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Designs and Syntheses of Oxathiin Carboxanilide Analogues and their Antiviral Activities

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Syntheses of new analogues of oxathiin carboxanilide (**UC84**) and their antiviral activities were described. The heterocyclic carboxylic acids including oxathiins (**4**), thiazines (**9**) and dithiins (**13**) in which the methyl was replaced either by lipophilic trifluoromethyl- or bulky phenyl-group were synthesized starting from β-keto esters (**5**). Reaction of **4**, **9** and **13** with thionyl chloride followed by treatment of the substituted aniline **22** gave the corresponding carboxanilides (**24a**~**24f**). The carboxanilides were subjected to Laweson's reagent the corresponding thiocarboxanilides (**24g**~**24k**). The antiviral activities of the synthesized compounds against human immunodeficiency virus type 1 (HIV-1), poliovirus type 1 (PV-1), coxsackie B virus type 3 (CoxB-3), vesicular stomatitis virus (VSV) and herpes simplex virus type 1 (HSV-1) were presented. The antiviral activity against HIV-1 of dithiin carboxanilide (**24e**) was similar with that of **UC84** (**24a**). The corresponding thiocarboxanilides (**24g**~**24k**) showed higher inhibitory activity against HIV-1 than the carboxanilides (**24a**, **24b**, **24d**, **24e**). The compounds in which ether the lipophilic trifluoromethyl substituents (**24d**, **24f**, **24i**, **24k**) or bulky phenyl substituent is present in the heterocyclic compounds showed lower inhibitory activity than that of the methyl substituents is present in the compounds against the HIV-1. But the trifluoromethylated dithiin (**24f**) showed higher inhibitory activity against PV-1 and CoxB-3 virus than commercial antiviral agents, ribavirin (RV).

Key words: Heterocyclic carboxanilide, Oxathiins, Thiazines, Dithiins, Thiocarboxanilides, Antiviral Activities

INTRODUCTION

HIV-1 reverse transcriptase (RT) inhibitors can be divided into two classes: nucleoside and non-nucleoside inhibitors (Levy, 1993). Many nucleoside analogues inhibit RT by function as chain terminators of newly synthesized proviral DNA during its transcription from viral RNA (Mitsuya *et al.*, 1985). In 1984, 3'-azido-2',3'-dideoxythymidine (AZT) was identified as active antiviral agents (Mitsuya *et al.*, 1986). There are currently nucleoside analogs approved for use in HIV disease (De Clercq, 1998). Although the nucleoside drugs are selective for HIV-1 RT, they are not highly specific and inhibit normal cellular polymerases, causing serious side effects. In addition, these are also relatively expensive to synthesize. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) have been recently developed for overcoming these problems. These do not function as chain terminators, and do not bind at the deoxyribonucleoside triphosphate (dNTP)-binding site (Pauwels *et al.*, 1990; Tucker *et al.*, 1996). Although the NNRTIs exhibit striking structural differences and fall into several chemical groups, they all contain aromatic moieties and bind to the same hydrophobic pocket in HIV-1 RT. It is therefore possible that they inhibit HIV-1 RT by similar mechanisms. These are HIV-1 RT specific and do not inhibit HIV-2 RT or host cell polymerases. In addition, these exhibit low cytotoxicity, and produce few side effects (Tantillo *et al.*, 1994). The U. S. National Cancer Institute (NCI) has undertaken a major initiator to discover novel anti-HIV agents from both synthetic and natural products. One new chemotype discovered by empirical screening was oxathiin carboxanilide, isopropyl 2-chloro-5-[(2-methyl-5,6-dihydro-1,4-oxathiin-3-carbonyl)amino] benzoate (**UC84**) (see Fig. 1). **UC84** originally synthesized as an analog of potential commercial

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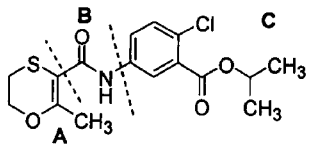


Fig. 1. Structure of **UC84**

fungicide carboxin (Vitavax®), was demonstrated to be highly active in preventing HIV-induced cell killing and in inhibiting HIV reproduction (Bader *et al.*,1991).

It is interesting to see the antiviral activities of new carboxanilides whose structures are modified based on the NNRTI, **UC84** (Fig. 1). Our study was focused on two synthetic approaches for the structural modification of **UC84** (Balzarini *et al.*, 1995): substitution of dihydro-1,4-oxathiin moiety to substituted heterocycles (part A in Fig. 1) and conversion of amide (part B in Fig. 1) to thioamide was designed.

MATERIALS AND METHODS

Materials

The reagents were purchased from Aldrich Chemical Company or TCI. The solvents, sulfuryl chloride, thionyl chloride and thiophosgene were distilled before use. The analytical solvents were used with analytical grade from Baker. All reactions were performed under nitrogen atmosphere. Melting points were determined on a digital melting point apparatus (WPA). ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian Gemini 300. Chemical shift (δ) are given in ppm and the coupling constants (*J*) are in Hz. IR spectra were obtained on Perkin Elmer 16F PC FT-IR. Mass (MS) spectra were recorded either on a Hewlett Packard 5890 Series II or 5972 Series with mass selective detector. All flash chromatographic isolation was accomplished on Kieselgel 60 GF254 (230-400 mesh).

