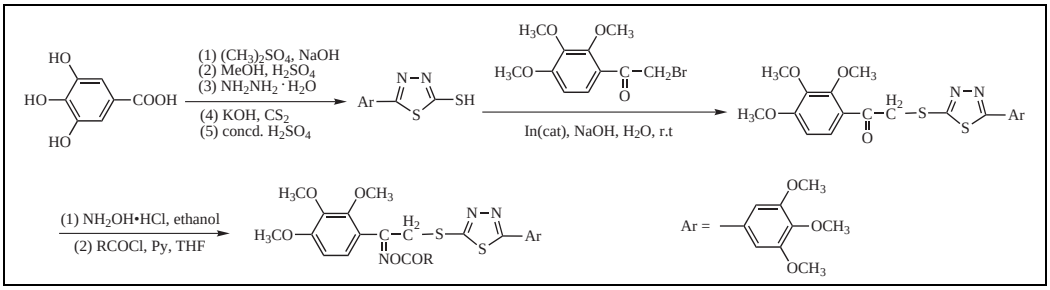
Wei Xue, Baoan Song*, Wei He, Hua Wang, Song Yang, Linhong Jin, Deyu Hu, Gang Liu, and Ping Lu

Key Laboratory of Green Pesticide and Agricultural Bioengineering, Ministry of Education, Center for Research and Development of Fine Chemicals, Guizhou University, Guiyang, P. R. China, 550025

songbaoan22@yahoo.com

Received August 8, 2005



In a search for new fungicide and anticancer agent with improved biological properties and different bioactivity spectrum, we designed and synthesized a series of novel oxime esters containing 1,3,4-thiadiazole group in moderate yield starting from gallic acid. The title compounds were identified by IR, ^{1}H NMR, ^{13}C NMR and elemental analysis. The bioassay tests showed that these title compounds exhibit weak to moderate anticancer activity *in vitro* by MTT method.

J. Heterocyclic Chem., **43**, 867 (2006).

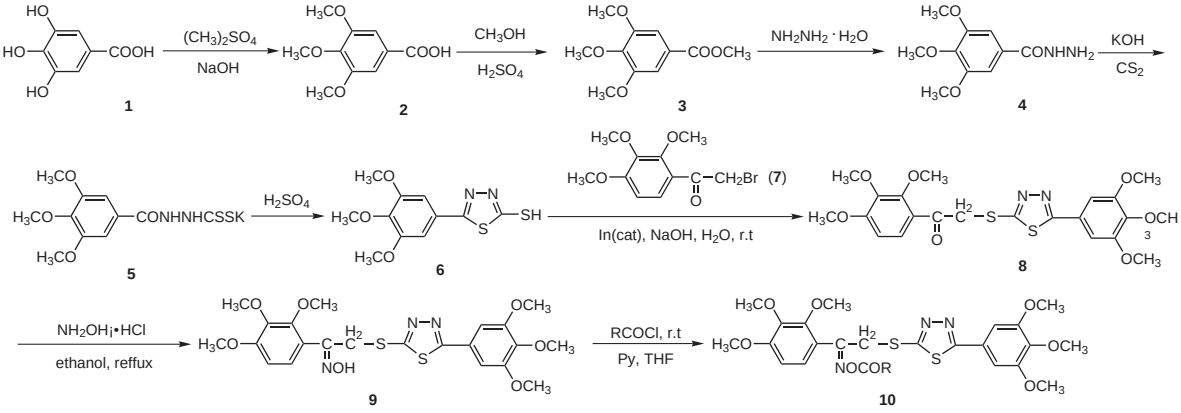
Introduction.

