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Derivatives of benzothiadiazole-7-carboxylates: synthesis and biological activity

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Abstract Salicylic acid (*SA*) and methyl jasmonate (*MJ*) are important plant signal molecules to cause systemic acquired resistance (*SAR*), while it's reported that they also have wide spectrum antitumor activities. Benzothiadiazole-7-carboxylates are plant activators which can cause *SAR* just like *SA* and *MJ*. To investigate whether the benzothiadiazole-7-carboxylate family is endowed with anticancer activities, several benzothiadiazole-7-carboxylate derivatives are synthesized and their inhibition to P388 murine leukemia cell and A549 human lung cancer cell compared with *MJ* are evaluated. The data indicated that benzo-1,2,3-thiadiazole-7-carboxylic acid 2-benzoyloxyethyl ester has a higher inhibition ability to the cancer cell P388 and A549, compared with *MJ*.

Keywords Jasmonate; Benzothiadiazole-7-carboxylates; Hydrogen peroxide; Biological activity.

Introduction

Plant stress hormones are defined as a group of naturally occurring organic substances, which influence physiological processes at low concentrations. Salicylic acid (*SA*), a kind of plant hormone, is a key compound that regulates resistance of fungal, bacterial, and viral pathogens and provides a signal for expression of pathogenesis-related proteins and other potentially protective factors induced following pathogen challenge [1]. Jasmonic acid (*JA*) and methyl jasmonate (*MJ*), also a kind of plant hormone and produced by the octadecanoid pathway *via* lipoxygenation of linolenic acid, serve as a signal for expression of a number of proteins inducing polyphenoloxidase and proteinase inhibitors [2–4]. *SA* and *MJ* are well known important plant signal molecules which can cause systemic acquired resistance (*SAR*) to pathogens and injury and they can elicit secondary metabolism in *Taxus* cell cultures [5–8]. *S*-Methyl benzo-1,2,3-thiadiazole-7-carboxylate (*BTH*), the potent synthetic salicylate mimic [9–11], has been invented by Ciba-Geigy Co. As the first commercial *SAR* activator for plants. It is noticed that *BTH* could induce the same set of defense response as salicylic acid in signal transduction. Recently, we have reported that the synthetic benzo-1,2,3-thiadiazole-7-carboxylates could be used for eliciting taxoid biosynthesis and resulted in nearly 40% increase in taxuyunnanine C content and production in comparison with methyl jasmonate, which was previously reported as the most powerful chemical elicitor for taxoid biosynthesis [12] Scheme 1.

SA has been reported to be able to induce intracellular biochemical events typical of a stress response in mammalian cells as well and induce apoptosis (programmed cell death) in human myeloid leuke-

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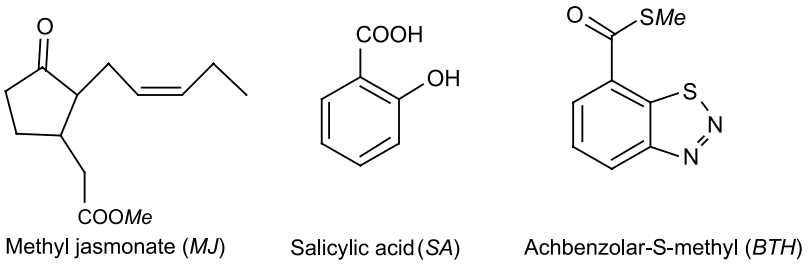
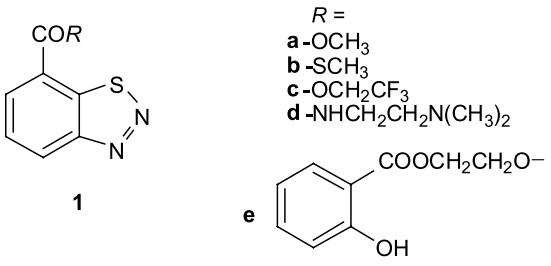