General work-up

"General work-up" means pouring the reaction mixture into brine, extraction with CH_2Cl_2, washing the combined organic layers successively with 1N HCl, and saturated aqueous $NaHCO_3$ solution and brine, drying with anhydrous $MgSO_4$, and then removal of the solvent *in vacuo*.

General hydrolysis

The ethanolic NaOH solution was refluxed until the two layers become homogeneous (about 2 h). The solution was cooled, diluted with water and acidified with 6N HCl until the pH reaches 2. The solution was extracted with CH_2Cl_2. The extract was dried over anhydrous $MgSO_4$ and then the solvent was removed *in vacuo*.

Synthesis of 2-methyl-5,6-dihydro-1,4-oxathiin-3-carboxylic acid (4a)

To a stirred and cooled solution of methyl 3-oxobutyrate (**5a**) (116 g, 1 mol) was added dropwise sulfuryl chloride (142 g, 1.05 mol) over 30 min at 0~5°C. After additional stirring of the reaction mixture for 1 h, the reaction mixture was evaporated. The residual oily liquid was distilled at reduced pressure (88~90°C/15 mmHg) to give methyl 2-chloro-3-oxobutyrate (**6a**) [89%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.43 (s, 3H, CH_3), 3.93 (s, 3H, OCH_3), 4.93 (s, 1H, CH)]. To a cooled and stirred benzene (50 ml) solution of **6a** (15 g, 0.10 mol) in an ice bath was added a mixture of triethylamine (15.3 ml, 0.11 mol) and 2-mercaptoethanol (7 ml, 0.10 mol) over 30 min at 20~25°C. Stirring was continued at room temperature for 1 h. After the insoluble precipitate was filtered off, the organic layer was washed with cold water and then dried over anhydrous $MgSO_4$. The solution was acidified with *p*-toluenesulfonic acid monohydrate (PTSA, 0.19 g) and the water was collected by refluxing using a Dean-Stark water trap for 2 h. The reaction mixture was cooled, washed with aqueous $NaHCO_3$ solution and water. The organic layer was dried over anhydrous $MgSO_4$. The benzene was removed *in vacuo* to give methyl 2-methyl-5,6-dihydro-1,4-oxathiin-3-carboxylate (**3a**) [77%; mp 58~60°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.33 (s, 3H, CH_3), 3.03-2.88 (m, 2H, 5-CH_2), 3.78 (s, 3H, OCH_3), 4.45-4.30 (m, 2H, 6-CH_2)] as a white solid . To a solution of **3a** (1.1 g, 6.5 mmol) in ethanol (2 ml) was added a solution of NaOH (0.5 g) in water (10 ml). The white solid prepared by the general hydrolysis was crystallized from *n*-hexane and ethyl acetate to afford 2-methyl-5,6-dihydro-1,4-oxathiin-3-carboxylic acid (**4a**) [72%; mp 176~178°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.34 (s, 3H, CH_3), 2.95-2.98 (m, 2H, 5-CH_2), 4.36-4.39 (m, 2H, 6-CH_2), 11.8 (s, 1H, OH); MS, m/z 355 (M^+); IR (KBr) 3274, 1712, 1656 cm^{-1}].

Synthesis of 2-phenyl-5,6-dihydro-1,4-oxathiin-3-carboxylic acid (4b)

By similar method described above, 2-phenyl-5,6-dihydro-1,4-oxathiin-3-carboxylic acid (**4b**) [84%; yellow solid; mp 137~138°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.06-3.09 (m, 2H, 5-CH_2), 4.48-4.51 (m, 2H, 6-CH_2), 7.31-7.40 (m, 5H, ArH), 11.04 (s, 1H, OH)] and its ethyl ester (**3b**) [63%; yellow solid; mp 114~117°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (t, *J*=7.1, 3H, CH_3), 3.11-3.14 (m, 2H, 5-CH_2), 4.00 (q, *J*=7.1, 3H, OCH_2), 4.50-4.53 (m, 2H, 6-CH_2), 7.35-7.36 (m, 5H, ArH)] were prepared starting from ethyl 3-oxo-3-phenylpropionate (**5b**).

Synthesis of 4-acetyl-3-methyl-5,6-dihydro-4*H*-1,4-thiazine-2-carboxylic acid (9)

