (MX-1, MCF-7) and Taxol-resistant (MX-1/T, MCF-7/T) breast cancer cells were treated with various concentrations of T03 (0.8–25.0 µmol/l) for 72 h. The CCK-8 assay results showed that T03 inhibited the growth of the breast cancer cells in dose-dependent manner (Fig. 1A). The T03 IC_{50} values were 8.46±0.28 (MX-1), 11.65±2.19 (MX-1/T), 6.39±1.15 (MCF-7), and 10.95±0.49 µmol/l (MCF-7/T; Table I). These results indicated that T03 effectively inhibited the growth of breast cancer cells, as well as the Taxol-resistant breast cancer cells. To establish whether T03 inhibits other drug-resistant cells, similar experiments on Doxorubicin-resistant breast cancer cells (MCF-7/ADM) were conducted. Similarly, T03 inhibited Adriamycin-resistant cells in a dose-dependent manner (Table II).

**T03 suppresses colony formation in breast cancer cells.** To confirm the suppressive ability of T03 on tumor cell proliferation, a colony formation assay was conducted in MCF-7 and MCF-7/T cells. The data revealed that T03 effectively inhibited the colony formation in MCF-7 and MCF-7/T colony cells in a dose-dependent manner (Fig. 1B and C). T03 inhibited colony formation by ~50.0% (IC_{50}) at a concentration of 7.61 µmol/l in MCF-7 and 7.45 µmol/l in MCF-7/T cells. The data were consistent with the results of the CCK-8 assay.
T03 treatment led to cell cycle arrest in breast cancer cells. T03 was observed to cause cell cycle arrest in breast cancer cells. T03 treatment led to elevated numbers of G2-phase cells and decreased G1-phase cells in a dose-dependent manner in MX-1 and MX-1/T cells (Fig. 2A and B). With a treatment of 5.0 µmol/l T03, the percentage of G1-phase cells increased to 15.23±0.83% (MX-1) and 29.30±1.37% (MX-1/T), while it was 11.37±0.90 and 11.47±1.26% in the untreated controls. Concomitantly, the percentage of G2 phase cells reduced to 60.37±1.01% (MX-1) and 40.60±1.06% (MX-1/T) compared with 68.10±1.65 and 56.33±0.86% in the untreated controls. Furthermore, while T03 treatment induced cell accumulation at the G2 and G0 phases, it caused the decrease of the S phase cells in MCF-7 and MCF-7/T (Fig. 2C and D). The percentage of G2 phase cells was 25.50±0.92 and 26.80±1.51% in MCF-7 and MCF-7/T cells, respectively, when they were treated with 5.0 µmol/l T03. By contrast, the percentages were 20.20±1.25% (MCF-7) and 21.73±1.66% (MCF-7/T) in the controls. The percentages of S phase cells decreased from 33.43±1.45 and 30.77±0.46% to 16.90±1.49 and 18.53±2.10% (following treatment with 5.0 µmol/l T03), respectively (Fig. 2C and D).

T03 induced apoptosis in breast cancer cells. T03 treatment was demonstrated to induce apoptosis in MX-1, MX-1/T, MCF-7, and MCF-7/T breast cancer cells. Upon treatment with 10.0 µmol/l T03, the early apoptosis increased by 4.16- and 2.90-fold, and late apoptosis increased by 67.25- and 3.23-fold in MX-1 and MX-1/T cells (Fig. 2E and F). In MCF-7 and MCF-7/T cells, early apoptosis increased from 1.28 and 4.51% (control) to 24.75 and 14.70%, respectively, while late apoptosis increased from 2.67 and 4.73% (control) to 24.05 and 18.00%, respectively (Fig. 2G and H).

T03 inhibited kinases in breast cancer cells. To investigate which kinases T03 inhibited, Caliper and ADP-Glo assays were performed on a panel of kinases in T03-treated breast cancer cells. The results indicated that various oncogetic kinases were susceptible to T03 inhibition. Of these kinases, c-Raf, PDGFRβ and RET proto-oncogene may be suppressed by T03, and the IC50s were 0.78, 0.23 and 0.71 µmol/l, respectively. In addition, T03 may inhibit the activity of PDGFRα, fms related tyrosine kinase 1 (FLT1), kinase insert domain receptor, FLT3, and c-kit with IC50 between 0.05 and 1.0 µmol/l. Furthermore, it was found that T03 downregulates FGFR1, FGFR2 and b-Raf at the micromole level. By contrast, T03 exerted little effect on other tested kinases, such as Aurora A, insulin like growth factor 1 receptor, ERK1, ERK2, Src, PI3K, erb-b2 receptor tyrosine kinase 2 and AMPK (Table III).