Recently oxime ester derivatives have attracted considerable attention in agrochemical and medicinal research, since these compounds were found to possess good bioactivities such as insecticidal, antiphytoviral, herbicidal, antitumor and fungicidal activities. A large number of researches on their synthesis and biological activities have been reported during the last twenty years [1-5]. However, little attention has been paid to the synthesis of oxime esters containing 1,3,4-thiadiazole moiety. Considered that 1,3,4-thiadiazole compounds tend to contain multi-structure in a molecule, the biological activity of these compounds may be improved by the promotion of these combination with the cell's microstructure and the accumulation of various biological activities resulting from the incorporation of different heterocyclic and non-heterocyclic nucleated changes on biological activity of the compounds [6-8]. In our previous work in it, we reported that some 2-(*1H*-1, 2, 4-triazol)-1-(2,3,4-trimethoxyphenyl)ethanoxime ester derivatives showed fungicidal activities [9-14]. Based on the theory of structure-activity relationship, we modified these structures by replacement of 1,2,4-triazole with benzothiazole group and found that some of them still kept high virucidal activities. Then we designed and synthesized a series of 2-(benzothiazol-2-thiol)-1-(2,3,4-trimethoxyphenyl)ethanoxime ester from which new anticancer agent and viricide was found [15-16]. In our search for new classes of compounds with high biological activity and low toxicity, a series of facile oxime ester containing 1,3,4-thiadiazole were synthesized from the starting material gallic acid **1**, followed by five reaction steps to obtain 5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazole-2-thiol (**6**). Thiadiazole compound **6** was then coupled with 2-bromo-1-(2,3,4-trimethoxyphenyl) ethanone (**7**) to give the key intermediate 1-(2,3,4-trimethoxyphenyl)-2-{[5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-yl]thio}ethanone (**8**). Acetophenone **8** was then condensed with hydroxylamine hydrochloride and the resulted acetophenoxime **9** was treated with acyl chloride in THF through which a number of new title compounds **10** were obtained. The synthetic route is shown in Scheme 1. And the structures of the compounds were confirmed by ^{1}H NMR, IR, and elemental analysis. Preliminary bioassay indicated that some of these compounds exhibit moderate anticancer activity *in vitro*.

Results and Discussion.

Our synthetic route leading to the title compound **10** is shown in Scheme 1. Methyl 3,4,5-trimethoxybezoate **3** was synthesized from the starting material gallic acid **1** through etherification and esterification reactions. In the following three reaction steps, the hydrazide **4**, potassium hydrazidecarbdithioate **5** [17-19], and finally thiadiazole thio compound **6** were obtained respectively.

Scheme 1



The synthetic route of oxime ester derivatives **10**.

The key intermediate, 1-(2,3,4-trimethoxyphenyl)-2-{[5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-yl]thio} ethanone (**8**), was prepared by thioetherification reaction through treating 2-bromo-1-(2,3,4-trimethoxyphenyl) ethanone (**7**) with thio compound **6** in aqueous media catalyzed by indium. The indium mediated aqueous organic reaction had been so attractive due to its excellent catalytic activity in water [20-24].

In order to optimize the reactions, the thioetherification reactions of compound **6** with compound **7** mediated by indium were studied, as shown in Table 1. When the reaction was carried out at 20-25 °C for 3 h catalyzed by 7 mol% indium, compound **8** was obtained in the yield of 83.7% (Table 1, entry 3). Reducing the amount of indium significantly lowered the product yield (Table 1, entries 1, 2). No obvious improvement could be found when indium was increased to 10 mol% (Table 1, entry 4). When the reaction was carried out at lower or higher temperature, the yield decreased remarkably (Table 1, entry 6-8). No reaction was observed when indium was not used (Table 1, entry 9). When the reactions were carried out from 1 h to 2 h in aqueous media catalyzed by indium at 20-25 °C, the yield of **8** was increased from 69.7% to 70.7% (Table 1, entries 5 and 10). Tiny improvement of the yield was obtained when the reaction time was prolonged to 4 h (Table 1, entry 11).