To a solution of methyl 2-chloro-3-oxobutyrate (**6a**) (15.1 g, 0.1 mol) in benzene (50 ml) was added dropwise a suspension of 2-aminoethanethiol HCl (12.5 g, 0.11 mol) in methanolic (40 ml) KOH (12.5 g, 0.22 mol) solution at 0~10°C over 1 h. The reaction mixture was stirred at room temperature over 30 min and filtered. The solvent was removed under vacuum to give oily residue, which was dissolved in CH_2Cl_2. The CH_2Cl_2 solution was washed with cold water and then dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to give methyl 3-methyl-5,6-dihydro-4*H*-1,4-thiazine-2-carboxylate (**7a**) [89%; mp 58~61°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.43 (s, 3H, 3-CH_3), 2.89-2.92 (m, 2H, 6-CH_2), 3.70-3.72 (m, 2H, 5-CH_2), 3.78 (s, 3H, OCH_3), 5.38 (br s, 1H, NH); IR (KBr) 3350, 1630, 1580 cm^{-1}] as a pale brown solid. A solution of **7a** (15.4 g, 0.089 mol) in acetic anhydride (20.9 ml) was refluxed over 24 h and then residual acetic anhydride was removed by fractional vacuum distillation. The residue was dissolved in ethyl acetate, washed with saturated aqueous sodium bicarbonate solution and cold water sequentially. The organic layer was dried (anhydrous $MgSO_4$). The dark brown solid was given by vacuum evaporation and then the solid was crystallized from ethyl acetate and petroleum ether to yield methyl 4-acetyl-3-methyl-5,6-dihydro-4*H*-1,4-thiazine-2-carboxylate (**8a**) [13.65 g, 71%; mp 74~76°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.16 (s, 3H, $COCH_3$), 2.45 (s, 3H, CH_3), 3.06-3.13 (m, 2H, 6-CH_2), 3.75-3.83 (m, 2H, 5-CH_2), 3.81 (s, 3H, OCH_3); IR (KBr) 1710, 1660 cm^{-1}] as a pale brown solid. To a solution **8a** (1.0 g, 46.5 mmol) in ethanol (2 ml) was added a solution of NaOH (0.5 g) dissolved in water (10 ml). The general hydrolysis of the reaction mixture gave 4-acetyl-3-methyl-5,6-dihydro-4*H*-1,4-thiazine-2-carboxylic acid (**9a**) [3.65 g, 44%; mp 168~169°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.20 (s, 3H, $COCH_3$), 2.49 (s, 3H, CH_3), 3.09-3.13 (m, 2H, 6-CH_2), 3.79-3.83 (m, 2H, 5-CH_2); MS, m/z 187 (M^+)] as a white solid.

Synthesis of 3-methyl-5,6-dihydro-1,4-dithiin-2-carboxylic acid (13c)

A solution of ethyl 2-chloro-3-oxobutyrate (**6c**) (69.7 ml, 0.5 mol), 1,2-ethanedithiol (46.2 ml, 0.55 mol) and PTSA (1.72 g) in benzene (750 ml) was refluxed for 3 h with a Dean-Stark water trap. The reaction mixture was cooled, washed with brine. The organic layer was dried over anhydrous magnesium sulfate. Evaporation of solvent gave a white solid, which was recrystallized from ether and petroleum ether to give ethyl 3-methyl-5,6-dihydro-1,4-dithiin-2-carboxylate (**12c**) [98%; mp 33~35°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (t, *J*=7.1, 3H, CH_3), 2.31 (s, 3H, CH_3), 3.07-3.10 (m, 2H, CH_2), 3.21-3.24 (m, 2H, CH_2), 4.20 (q, *J*=7.1, 2H, CH_2); MS, m/z 204 (M^+)] as a white solid. A mixture of ethyl ester **12c** (40 g, 0.2 mol) was refluxed for 2 h in a NaOH (11.8 g, 0.3 mol) solution in ethanol (5 ml)-water (150 ml) cosolvent. The white solid prepared by the general hydrolysis was crystallized from ether and petroleum ether to afford 3-methyl-5,6-dihydro-1,4-dithiin-2-carboxylic acid (**13**) [72%; white solid; mp 173~175°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.40 (s, 3H, CH_3), 3.10-3.14 (m, 2H, CH_2), 3.26-3.29 (m, 2H, CH_2), 8.92 (1H, OH); MS, m/z 176 (M^+)].

Synthesis of 2-trifluoromethyl-5,6-dihydro-1,4-oxathiin-3-carboxylic acid (17a)