T03 inhibited xenograft tumor growth of breast cancer cells. Based on the above results obtained in vitro, further experiments were performed to determine whether T03 inhibits xenograft tumor growth from the breast cancer cells. To obtain xenograft tumors, the MX-1, MX-1/T, MCF-7, and MCF-7/T human breast cells were inoculated into BALB/c nu/nude mice. While T03 was used to treat the xenograft tumors, Taxol was adopted as a reference compound. When 5 mg/kg Taxol was applied to MX-1 and MX-1/T xenografts, the treated/control (T/C) ratio were 55.56 and 95.13%, respectively, according to the relative tumor volume (RTV).

Furthermore, the inhibition ratios were 41.56% in MX-1 and 0% in MX-1/T based on the relative tumor weight, indicating that MX-1/T was less sensitive to Taxol at a dose of 5 mg/kg. For T03, the T/C ratio was 46.99% (50 mg/kg) and 34.68% (100 mg/kg) according to RTV, and the inhibition ratio of tumor weight reached 50.00 and 62.90% in MX-1 xenografts (Table IV and Fig. 3A). Furthermore, in MX-1/T xenografts, the T/C ratio of RTV was 45.06% (50 mg/kg) and 31.47% (100 mg/kg), and the inhibition ratio of the tumor weight was 51.02 and 59.98%, respectively (Table V and Fig. 3B).
Table IV. Antitumor activity of T03 on the breast cancer MX-1 xenograft model.

| Compound | Dose, mg/kg | Animals, n | Body weight, g  | Relative tumor volume | Tumor weight |
|----------|-------------|------------|-----------------|-----------------------|--------------|
|          |             |            | Initial | Final | % | Initial | Final | % | Initial | Final | % | Initial | Final | % |
| Control  | 5/5         | 16.0±1.0   | 21.8±1.9 | 22.09±5.12 | 1.61±0.45 |
| Taxol    | 5/5         | 16.0±0.7   | 20.8±2.2 | 12.27±2.33 | 0.94±0.20  |
| T03      | 50/50       | 15.0±1.0   | 19.2±1.3 | 10.38±5.48 | 0.81±0.49  |
|          | 100/50      | 15.2±0.5   | 18.9±1.4 | 7.66±0.48  | 0.60±0.03  |

*P<0.05, *P<0.01 and *P<0.001 vs. control. Means ± standard deviation.

Table V. Antitumor activity of T03 on the breast cancer MX-1/T xenograft model.

| Compound | Dose, mg/kg | Animals, n | Body weight, g  | Relative tumor volume | Tumor weight |
|----------|-------------|------------|-----------------|-----------------------|--------------|
|          |             |            | Initial | Final | % | Initial | Final | % | Initial | Final | % | Initial | Final | % |
| Control  | 5/5         | 16.0±1.0   | 20.8±2.9 | 23.87±10.22 | 1.57±0.47 |
| Taxol    | 5/5         | 15.6±0.6   | 20.4±0.9 | 22.71±11.09 | 1.58±0.79  |
| T03      | 50/50       | 15.2±0.5   | 19.0±2.5 | 10.76±8.66  | 0.77±0.62  |
|          | 100/50      | 15.8±0.8   | 18.8±2.2 | 7.51±0.86  | 0.63±0.06  |