The synthesis of 1-(2,3,4-trimethoxyphenyl)-2-{[5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-yl]thio}ethanone oxime (**9**) was investigated with different base (including inorganic base such as NaOH, KOH, Na_2CO_3, K_2CO_3, and organic base such as pyridine and triethylamine) and different solvents (anhydrous methanol and ethanol). The results are given in Table 2. Firstly, the reaction temperature should be reflux condition. At room temperature, no ketoxime **9** could be obtained. Secondly, the results demonstrated that the presence of pyridine could accelerate the oximation reaction. Moreover, when other base instead of pyridine was used in the present reaction, the yield could not be further improved. Further, the effects of solvents, molar ration of the substrates on the oximation reaction were also examined. The best result was obtained when acetophenone **8** was treated with 3 equiv of hydroxylamine and 18.2 equiv of pyridine in anhydrous ethanol at reflux temperature for 8 h.

The esterification of ketoxime **9** with acyl chloride produced **10** in moderate yield. In order to optimize the reaction conditions, the esterification reactions were carried out under several conditions. It was found that the reaction temperature should be maintained between 0 °C and 25 °C. Below 0 °C, the reaction was very slow. Conversely, on increasing the reaction temperature over 25 °C, side reactions occurred and the yield of **10** was significantly reduced. In addition, different bases such as pyridine, triethylamine, sodium carbonate, and potassium carbonate were studied. The results demonstrated that the presence of pyridine could accelerate the esterification reaction. Further, the effects of solvents on the esterification reaction were also examined. The best result was obtained when the ketoxime **9** was treated with 1.1 equiv. of acyl choride and 1.6 equiv. of pyridine in THF at 0-25 °C for 8 h.

The antitumor activity was assayed by the MTT method [25]. The results are listed in Table 3. It was found that some of these compounds exhibit certain activities against PC3 cancer cell line *in vitro*. The data listed in Table 3 indicate that the nature of substituent dramatically affects the antitumor activity. As can be easily seen, the antiproliferative bioactivity of three ketoxime benzoates, that are benzoate **10b** (41.5%), (3,4,5-trimethoxy) benzoate **10c** (66.1%) and 3,5-dinitrobenzoate **10g** (70.2%) at 10 μM are all higher than the other compounds. Hence we may infer that the aryl group is very important to their bioactivity and the substituted aryl group is even more favorable. For example, the antiproliferation activity of compound **10g** at the concentration of 5 and 10 μM against PC3 cells are 56.7% and 70.2%, respectively.

Conclusion.

In summary, the present method for the formation of key intermediate **8** in aqueous media catalyzed by indium offers several advantages: faster reaction rates, fewer by-products, high yields, and the use of water as reaction medium. Some of the target compounds have moderate antiproliferative bioactivity against cancer cell. For example, compounds **10c** and **10g** can inhibit 66.1% and 70.2% of PC3 cell proliferation at 5 and 10 μM.

EXPERIMENTAL

Unless otherwise indicated, all common reagents and solvents were used as obtained from commercial supplies without further purifications. All melting points of the products were determined on a XT-4 binocular microscope (Beijing Tech Instrument Co., China) and are not corrected. The infrared spectra were recorded on a Bruker VECTOR22 spectrometer in KBr disks. ^{1}H NMR (solvent $CDCl_3$) and ^{13}C NMR spectra (solvent $CDCl_3$) were performed on a Varian-INOVA 400 MHz spectrometer at room temperature using TMS as internal standard. The mass spectra were taken on an HP5988A spectrometer. Elemental analysis was performed by an Elementar Vario-III CHN analyzer.

3,4,5-Trimethoxybenzohydrazide (**4**).

A mixture of methyl 3,4,5-trimethoxybezoate (8.2 g, 3.6 mmol) and 20 mL hydride hydrazide in 10 mL methanol were refluxed for 5 h. The product was collected by filtration. The white solid was washed with water and recrystallized from water to give colorless needles, yield 89.0%, m.p. 152-154 °C. (lit. ref. [26], m.p. 158-159 °C).