Ethyl 4,4,4-trifluoro-3-oxobutyrate (**14**) (98.35 g, 0.45 mol) was treated with chlorine while stirring at 20°C until the weight of the reaction mixture reaches to 15% increase. Nitrogen gas was purged to remove excess HCl, and then fractional vacuum distillation gave a transparent oil, ethyl 2-chloro-4,4,4-trifluoro-3-oxobutyrate (**15**) [83.9 g, 84%; 92°C/30 mmHg; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (t, *J*=7.2, 3H, CH_3), 4.35 (q, *J*=7.2, 2H, CH_2), 5.22 and 12.52 (2s, 1H, CH and OH)]. To a solution of **15** (22.4 g, 0.102 mol) and triethylamine (15.69 ml, 0.112 mol) in benzene (110 ml) cooled under the cold water bath (5°C) was added dropwise 2-mercaptoethanol (7.4 ml, 0.105 mol) over 1 h. Stirring was continued for 2 h at the same bath and insoluble precipitate was filtered off. The general work-up gave ethyl 2-hydroxy-2-trifluoromethyl-1,4-oxathiane-3-carboxylate (**20a**) [25.9 g, 97%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30 (t, *J*=7.2, 3H, CH_3), 2.42-3.06 (m, 2H, 5-CH_2), 4.07 (s, 1H, CH), 4.18-4.46 (m, 2H, 6-CH_2), 5.54 (s, 1H, OH); MS, m/z 260 (M^+); IR (KBr) 1720, 3442 cm^{-1}] as a yellow oil. To a solution of **20a** (25.9 g, 0.100 mol) and pyridine (8.9 ml, 0.110 mol) in benzene (100 ml) was added thionyl chloride (12.82 ml, 0.110 mol) at 20°C over 2 h. Stirring was continued at room temperature for 5 h. The insoluble precipitates were filtered off. To the reaction mixture was added dropwise triethylamine (20.69 ml, 0.150 mol) at 10°C over 1 h. The cooling bath was removed and the reaction mixture was stirred at room temperature for 48 h. The insoluble precipitates were filtered off. The general work-up of the reaction mixture gave a dark brown oily residue. The residue was flash-chromatographied by using a mixture of *n*-hexane:ethyl acetate (4:1) as an eluent to give ethyl 2-trifluoromethyl-5,6-dihydro-1,4-oxathiin-3-carboxylate (**18a**) [14.05 g, 58%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32 (t, *J*=7.2, 3H, CH_3), 3.09-3.12 (m, 2H, 5-CH_2), 4.26 (q, *J*=7.2, 2H, OCH_2), 4.39-4.42 (m, 2H, 6-CH_2); ^{13}C NMR (78.5 MHz, $CDCl_3$) δ 13.5 (s, OCH_2CH_3, CH_3), 25.1 (s, S-CH_2), 62.3 (s, OCH_2CH_3, CH_2), 65.8 (s, O-C), 117.4 (s, C=C), 121.0 (s, C=C), 140.3 (q, CF_3, *J*=147), 163.5 (s, CO); IR (KBr) 1730 cm^{-1}] as a dark brown oil. A suspended solution of the **18a** (12.11 g, 0.050 mol) in ethanol (5 ml) was added a solution of NaOH (3.00 g, 0.075 mol) in water (25 ml). The general hydrolysis of the reaction mixture gave 2-trifluoromethyl-5,6-dihydro-1,4-oxathiin-3-carboxylic

acid (**17a**) [63%; mp 143~144°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.09-3.12 (m, 2H, 5-CH_2), 4.43-4.46 (m, 2H, 6-CH_2), 5.35 (1H, OH); IR (KBr) 1700 cm^{-1}] as a white solid.

Synthesis of 3-trifluoromethyl-5,6-dihydro-1,4-dithiin-2-carboxylic acid (17b)

To a solution of ethyl 2-chloro-4,4,4-trifluoro-3-oxobutanoate (**15**) (48.1 g, 0.22 mol) in benzene (200 ml) were added dropwise a mixture of 1,2-ethanedithiol (19.3 ml, 0.23 mol) and triethylamine (27.0 ml, 0.19 mol) under ice bath over 30 min. Stirring was continued for 2 hr at room temperature. The insoluble white solid was filtered off. To the filtrate was added pyridine (18.4 ml, 0.23 mol), and then dropwise thionyl chloride (16.8 ml, 0.23 mol) at 20°C below over 20 min. The reaction mixture was stirred for 2 h at room temperature and filtered, and then removed the solvent under vacuum. The solid was dissolved with dried benzene (200 ml) and then treated with triethylamine (29.0 ml, 0.209 mol). The reaction mixture was refluxed for 17 h. The solution was cooled to room temperature. Insoluble solid was filtered off. The general work-up gave a brown oily liquid (54.6 g). The product was purified by flash-chromatography using *n*-hexane:ethyl acetate (10:1) to afford ethyl 3-trifluoromethyl-5,6-dihydro-1,4-dithiin-2-carboxylate (**18b**) [37.2 g, 63.3%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32 (t, *J*=7.2, 3H, CH_3), 3.20-3.36 (m, 4H, 5-CH_2 and 6-CH_2), 4.23-4.29 (q, *J*=7.2, 2H, OCH_2)] as a brown oil. To a solution of **18b** (7.2 g, 0.144 mol) in ethanol (5 ml) was added a solution of NaOH (5.21 g, 0.131 mol) in water (50 ml). By the general hydrolysis, 3-trifluoromethyl-5,6-dihydro-1,4-dithiin-2-carboxylic acid (**17b**) was prepared. Crystallization from ether and petroleum ether gave **17b** [23.5 g, 70.8%; mp 119~120°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.19-3.37 (m, 4H, 5-CH_2 and 6-CH_2), 8.93 (s, 1H, OH)] as a yellow solid.

Synthesis of isopropyl 5-amino-2-chlorobenzoate (22)

To a suspension of 5-amino-2-chlorobenzoic acid (**21**) (34 g, 0.2 mol) in 2-propanol (100 ml) was added dropwise thionyl chloride (71 g, 0.6 mol) over 30 min. The reaction mixture was refluxed for 6 h while stirring. The excess 2-propanol was evaporated under reduced pressure. The reaction mixture was dissolved with CH_2Cl_2, and washed with 10% aqueous NaOH solution (about 30 ml) water twice, dried over anhydrous $MgSO_4$. Evaporation of the solvent gave a dark purple oil, which was crystallized on seeding in refrigerator to give isopropyl 5-amino-2-chlorobenzoate (**22**) solid [39 g, 92%; mp 51-52°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (d, *J*=6.5, 6H, 2 × CH_3), 3.74 (s, 2H, NH_2), 5.18-5.27 (m, 1H, CH), 6.64-7.16 (m, 3H, ArH); MS, m/z 213 (M^+)] as a dark purple solid.