Scheme 1



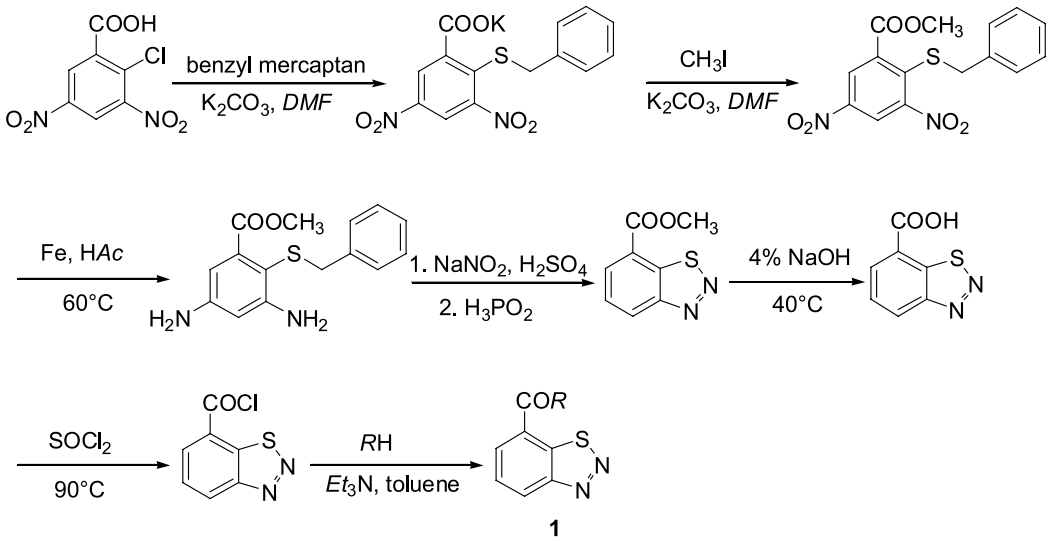
Scheme 2

mia, FS-4 fibroblasts and pancreatic cancer [13]. Recently, *Flescher et al.* reported that jasmonates induce suppression of cellular proliferation and death in various human and mouse cancer lines, including breast, prostate, melanoma, lymphoblastic leukemia, and lymphoma cells [14–19]. As the mimic of *SA*, *BTH* has some similar biological activities in plant cells in comparison with *MJ* and there are some similarities between jasmonate actions in plants cells, we hypothesized that *BTH* and its derivatives may be effective to some mammalian cancer cells. To verify this inference, several benzothiadiazole-7-carboxylate derivatives (Scheme 2) were synthesized and their cytotoxicity to P388 murine leukemia cell and A549 human lung cancer cell are evaluated.

Results and Discussion

Benzothiadiazole-7-carboxylate derivatives are prepared from 2-chlorobenzonic acid by nitration, substitution of Cl by benzyl mercaptan, then reduction and diazotization and cyclization [20]. The series of compounds are then prepared from benzothiadiazole-7-carboxylic acid chloride and the corresponding alcohol or amine (Scheme 3) [10] and purified by recrystallization and silica gel chromatography. Their structures were confirmed with ^{1}HNMR, HR-MS, and elemental analyses.

Compound **1b** was prepared by acylation of benzothiadiazole. However, esterification of benzothia-



Scheme 3

diazole-7-carboxylic acid with trifluoroethanol did not work in the preparation of **1c** under the same conditions because of the acidity of trifluoroethanol, so condensation of benzothiadiazole-7-carboxylic acid chloride and trifluoroethanol in the presence of triethylamine was adopted. In the process of preparing **1e**, the protection of hydroxyl group with acetic anhydride was not required. Because of steric factors and intramolecular hydrogen bond, the free phenolic hydroxyl group would not participate in the reaction as long as no excess acid chloride was added.

The *in vitro* antitumor activities of these compounds were evaluated by *MTT* tetrazolium dye assay against P388 (murine leukemia cell) (Table 1) and Sulforhodamine B (*SRB*) assay against A549 (human lung cancer cell) (Table 2).

As shown in Tables 1 and 2, compounds **1a**–**1e** show some ability for cancer cell inhibition, especially **1e** has a higher inhibition ability than *MJ*. Actually, **1e** was found to induce plant defense responses as effectively as *MJ*, and it could be used for eliciting taxoid biosynthesis and resulted in nearly 40% increase in taxuyunnanine C content and production in comparison with *MJ* [12].

It is well-known that jasmonates are plant lipid derivatives, similar to mammalian eicosanoid, that play a critical role in plant defenses against herbivores and pathogens through up-regulating the expression of defense-related genes. Recently, jasmonates were shown to induce cell cycle arrest or apoptosis in human leukemia, prostate and breast cancer cells, but not in normal lymphocytes. *Kim et al.* reported that *MJ* induces apoptosis through induction of Bax/Bcl-XS and activation of caspase-3 *via* hydrogen peroxide (H_2O_2) generation in A549 human lung adenocarcinoma cells [21]. *Flescher et al.* even thought that jasmonates can induce similar metabolic, signaling, and stress-associated modifications (that could lead to decreased growth and viability) in plant cells as well as in cancerous animal cells [19]. In order to find some biological evidences for the above results, *MJ* and **1e** were selected to demonstrate as typical examples the elicitor-induced plant defense responses, while the cultures with non-elicitor addition was used as controls (Fig. 1).