Potassium 2-(3,4,5-trimethoxybenzoyl)hydrazidecarbdithioate (**5**).

To a three-necked 100 mL flask was added 3,4,5-trimethoxybenzohydrazide (**4**) (6.8 g, 30 mmol), potassium hydroxide (2 g) and methanol (80 mL). With stirring carbon disulfide (2.28 g, 30 mmol) was added dropwise and the reactants were refluxed for 2 h monitored by TLC (water ethanol = 1:3). The reaction mixture was cooled to room temperature. The crude product was collected by filtration, washed with methanol, and dried to give compound **5** as colorless crystal, yield 66.5%, m.p. 213-214 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.805 (s, 3H, CH_3O), 3.889 (s, 6H, $2CH_3O$), 6.798 (s, 2H, ArH), 9.980 (s, 1H, NH), 10.951(s, 1H, CONH).

5-(3,4,5-Trimethoxyphenyl)-1,3,4-thiadiazole-2-thiol (**6**).

To an oven-dried three-necked 250 mL round-bottom flask fitted with a magnetic stirring bar was added 90 mL concentrated sulfuric acid (purity 98%). The reactant was cooled to -10 °C and then potassium salt **5** (41.0 g, 0.120 mol) was slowly added. After reacted at -5-0 °C for 1.5 h, the resulting solution was poured into ice-cold water and dissolved in dilute aqueous sodium hydroxide. The insoluble solid was filtered and the filtrate was acidified with dilute hydrochloric acid to pH 2. The mixture was then kept at room temperature for 8 h. The precipitate was then collected by filtration, washed twice with water, dried, and recrystallized from DMF/Ethanol (*V*/*V*, 1/3) to obtain compound **6**, white solid, yield 43.8%, m.p. 257-258 °C ; IR: 3069, 3000, 2953, 1585, 1560, 1506, 1433, 1242, 1047, 833 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.772 (s, 3H, CH_3O), 3.800 (s, 6H, $2\times CH_3O$), 6.977 (d, 2H, ArH, *J* = 8.8 Hz), 13.981 (s, 1H, SH).

Anal. Calcd. For $C_{11}H_{12}N_2O_3S_2$: C, 46.46; H, 4.25; N, 9.85. Found: C, 46.53; H, 4.30; N, 9.81.

1-(2,3,4-Trimethoxyphenyl)-2-{[5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-yl]thio}ethanone (**8**).

To a three-necked 100 mL round-bottom flask fitted with a magnetic stirring bar was added compound **6** (4 mmol), 3.5% NaOH aqueous solution (4 mmol) and 30 mL of water. The mixture was stirred at room temperature for 10 min. Then 4 mmol 2-bromo-1-(2,3,4,-trimethoxyphenyl) ethanone (**7**) [14] and indium (0.28 mmol) were added. The resulting mixture was then stirred for 3 h at room temperature. The reaction was followed and monitored by TLC (petroleum ether:acetone = 2:1), and the solid material collected by filtration. The solid was washed with acetone, dried, and crystallized from acetone and petroleum ether (*V*/*V*, 2/1) to give **8** as white solid, yield 83.7%, m.p. 185-187 °C; IR: 2960, 2937, 2800, 1655, 1591, 1495, 1236, 1047, 837, 798 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.884 (s, 3H, 3-CH_3O), 3.926(s, 6H, 3,5-di-CH_3O), 3.906(s, 6H, 2×4-CH_3O), 4.110 (s, 3H, 2-CH_3O), 4.969 (s, 2H, SCH_2), 6.744-7.693 (m, 4H, Ar-H); ^{13}C NMR ($CDCl_3$):191.810, 168.455, 164.111, 158.479, 154.519, 153.596, 141.987, 140.048, 126.492, 125.164, 123.066, 107.286, 104.836, 60.999, 56.321.