Syntheses of carboxanilides : *General procedure*

To a suspension of the carboxylic acid (5 mmol) in benzene (10 ml) was added sequentially trietylamine (5.5 mmol) and thionyl chloride (5.5 mmol) under nitrogen atmosphere. The reaction mixture was refluxed for 1 h. The solution was cooled to room temperature and then evaporated under the reduced pressure. To the residue dissolved in benzene (10 ml) was added isopropyl 5-amino-2-chlorobenzoate (**22**) (5.5 mmol) was added to it. The solution was stirred at room temperature for overnight. The general work-up gave the corresponding carboxanilide.

Isopropyl 2-chloro-5-[(2-methyl-5,6-dihydro-1,4-oxathiin-3-carbonyl)amino]benzoate (24a)

yield: 70%; pale yellow solid; mp 132~133°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (d, *J*=5.9, 6H, 2 × CH_3), 2.27 (s, 3H, CH_3), 2.98-3.01 (m, 2H, 5-CH_2), 4.41-4.44 (m, 2H, 6-CH_2), 5.24-5.29 (m, 1H, CH), 7.37-7.79 (m, 3H, ArH), 8.00 (br s, 1H, NH); MS, m/z 355 (M^+); IR (KBr) 3274, 1712, 1656 cm^{-1}.

Isopropyl 2-chloro-5-[(2-phenyl-5,6-dihydro-1,4-oxathiin-3-carbonyl)amino]benzoate (24b)

yield: 64%; pale yellow solid; mp 117~118°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36 (d, *J*=6.5, 6H, 2 × CH_3), 3.15-3.18 (m, 2H, 5-CH_2), 4.51-4.54 (m, 2H, 6-CH_2), 5.18-5.24 (m, 1H, CH), 6.99 (br s, 1H, NH), 7.24-7.49 (m, 3H, ArH); MS, m/z 417 (M^+); IR (KBr) 3262, 1720, 1640 cm^{-1}.

Isopropyl 5-[(4-acetyl-3-methyl-5,6-dihydro-4*H*-1,4-thiazine-2-carbonyl)amino]-2-chlorobenzoate (24c)

yield: 56%; pale brown solid; mp 150~150°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (d, *J*=6.2, 6H, 2 × CH_3), 2.18 (s, 3H, CH_3), 2.42 (s, 3H, $COCH_3$), 3.19-3.23 (m, 2H, 6-CH), 3.79-3.83 (m, 2H, 5-CH), 5.22-5.31 (m, 1H, CH), 7.39-7.85 (m, 3H, ArH), 8.36 (br s, 1H, NH); IR (KBr) 3296, 1720, 1670 cm^{-1}.

Isopropyl 2-chloro-5-[(2-trifluoromethyl-5,6-dihydro-1,4-oxathiin-3-carbonyl) amino]benzoate (24d)

yield: 56%; white solid; mp 112~113°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 (d, *J*=6.4, 6H, 2 × CH_3), 3.12-3.15 (m, 2H, 5-CH_2), 4.41-4.44 (m, 2H, 6-CH_2), 5.24-5.29 (m, 1H, CH), 7.39-7.81 (m, 3H, ArH), 7.70 (br s, 1H, NH); MS, m/z 409 (M^+); IR (KBr) 3266, 1714, 1650 cm^{-1}.

Isopropyl 2-chloro-5-[(3-methyl-5,6-dihydro-1,4-dithiin-2-carbonyl)amino] benzoate (24e)

yield: 85%; pale yellow solid; mp 134~135°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (d, *J*=6.2, 6H, 2 × CH_3), 2.36 (s, 3H, CH_3), 3.12-3.15 (m, 2H, CH_2), 3.30-3.34 (m, 2H, CH_2), 5.23-5.32 (m, 1H, CH), 7.38-7.83 (m, 3H, ArH), 8.24 (br s, 1H, NH); MS, m/z 371 (M^+); IR (KBr)

3274, 1712, 1648, 1580 cm^{-1}.

Isopropyl 2-chloro-5-[(3-trifluoromethyl-5,6-dihydro-1,4-dithiin-2-carbonyl)amino]benzoate (24f)

yield: 93%; pale yellow solid; mp 117~118°C; ^1H NMR (300 MHz, $CDCl_3$) δ 1.38 (d, J=6.2, 6H, 2 × CH_3), 3.20-3.24 (m, 2H, CH_2), 3.35-3.38 (m, 2H, CH_2), 5.22-5.31 (m, 1H, CH), 7.40-7.83 (m, 3H, ArH), 7.67 (br s, 1H, NH); MS, m/z 425 (M^+); IR (KBr) 3320, 1690, 1538 cm^{-1}.