Table 1 Inhibition ratio to P388 %

Compound	$10^{-4}M$	10^{-5} *M*	10^{-6} *M*	10^{-7} *M*	10^{-8} *M*
MJ	8.6	5.7	14.4	10.2	8.6
1a	67.0	0	0	0	0
1b	44.9	9.3	9.9	0	0
1c	54.0	11.4	6.9	1.0	0
1d	44.5	0	0	0	0
1e	85.8	14.2	11.4	8.0	9.2

Table 2 Inhibition ratio to A549 %

Compound	10^{-4} *M*	10^{-5} *M*	10^{-6} *M*	10^{-7} *M*	10^{-8} *M*
MJ	23.9	11.9	28.1	28.8	19.0
1a	8.9	0	29.9	20.9	7.0
1b	37.8	0	23.7	19.1	6.3
1c	28.3	0	22.2	17.5	6.9
1d	34.9	0	0	0	0
1e	41.0	13.6	15.5	29.9	22.2

Figure 1 shows that an increased level of H_2O_2 production was observed in *MJ*- and **1e**-elicited *T. chinensis* suspension cultures. It can be seen that the time course of H_2O_2 production of **1e**-elicited cells was almost the same as that of *MJ*-treated cells and in the first hour the H_2O_2 production of **1e**-elicited cells was higher than that of *MJ*-treated cells, which is in accordance with the cancer cell inhibition ability shown in Tables 1 and 2.

To the best of our knowledge, benzothiadiazole-7-carboxylates were never studied as antitumor agents. The mechanism of plant activator is to activate the

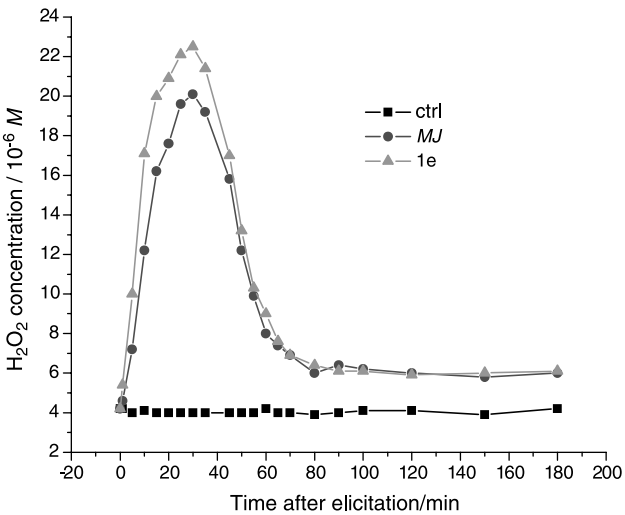


Fig. 1 Methyl jasmonate (*MJ*) and **1e** elicitor-induced H_2O_2 production in *T. chinensis* suspension cultures. *MJ* or **1e** (at 100 μM) was added to the cultures in 1 mm^3 ethanol per 1 cm^3 culture medium on day 7 of cultivation and elicited cell cultures were sampled at certain intervals for H_2O_2 analysis. Data are the means of three flasks and vertical bars show standard deviations.

plants' own immune system which is called *SAR*, they do not have direct antimicrobial activity, and work by inducing resistance to the same spectrum of pathogens and lead to expression of the same biochemical markers, such as the PR-proteins in the plant [22]. They usually have lower cytotoxicity, and the same function mechanism as *SA* and *MJ* to some extent. So, as one kind of unnatural plant stress hormones, benzothiadiazole-7-carboxylates may be further evaluated as novel anticancer agents, and it is much easier to synthesize these compounds than to synthesize methyl jasmonate derivatives [23–27].

Experimental

Triethylamine was dried over KOH and distilled. Methyl jasmonate was purchased from Tokyo Kasei Kogyo Co., Ltd (Tokyo, Japan). Trifluoroethanol was purchased from Fluka Chem. Co. (Switzerland). All other solvents and chemicals were reagent grade and used without further purification. Melting points were recorded by an electrothermal digital apparatus. The ^{1}HNMR spectra were recorded with a Brucker AM-500 spectrometer. The MS spectra were measured with a HR-MS Micromass GCT CA 055 spectrometer. Elemental analyses (C, H, and N) were conducted using the Elemental Vario EL III Analyzer, their results were found to be in good agreement (±0.3%) with the calculated values. Compound **1a** (methyl benzo-1,2,3-thiadiazole-7-carboxylate), benzo-1,2,3-thiadiazole-7-carboxylic acid, and benzo-1,2,3-thiadiazole-7-carboxylic acid chloride were prepared according to Ref. [19].