Anal. Calcd. for $C_{22}H_{24}N_2O_7S_2$: C, 53.60; H, 4.91; N, 5.69. Found C, 53.50; H, 4.88; N, 5.46.

1-(2,3,4-Trimethoxyphenyl)-2-{[5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-yl]thio}ethanone oxime (**9**).

To an oven-dried three-necked 250 mL round-bottom flask fitted with a magnetic stirring bar was added compound **8** (2.51 g, 5 mmol), pyridine (7.20 g, 91 mmol) and 80 mL absolute ethanol. The resulting mixture was stirred for 30 min. A solution of $NH_2OH\cdot HCl$ (1.05 g, 15.0 mmol) in anhydrous ethanol (20 mL) was added and the mixture was refluxed for 8 h. The reaction was followed and monitored by TLC (petroleum ether:acetone = 1:4). The mixture was then poured into ice water (100 mL). The crude solid was collected by filtration, washed with water, dried, and crystallized from acetone to give **9** as white solid, yield 66.7%, m.p. 135-137 °C; IR: 2938, 2839, 1585, 1105, 972, 846 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): 3.777 (s, 3H, MeO), 3.800 (s, 3H, MeO), 3.805 (s, 3H, MeO), 3.825 (s, 3H, MeO), 3.903 (s, 6H, 2MeO), 4.604 (s, 2H, CH_2S), 6.703 (d, 1H, ArH, *J* = 8.8 Hz), 6.966 (d, 1H, ArH, *J* = 8.8 Hz), 7.140 (s, 2H, ArH), 10.900 (s, 1H, NOH).

Anal. Calcd. for $C_{22}H_{25}N_3O_7S_2$: C, 52.06; H, 4.96; N, 8.28. Found: C, 52.00; H, 5.15; N, 8.09.

1-(2,3,4-Trimethoxyphenyl)-2-{[5-(3,4,5-trimethoxyphenyl)-1,3,4- thiadiazol-2-yl]thio}ethanone oxime ester **10a-g**.

To a 50 mL three-necked-round-bottomed flask, ketoxime **9** (0.51 g, 1 mmol) was mixed with pyridine (0.13 g, 1.6 mmol), and then dry THF (10 mL) was added. The resulting solution was stirred for 30 min and cooled to 0 °C with an ice bath. A solution of acyl chloride (1.1 mmol) in THF (6 mL) was added dropwise during 20 min. After having been stirred at 0 °C for 1 h, the reaction was warmed to 20-25 °C for 8 h. Then the solution was poured into 20 mL water, and the mixture was

neutralized by 5% potassium carbonate to pH 7.0. The crude solid was collected by filtration, washed with water, dried, and crystallized from acetone to give title compounds **10**.

1-(2,3,4-Trimethoxyphenyl)-2-{[5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-yl]thio}ethanone *O*-(3-phenylprop-2-enolyl)-oxime (**10a**).

This compound was obtained as white solid, yield 70.3%, m.p. 144-146 °C; IR: 2932 1746, 1630, 1585, 1238, 1103, 989, 864 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.776 (s, 3H, CH_3O), 3.843 (s, 6H, 2×CH_3O), 3.845 (s, 3H, CH_3O), 3.871 (s, 3H, MeO), 4.002 (s, 3H, CH_3O), 4.772 (s, 2H, CH_2S), 6.514 (d, 1H, ArH, J = 8.8 Hz), 6.822 (d, 1H, ArH, J = 8.8 Hz), 7.129 (s, 2H, Ar), 7.342 (t, 2H, ArH, J = 11.2 Hz), 7.405 (t, 1H, ArH, J = 14.4 Hz), 7.169 (d, 1H, =CH, J = 8.4 Hz), 7.635 (d, 2H, =CH, J = 7.6 Hz), 7.765 (d, 1H, ArH, J = 16 Hz); EIMS: m/z 637 (M^+, 10.2).