Syntheses of thiocarboxailides : *General procedure*

A suspension of oxathiin carboxanilide (1 mmol), Lawesson's reagent (1.4 mmol) and sodium bicarbonate (9.7 mmol) in toluene (20 ml) was refluxed for 5~15 h. The reaction mixture was cooled to room temperature and filtered. Evaporation of the filtrate gave a solid, which was dissolved in CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ solution and then water. The organic layer was dried over anhydrous $MgSO_4$, and then flash column chromatographed by using benzene : ethyl acetate (20:1) as an eluent.

Isopropyl 2-chloro-5-[(2-methyl-5,6-dihydro-1,4-oxathiin-3-carbothionyl)amino]benzoate (24g)

yield: 73%; yellow solid; mp 91~92°C; ^1H NMR (300 MHz, $CDCl_3$) δ 1.39 (d, J=6.2, 6H, 2 × CH_3), 2.09 (s, 3H, CH_3), 3.03-3.06 (m, 2H, 5-CH_2), 4.33-4.36 (m, 2H, 6-CH_2), 5.23-5.32 (m, 1H, CH), 7.44-7.97 (m, 3H, ArH), 9.00 (br s, 1H, NH); MS, m/z 371 (M^+); IR (KBr) 3448, 1720, 1298 cm^{-1}.

Isopropyl 2-chloro-5-[(2-phenyl-5,6-dihydro-1,4-oxathiin-3-carbothionyl)amino] benzoate (25h)

yield: 79%; yellow solid; mp 166~167°C; ^1H NMR (300 MHz, $CDCl_3$) δ 1.39 (d, J=5.9, 6H, 2 × CH_3), 3.21-3.24 (m, 2H, 5-CH_2), 4.49-4.53 (m, 2H, 6-CH_2), 5.20-5.31 (m, 1H, CH), 7.36-7.57 (m, 3H, ArH), 8.27 (br s, 1H, NH); MS, m/z 433 (M^+); IR (KBr) 3126, 1722, 1558, 1498, 1296 cm^{-1}.

Isopropyl 2-chloro-5-[(2-trifluoromethyl-5,6-dihydro-1,4-oxathiin-3-carbothionyl) amino]benzoate (24i)

yield: 88%; yellow solid; mp 135~136°C; ^1H NMR (300 MHz, $CDCl_3$) δ 1.40 (d, J=6.3, 6H, 2 × CH_3), 3.18-3.21 (m, 2H, 5-CH_2), 4.43-4.46 (m, 2H, 6-CH_2), 5.20-5.31 (m, 1H, CH), 7.36-7.57 (m, 3H, ArH), 8.27 (br s, 1H, NH); MS, m/z 425 (M^+); IR (KBr) 3268, 1694, 1324 cm^{-1}.

Isopropyl 2-chloro-5-[(3-methyl-5,6-dihydro-1,4-dithiin-2-carbothionyl)amino]benzoate (24j)

yield: 79%; yellow oil; ^1H NMR (300 MHz, $CDCl_3$) δ 1.40 (d, J=6.3, 6H, 2 × CH_3), 2.15 (s, 3H, CH_3), 3.26-3.28 (m, 4H, 2CH_2), 5.26-5.33 (m, 1H, CH), 7.45-8.11 (m, 3H, ArH), 8.11 (br s, 1H, NH); MS, m/z 387 (M^+); IR (KBr) 3260, 1706, 1296 cm^{-1}.

Isopropyl 2-chloro-5-[(3-trifluoromethyl-5,6-dihydro-1,4-dithiin-2-carbothionyl)amino]benzoate (24k)

yield: 64%; yellow solid; mp 160~161°C; ^1H NMR (300 MHz, $CDCl_3$) δ 1.39 (d, J=6.2, 6H, 2 × CH_3), 3.22-3.26 (m, 2H, 5-CH_2), 3.39-3.42 (m, 2H, 6-CH_2), 5.23-5.32 (m, 1H, CH), 7.45-8.02 (m, 3H, ArH), 8.87 (br s, 1H, NH); MS, m/z 441 (M^+); IR (KBr) 3258, 1692, 1268 cm^{-1}.

Antiviral activity tests

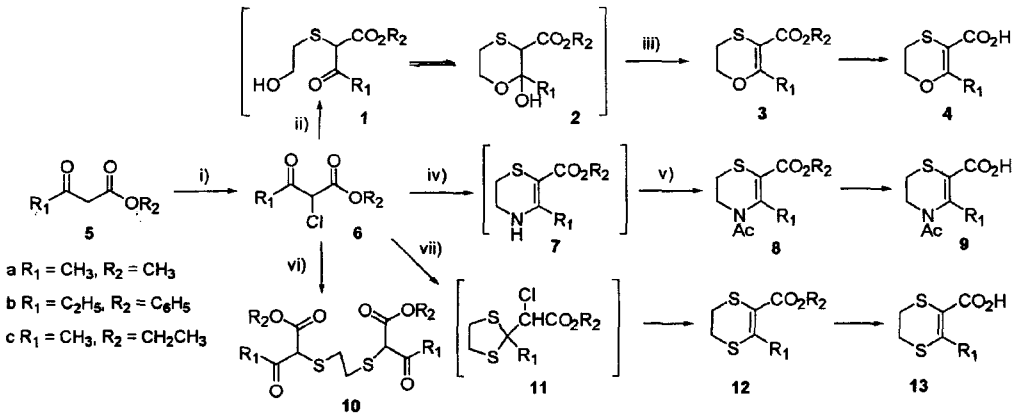
In vitro evaluation of antiviral acitivity for the synthesized thiocarbamate analogues was performed against herpes simplex virus type 1 (HSV-1), poliovirus type 1 (PV-1), Coxsackie B virus type 3 (CoxB-3), vesicular stomatitis virus strain (VSV) and human immunodeficiency virus type 1 (HIV-1) by general methods. (Pauwels *et al.*, 1988; Lee *et al.*, 1997)