*P<0.05 and *P<0.01 vs. control. Means ± standard deviation.
indicating that T03 exerted more effective inhibition on the
MX-1 and MX-1/T xenografts.
In addition, it was observed that T03 presented even
higher inhibitory ability to MCF-7 and MCF-7/T xenografts
compared with MX-1 or MX-1/T models. While Taxol was
applied to the MCF-7 xenograft, the T/C ratio of the RTV was
6.75% (5 mg/kg) and the inhibition rate of the tumor weight
was 96.36%. By contrast, Taxol exerted almost no inhibi-
tory effect on the MCF-7/T xenograft model. In contrast to
this, when T03 was applied to MCF-7 xenografts, the RTV
T/C ratio was 45.63% (50 mg/kg) and 32.55% (100 mg/kg),
and the inhibition rate of the tumor weight reached 57.09 and
62.60% (*Table VI and *Fig. 3C). Furthermore, in MCF-7/T
xenografts, the RTV T/C ratios were 41.21% (50 mg/kg) and
25.52% (100 mg/kg) while the inhibition rate of the tumor
weight attained 44.06 and 60.22%, respectively (*Table VII
and *Fig. 3D). These data indicated that T03 inhibits Taxol-sensitive
and -resistant breast cancer tumors.
T03 downregulated the Raf/MEK/ERK and PDGFRβ/Akt
signaling pathway in MCF-7 and MCF-7/T xenograft nude
model mice. Subsequently, whether T03 inactivated the
above-mentioned kinase-associated signaling pathways was
evaluated. As expected, the phosphorylated levels of PDGFRβ
decreased in T03-treated tumors of MCF-7 and MCF-7/T
xenografts (Fig. 4A and B). As PDK is an important down-
stream target of PDGFRβ/PI3K signaling and a key upstream
kinase of the AKT/mTOR signaling pathway, its activation
promotes proliferation and inhibits apoptosis in numerous
human cancer types (9,22,23). The current study observed that
the activity of PDK, AKT, and mTOR decreased in MCF-7
and MCF-7/T treated with T03 compared with the controls
(Fig. 4A and B), indicating that T03 may downregulate
PDGFRβ and PDK/AKT/mTOR and in MCF-7 and MCF-7/T
xenograft models.
As T03 inhibited c-Raf in the current study, whether
T03 downregulates the Ras/Raf/ERK signaling pathway in
MCF-7 and MCF-7/T xenografts was examined. Fig. 4C
and D demonstrate that T03 treatment significantly and
dose-dependently decreased p-c-Raf, p-MEK, and p-ERK in
MCF-7 and MCF-7/T tumors, while the basal levels of c-Raf,
MEK and ERK were only reduced in the group treated with
100 mg/kg T03.
Furthermore, T03 downregulated Aurora A, a downstream
effector of the Ras/Raf/MEK/ERK signaling pathway (24), in
MCF-7 and MCF-7/T xenografts. Aurora A and p-Aurora A
were markedly downregulated upon T03 treatment, particu-
larly in MCF-7 tumors, which were consistent with the previous
study (24) that Aurora A and p-Aurora A were frequently
activated by the Ras/Raf signaling pathway (Fig. 4E and F).

**Discussion**

Despite improvements in prevention, early detection, and
treatment, breast cancer remains one of the most common
malignant tumors affecting women in western countries (25).
Drug-resistance of breast cancer to Taxol has limited its effect
and application in clinical treatment. In the present study, the
anti-tumor activity of the novel multi-kinases inhibitor, T03 was
investigated, as well as its potential in breast cancer treatment.
Table VI. Antitumor activity of compounds on the breast cancer MCF-7 xenograft model.

| Compound | Dose, mg/kg | Animals, n | Initial | Final | Initial | Final | Relative tumor volume treated/ | Tumor weight |
|----------|-------------|-------------|---------|-------|---------|-------|-----------------------------|-------------|
| Control  | 5/5         | 21.8±0.8    | 26.0±1.2| 237.5±32.6 | 1,163.8±503.2 | 5.06±2.46 | 1.02±0.46 |
| Taxol    | 5           | 21.0±1.6    | 22.8±2.3| 227.4±74.6  | 68.3±32.5  | 0.34±0.22  | 6.75       |
| T03      | 50          | 20.6±1.1    | 26.0±4.6| 230.6±65.3  | 521.2±658.1| 2.31±2.50 | 45.63     |
|          | 100         | 21.0±2.2    | 23.2±3.1| 240.9±38.6  | 419.3±413.6| 1.65±1.55 | 32.55     |

Table VII. Antitumor activity of compounds on the breast cancer MCF-7/T xenograft model.

| Compound | Dose, mg/kg | Animals, n | Initial | Final | Initial | Final | Relative tumor volume treated/ | Tumor weight |
|----------|-------------|-------------|---------|-------|---------|-------|-----------------------------|-------------|
| Control  | 5/5         | 18.0±1.4    | 18.6±2.4| 112.0±5.8 | 1,373.5±363.1 | 12.19±2.78 | 1.26±0.24 |
| Taxol    | 5           | 16.8±1.9    | 17.5±2.1| 132.5±45.3 | 1,871.0±981.5 | 12.59±3.17 | 1.36±0.48 |
| T03      | 50          | 17.4±1.2    | 17.4±2.5| 142.4±38.7 | 621.2±243.3  | 5.02±2.11  | 41.21     |
|          | 100         | 17.3±1.1    | 15.6±2.7| 148.1±37.1 | 442.6±266.0  | 3.11±2.19  | 25.52     |