Anal. Calcd. for $C_{31}H_{31}N_3O_8S_2$ (637): C, 58.38; H, 4.90; N, 6.59. Found: C, 58.33; H, 4.92; N, 6.58.

1-(2,3,4-Trimethoxyphenyl)-2-{[5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-yl]thio}ethanone *O*-benzoyloxime (**10b**).

This compound was obtained as white solid, yield 53.7%, m.p. 144-145 °C; IR: 2932, 1742, 1586, 1238, 1109, 965, 861 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 3.869 (s, 3H, CH_3O), 3.883 (s, 3H, CH_3O), 3.906 (s, 6H, 2×CH_3O), 3.936 (s, 3H, CH_3O), 4.001 (s, 3H, CH_3O), 4.834 (s, 2H, CH_2S), 6.662 (d, ArH, 1H, J = 8.8 Hz), 7.029 (s, 2H, ArH), 7.561 (d, 1H, ArH, J = 8.8 Hz), 7.256 (t, 1H, ArH, J = 13.6 Hz), 7.447 (t, 2H, ArH, J = 14.8 Hz), 8.089 (d, 2H, ArH, J = 7.6 Hz); EIMS: m/z 611(M^+, 18.0).

Anal. Calcd. for $C_{29}H_{29}N_3O_8S_2$ (611): C, 56.94; H, 4.58 N, 6.87. Found: C, 56.97; H, 4.74; N, 6.98.

1-(2,3,4-Trimethoxyphenyl)-2-{[5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-yl]thio}ethanone *O*-(3,4,5-trimethoxybenz-oyl) oxime (**10c**).

This compound was obtained as white solid, yield 42.1%, m.p. 160-162 °C; IR: 2939, 1732, 1589, 1234, 1105, 993, 840 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 3.867 (s, 3H, CH_3O), 3.882 (d, 6H, 2× CH_3O, J = 8.2 Hz), 3.926 (s, 6H, 2× CH_3O), 3.929 (s, 9H, 3× CH_3O), 4.011 (s, 3H, CH_3O), 4.79 (s, 2H, CH_2S), 6.664 (d, 1H, ArH, J = 8.8 Hz), 7.021 (s, 2H, ArH), 7.221-7.334 (m, 2H, ArH), 8.210 (q, 1H, ArH, J = 9.6 Hz), 8.466 (q, 1H, ArH, J = 6.0 Hz); EIMS: m/z 701 (M^+, 13.1).

Anal. Calcd. for $C_{32}H_{35}N_3O_{11}S_2$ (701): C, 54.77; H, 5.03; N, 5.99. Found: C, 54.96; H, 5.04; N, 5.93.

1-(2,3,4-Trimethoxyphenyl)-2-{[5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-yl]thio}ethanone *O*-[(2-chloropyridin-3-yl)-carbonyl]oxime (**10d**).

This compound was obtained as white solid, yield 52.1%, m.p. 152-154 °C; IR: 2930, 1767, 1584, 1267, 1111, 1028, 922 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 3.867 (s, 3H, CH_3O), 3.882 (s, 6H, 2× CH_3O), 3.910 (s, 3H, CH_3O), 3.926 (s, 3H, CH_3O), 4.011 (s, 3H, CH_3O), 4.790 (s, 2H, CH_2S), 6.653 (d, ArH, 1H, J = 8.8Hz), 7.021 (s, 2H, ArH), 7.232 (d, 1H, ArH, J = 8.8 Hz), 7.331 (t, 1H, PyH, J = 12.4 Hz), 8.222 (d, 1H, PyH, J = 9.6 Hz), 8.474 (d, 1H, PyH, J = 6.8 Hz); EIMS: m/z 647 (M^+, 10.9).

Anal. Calcd. for $C_{28}H_{27}ClN_4O_8S_2$ (647): C, 51.87; H, 4.10; N, 8.60. Found: C, 51.97; H,4.21; N, 8.66.