H_2O_2 produced by the cells and released into the medium was determined by the scopoletin fluorescence oxidative quenching method (excitation wavelength: 350 nm, emission: 460 nm) according to Ref. [28]. To measure H_2O_2 accumulation, samples were taken at various intervals over the 180-min period following elicitation. Aliquots of 4 cm^3 extracellular medium were mixed with 40 mm^3 5 m*M* stock solution of scopoletin in *DMSO* and 40 mm^3 1 mg/cm^3 stock solution of peroxidase (Sino-American Biotechnology Co., Shanghai). The concentration of H_2O_2 in the medium was calculated from the fluorescence decrease using a calibration curve established in the presence of H_2O_2. A standard curve by adding scopoletin to the solutions at different H_2O_2 concentrations was prepared by using cell-free medium. Effects of various jasmonate elicitors on peroxidase-dependent assay for H_2O_2 determination were tested. Various jasmonates were added to cell-free medium to obtain final concentrations of 10, 50, or 100 μ*M*. Under the assay conditions, an addition of jasmonate elicitors had no obvious effect on the decrease of scopoletin fluorescence because of H_2O_2 addition.

S-Methyl benzo-1,2,3-thiadiazole-7-carboxylate (**1b**, $C_8H_6N_2OS_2$)
White solid; mp 134–135°C; ^{1}HNMR (500 MHz, *DMSO-d*$_6$): $\delta = 2.59$ (s, SCH_3), 7.98 (dd, 1H, $J = 7.26$, 7.89 Hz, 5-*Ar*-H), 8.60 (d, $J = 7.26$ Hz, 4-*Ar*-H), 9.08 (d, $J = 7.88$ Hz, 6-*Ar*-H) ppm; IR (KBr): $\bar{\nu} = 3000$, 1625, 1540, 1470, 1398, 1287, 1071, 908, 780, 730 cm^{-1}; HRMS (EI): m/z (%) = 209.9920 [M^+], $C_8H_6N_2OS_2$ requires 209.9922.

2,2,2-Trifluoroethyl benzo-1,2,3-thiadiazole-7-carboxylate (**1c**, $C_9H_5F_3N_2O_2S$)
Light yellow crystals; mp 119–121°C; ^{1}HNMR (500 MHz, *DMSO-d*$_6$): $\delta = 5.16$ (q, $J = 9.0$ Hz, CH_2CF_3), 7.98 (dd, $J =$ 8.35, 7.36 Hz, 5-*Ar*-H), 8.49 (d, $J = 7.37$ Hz, 4-*Ar*-H), 9.10 (d, $J = 8.35$ Hz, 6-*Ar*-H) ppm; IR (KBr): $\bar{\nu} = 3074$, 2978, 1718, 1558, 1410, 1305, 1180, 1135, 1050, 980, 820, 753 cm^{-1}; HRMS (EI): m/z (%) = 262.0026 [M^+], $C_9H_5F_3N_2O_2S$ requires 262.0024.

N-(2-(Dimethylamino)ethyl)benzo-1,2,3-thiadiazole-7-carboxamide (**1d**, $C_{11}H_{14}N_4OS$)
White solid; mp 172–174°C; ^{1}HNMR (500 MHz, *DMSO-d*$_6$): $\delta = 2.18$ (s, $N(CH_3)_2$), 2.46(t, $J = 6.80$ Hz, CH_2), 3.47 (dt, $J_1 = 6.79$, $J_2 = 5.37$ Hz, CH_2), 7.92(dd, $J_1 = 8.21$ Hz, $J_2 =$ 7.36 Hz, *Ar*-H-5), 8.47(d, $J = 7.35$ Hz, *Ar*-H-4), 8.89(d, $J =$ 8.21 Hz, *Ar*-H-6), 9.14(t, NH, $J = 5.37$ Hz) ppm; IR (KBr): $\bar{\nu} = 3563$(NH), 3259, 2948, 1636, 1540, 1465, 1313, 1170, 1050, 940, 770 cm^{-1}; HRMS (EI): m/z (%) = 250.0884 [M^+] $C_{11}H_{14}N_4OS$ requires 250.0888.

Benzo-1,2,3-thiadiazole-7-carboxylic acid 2-benzoyloxyethyl ester (**1e**, $C_{16}H_{12}N_2O_5S$)
White crystals; mp 92–94°C; ^{1}HNMR (500 MHz, *DMSO-d*$_6$): $\delta = 4.71$–4.80 (m, OCH_2-CH_2O), 6.88–6.91 (m, *Ar*-H), 6.95–6.97 (m, *Ar*-H), 7.48–7.51 (m, *Ar*-H), 7.77–7.80 (m, *Ar*-H), 7.94 (dd, $J = 7.34$ and 8.34 Hz, 5-*Ar*-H), 8.45 (d, $J = 7.33$ Hz, 4-*Ar*-H), 9.04 (d, $J = 8.34$ Hz, 6-*Ar*-H), 10.38 (s, OH) ppm; IR (KBr): $\bar{\nu} = 3280$ (OH), 3059, 2963, 1722, 1666, 1606, 1580, 1480, 1400, 1290, 1250, 1161, 1080, 860, 756, 700 cm^{-1}; HRMS (EI): m/z (%) = 344.0451 [M^+], $C_{16}H_{12}N_2O_5S$ requires 344.0467.

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