*P<0.01 and *P<0.05 vs. control. Means ± standard deviation.
As with Taxol, T03 displayed similar antitumor effects on MX-1 and MCF-7 breast cancer cells. Furthermore, T03 inhibited the growth of Taxol-resistant MX-1/T and MCF-7/T breast tumors in vitro and in vivo. It caused G2/M-phase cell accumulation and induced apoptosis, thus resulting in cell growth inhibition. In addition, T03 resulted in tumor regression in MX-1- and MCF-7-derived xenografts, as well as in Taxol-resistant MX-1/T and MCF-7/T tumors, indicating that T03 may exert effects on Taxol-sensitive and -resistant breast cancer cells. In addition, T03 was demonstrated to exert efficient effects on other types of drug-resistant breast cancer cells, such as Doxorubicin (Adriamycin)-resistant cells, which indicated that T03 may be used for treatment of other types of drug-resistant breast cancer.

Previous studies revealed that PDGFRβ was overexpressed in breast cancer (26,27). Highly activated PDGFRβ promoted tumor cell proliferation via PI3K/Akt and Ras/MEK/ERK signaling pathways (28,29), and resulted in distant metastasis and insensitivity to chemotherapy (30). The current study demonstrated that T03 may downregulate the Akt/mTOR and Ras/MEK/ERK signaling pathways, as well as PDGFRβ in MCF-7 and MCF-7/T tumors in vivo. In MCF-7 and MCF-7/T xenograft tumors, T03 significantly reduced p-PDGFRβ, which was further confirmed by performance of the biochemical assay. Furthermore, T03 downregulated PDK, AKT and mTOR. As PDK-1, Akt and mTOR are the downstream components of PDGFRβ, and are involved in cell growth and apoptosis, T03 may cause cell growth inhibition and apoptosis via downregulation of the PDGFRβ/Akt signaling pathway. PDGFRβ activation is known to induce Taxol-resistance in breast cancer (31,32). Therefore, T03 may be used for inhibiting Taxol-resistant breast cancer.

Raf kinase is an upstream member of the Raf/MEK/ERK signaling cascade (33). Activation of the Raf/MEK/ERK signaling pathway has been associated with chemoresistance of breast cancer (34-36). The present data indicated that T03 inhibited p-c-Raf, and consequently resulted in downregulation of p-MEK and p-ERK. These results indicate that T03 may inhibit the cell cycle and induce apoptosis via downregulation of the Raf/MEK/ERK signaling pathway in MCF-7 and MCF-7/T breast cancer.

Previous studies observed that activation of c-Raf signaling led to stabilization and accumulation of Aurora A mitotic kinase in breast cancer cells, and deduced that c-Raf may regulate the expression levels of Aurora A (24,37). The current study found that T03 treatment led to inhibition of c-Raf and decreased Aurora A in MCF-7 and MCF-7/T xenografts.

Previous studies have demonstrated that over-expressed Aurora A inhibits apoptosis, promotes cell cycle progression.
and metastasis, and mediates Taxol-resistance in breast cancer and other types of cancer (38,39). Inhibition of Aurora A by T03 may cause G2/M cell accumulation, apoptosis and sensitivity to Taxol in breast cancer. Based on the current results, the therapeutic efficacy of T03 on breast cancer may be partially attributed to the inhibition of c-Raf, PDGFRβ and the associated signaling pathways (40,41).

Although T03 presented similar anti-tumor activity in Taxol-sensitive and -resistant breast cancer, and Doxorubicin-resistant breast cancer as well, there are certain efficacy differences, which merit further investigation. In addition, based on our existing data, whether the anti-tumor effects are transient or permanent could not be determined.

In conclusion, the current study demonstrated that T03 induces cell cycle arrest and apoptosis, and inhibits cell proliferation in MX-1, MCF-7, MX-1/T, and MCF-7/T breast cancer in vitro and in vivo. These results demonstrate that T03 inhibits breast cancer growth by downregulating PDGFRβ/Akt and Ras/Raf/ERK signaling pathways, which are regulators of apoptosis, proliferation and chemoresistance. These findings indicate that T03 may be a potential candidate for effective chemotherapy of breast cancer, particularly for Taxol-resistant breast cancer.

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