RESULTS AND DISCUSSION

Dihydro-1,4-oxathiin carboxylic acid **4**, dihydro-1,4-thiazine carboxylic acid **9** and dihydro-1,4-dithiin carboxylic acid **13** were prepared by application of previously reported methods (Scheme 1) (Harrison *et al.*, 1991; Mah *et al.*, 1997, Lee *et al.*, 1991).

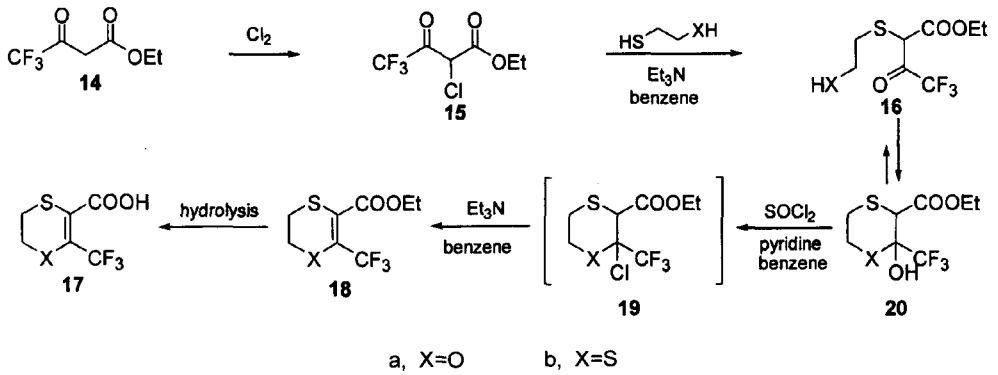
Chlorination of β-keto ester **5** by sulfuryl chloride gave α-chloro-β-keto ester **6**. Treatment of **6** with 2-mercaptoethanol in the presence of triethylamine afforded β-hydroxysulfide **1** which exists in equilibrium with 1,4-oxathiane **2**. Without isolation of **1** an acid(PTSA) catalyzed dehydration gave dihydro-1,4-oxathiin carboxylate **3** in moderate yield. Dihydro-1,4-thiazine carboxylate **8** was prepared by the similar procedure described above. Thus, **6** was subjected with 2-aminoethanethiol hydrochloride in the presence of methanolic potassium hydroxide solution followed by acetylation with acetic anhydride at room temperature to yield **8**. Attempt to the synthesis of dihydro-1,4-dithiin carboxylate **12** by the similar method described above failed to give dimeric compound **10** (Lee *et al.*, 1991). Instead, refluxing of **6c** with 1,2-ethanedithiol in the presence of the catalytic amount of PTSA with Dean-Stark water trap afforded dihydro-1,4-dithiin ester **12c** in high yield (98%) through probable intermediate **11c**. Carboxylic acids **4**, **9** and **13** were obtained by the hydrolysis of the ester **3**, **8** and **12**, respectively.

Trifluoromethyl group has received increasing interest owing to its unique nature for material sciences and potential biological activities for pharmaceuticals and agrochemicals. Trifluoromethylated carboxylic acids were synthesized from commercially available trifluoromethylated β-keto ester **14** (Scheme 2).



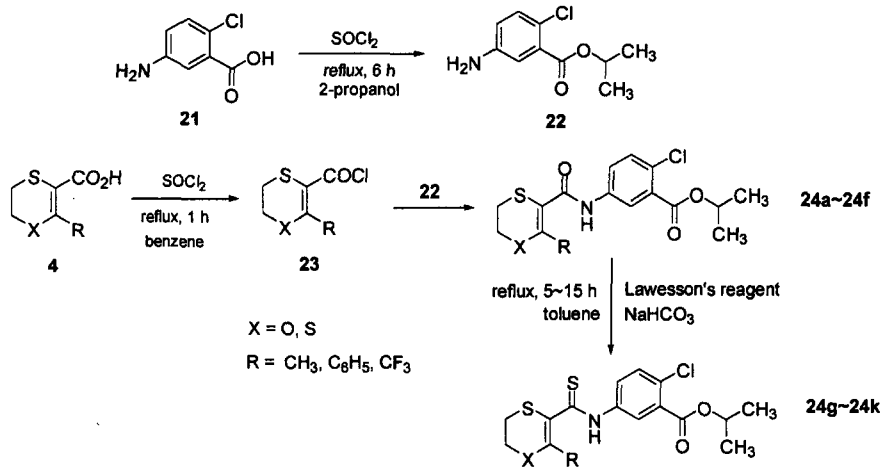
i) SO_2Cl_2, 20~25°C, 30 min, 89%, ii) $HSCH_2CH_2OH$, Et_3N, benzene, RT, 1h, iii) PTSA (0.05 equiv.), benzene, -H_2O, 2h, 77%, iv) $NH_2CH_2CH_2SH$. HCl, KOH, MeOH, benzene, 30 min, 89%, v) Ac_2O (2.5 equiv.), RT, 24h, 71%, vi) $HSCH_2CH_2SH$.HCl, Et_3N, benzene, RT, 1h, vii) $HSCH_2CH_2SH$, PTSA(0.05 equiv.), benzene, -H_2O, 2h, 98%