1-(2,3,4-Trimethoxyphenyl)-2-{[5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-yl]thio}ethanone *O*-nonanoyloxime (**10e**).

This compound was obtained as white solid, yield 80.2%, m.p. 88-89 °C; IR: 2928 1773, 1586, 1252, 1076, 1009, 868 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.865 (t, 3H, CH_3, J = 14 Hz), 1.239~1.329 (m, 10H, $5CH_2$), 1.678 (s, 2H, CH_2), 2.404 (t, 2H, CH_2, J = 15.2 Hz), 3.852 (s, 3H, CH_3O), 3.86 (s, 3H, CH_3O), 3.907 (s, 3H, CH_3O), 3.929 (s, 6H, 2× CH_3O), 3.971 (s, 3H, CH_3O), 4.689 (s, 2H, CH_2S), 6.629 (d, 1H, ArH, J = 8.8Hz), 7.086 (s, 2H, ArH), 7.171 (d, 1H, ArH, J = 8.8 Hz); EIMS: m/z 647(M^+, 14.1).

Anal. Calcd. for $C_{31}H_{41}N_3O_8S_2$ (647): C, 57.58; H, 6.40; N, 6.55. Found: C, 57.48; H, 6.38; N, 6.49.

1-(2,3,4-Trimethoxyphenyl)-2-{[5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-yl]thio}ethanone *O*-(2-methylheptanoyl)-oxime (**10f**).

This compound was obtained as white solid, yield 65.5%, m.p. 95-96°C; IR: 2959, 1757, 1585, 1260, 1074, 1001, 924, 841 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.865 (t, 3H, CH_3, J = 14 Hz), 1.239~1.329 (m, 8H, $4CH_2$), 1.678 (s, 3H, CH_3), 2.406 (t, 1H, CH, J = 15.2 Hz), 3.852 (s, 3H, CH_3O), 3.860 (s, 3H, CH_3O), 3.907 (s, 3H, CH_3O), 3.929 (s, 6H, 2× CH_3O), 3.971 (s, 3H, CH_3O), 4.689 (s, 2H, CH_2S), 6.629 (d, 1H, ArH, J = 8.8Hz), 7.086 (s, 2H, ArH), 7.171 (d, 1H, ArH, J = 8.8 Hz); EIMS: m/z 633 (M^+, 12.0).

Anal. Calcd. for $C_{30}H_{39}N_3O_8S_2$ (633): C, 56.80; H, 6.11; N, 6.59. Found: C, 56.85; H, 6.20; N, 6.63.

1-(2,3,4-Trimethoxyphenyl)-2-{[5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-yl]thio}ethanone *O*-(3,5-dinitrobenzoyl)-oxime (**10g**).

This compound was obtained as white solid, yield 55.5%, m.p. 155-156 °C; IR: 2943 1759, 1587, 1262, 1072, 1009, 861 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 3.884 (s, 3H, CH_3O), 3.905 (s, 3H, CH_3O), 3.915 (s, 6H, 2× CH_3O), 3.930 (s, 3H, CH_3O), 4.053 (s, 3H, CH_3O), 4.852 (s, 2H, CH_2S), 6.709 (d, 1H, ArH, J = 8.8 Hz), 6.910 (s, 2H, ArH), 7.290 (d, 1H, ArH, J = 8.8 Hz), 9.066 (s, 1H, ArH), 9.190 (s, 2H, ArH); EIMS: m/z 701 (M^+, 14.1).

Anal. Calcd. for $C_{29}H_{27}N_5O_{12}S_2$ (701): C, 49.94; H, 3.99; N, 9.58. Found: C, 50.05; H, 4.02; N, 9.64.

MTT Assay Against Cell Proliferation.

All compounds tested were dissolved in DMSO (1-100 μM solutions) and subsequently diluted in the culture medium before treatment of the cultured cells. Tested cells were plated in 96-well plates at a density of 3×10^3 cells/well/100 μL of the proper culture medium and treated with the compounds at concentration of 1-100 μM for 48 h. In parallel, the cells were treated with 0.1% of DMSO as control. A MTT [3-(4,5-dimethylthiazol-2yl)-2, 5-diphenyltetrazolium bromide] assay (Roche Molecular Biochemicals) was performed according to the instructions provided by Roche. This assay is based on the cellular cleavage of the tetrazolium salt, MTT, into a formazan that is insoluble in the cell culture medium and is measured at 550 nm directly in 96-well assay plates. Absorbance is directly proportional to the number of living cells in culture. PC3 (prostate cancer) cell lines (provided by Cell Bank of Committee on Type Culture Collection of Chinese Academy of Science) were cultivated in F-12 medium supplemented with 10% fetal bovine serum (provided by TBD & HY Bio. Co.) and 2 mM of L-glutamine. Tissue culture reagents were obtained from Gibco Co.

Table 1

Indium-catalyzed synthesis of key intermediate **8** [a].

Entry	Indium	Temp / °C	Time / h	Yield / %
1	1 mol%	20~25	3	53.0
2	5 mol%	20~25	3	67.8
3	7 mol%	20~25	3	83.7
4	10 mol%	20~25	3	84.1
5	7 mol%	20~25	1	69.7
6	7 mol%	10~15	3	69.2
7	7 mol%	30~35	3	71.1
8	7 mol%	40~45	3	69.9
9	0 mol%	20~25	3	---
10	7 mol%	20~25	2	70.7
11	7 mol%	20~25	4	83.9

[a] Reaction conditions: 4 mmol of 5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazole-2-thiol (**6**), 4 mmol of 2-bromo-1-(2,3,4-trimethoxyphenyl) ethanone (**7**), 30 mL of water, 3.5 % NaOH (4 mmol), appropriate amount of indium, at 20-25 °C for 3 h.

Table 2

Effect of different experimental condition on the yields of compound **9** [a].

Entry	Molar ratio [b]	Base	Solvent	Temp / °C	Yield %
1	1 : 1	pyridine	ethanol	reflux	13.2
2	1 : 2	pyridine	ethanol	reflux	34.5
3	1 : 3	KOH	ethanol	reflux	32.5
4	1 : 3	pyridine	ethanol	reflux	66.7
5	1 : 3	Et_3N	ethanol	reflux	35.6
6	1 : 3	K_2CO_3	ethanol	reflux	35.6
7	1 : 4	pyridine	ethanol	reflux	66.9
8	1 : 3	pyridine	methanol	reflux	51.2
9	1 : 3	pyridine	ethanol	20-25	0

[a] Reaction condition: stirred for 8 h in anhydrous solvent; [b] Molar ratio of compound **8** to $NH_2OH{\cdot}HCl$.

Table 3

Inhibition rate (%) of the title compounds **10a**-**10g** against PC3 cell at 5 and 10 μM.

Comp.	5 μM	10 μM
10a	8.9	5.2
10b	3.9	41.5
10c	62.5	66.1
10d	10.1	33.2
10e	3.3	28.7
10f	6.7	33.1
10g	56.7	70.2

Acknowledgement.

This work was financially supported by the National Basic Research Program of China (Grant No. 2003CB114404), High-Tech Research and Development Program of China (Grant Nos. 2003AA2Z3542 and 2002AA217131), the National Nature Science Foundation of China (Grant No. 20362004), the Foundation for New Century Talent in University of China (Grant No. NCET-06-0913), the Excellent Youth Scientist Foundation of Guizhou Province of China (Grant No. 20050515), and the Nature Science Foundation of Guizhou Province in China (Grant No. 20043019).

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*To whom correspondence should be addressed. Tel: +86-851-3620521, Fax: +86-851- 3622211, E-mail: songbaoan22@yahoo.com

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