Scheme 1. Syntheses of heterocyclic carboxylic acids



a, X=O b, X=S

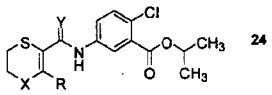
Scheme 2. Syntheses of trifluoromethylated carboxylic acids



Scheme 3. Syntheses of heterocyclic carboxanilides

Chlorination of β-keto ester **14** with sulfuryl chloride gave **15** in low yield (10%). Successful result (84% isolated yield) was obtained from the treatment of chlorine with **14** under the ice bath. The reaction of **15** either with 2-mercaptoethanol or ethanedithiol in the presence of triethylamine afforded corresponding β-hydroxy sulfide

Table I. Melting points and yields of carboxanilides and thiocarboxanilides prepared



Comp.	X	R	Y	mp(°C)	Yield (%)	Compd.	X	R	Y	mp(°C)	Yield(%)
24a	O	CH_3	O	132~133	70	24g	O	CH_3	S	91~92	73
24b	O	C_6H_5	O	117~118	64	24h	O	C_6H_5	S	166~167	79
24c	NAc	CH_3	O	150~151	56	24i	O	CF_3	S	135~136	88
24d	O	CF_3	O	112~113	56	24j	S	CH_3	S	oil	79
24e	S	CH_3	O	134~135	85	24k	S	CF_3	S	160~161	64
24f	S	CF_3	O	117~118	93						

Table II. Antiviral activities of the carboxanilides and the thiocarboxanilides

Compd.	Virus	EC_{50}	CC_{50}	SI	Compd.	Virus	EC_{50}	CC_{50}	SI
	HIV-1	1.28	21.2	16		HIV-1	0.0635	13.431	212
	PV-1	>82.5	82.5	<1		PV-1	>8.0	8.0	<1
24a	CoxB-3	>82.5	82.5	<1	**24g**	CoxB-3	>8.0	8.0	<1
	VSV	20.1	82.5	4.1		VSV	>8.0	8.0	<1
	HSV-1	>28.1	28.1	<1		HSV-1	>16.0	16.0	<1
	HIV-1	>13.0	13.0	<1		HIV-1	2.15	7.79	3.63
	PV-1	>62.8	62.8	<1		PV-1	>8.4	8.4	<1
24b	CoxB-3	>62.8	62.8	<1	**24h**	CoxB-3	>8.4	8.4	<1
	VSV	33.3	62.8	1.89		VSV	>8.4	8.4	<1
	HSV-1	>18.0	18.0	<1		HSV-1	>100	>100.0	ND
	HIV-1	>11.0	11.0	<1		HIV-1	1.68	9.02	5.38
	PV-1	>68.8	68.8	<1		PV-1	>6.5	6.5	<1
24c	CoxB-3	>68.8	68.8	<1	**24i**	CoxB-3	>6.5	6.5	<1
	VSV	25.7	68.8	3.0		VSV	>6.5	6.5	<1
	HSV-1	>33.6	33.6	<1		HSV-1	>17.4	17.4	<1
	HIV-1	>9.1	9.1	<1		HIV-1	0.32	6.07	18.94
	PV-1	>30.9	30.9	<1		PV-1	>5.2	5.2	<1
24d	CoxB-3	>30.9	30.9	<1	**24j**	CoxB-3	>5.2	5.2	<1
	VSV	11.3	30.9	2.7		VSV	>5.2	5.2	<1
	HSV-1	>15.8	15.8	<1		HSV-1	>18.7	18.7	<1
	HIV-1	1.4	9.3	6.5		HIV-1	>7.9	7.9	<1
	PV-1	>6.8	6.8	<1		PV-1	>6.3	6.3	<1
24e	CoxB-3	>6.8	6.8	<1	**24k**	CoxB-3	>6.3	6.3	<1
	VSV	>6.8	6.8	<1		VSV	>6.3	6.3	<1
	HSV-1	>5.7	5.7	<1		HSV-1	>32.9	32.9	<1
	HIV-1	>9.7	9.7	<1	AZT	HIV-1	0.0018	1.1552	996
	PV-1	22.06	>100.0	>4.53		PV-1	162.16	>300	>1.68
24f	CoxB-3	22.213	>100.0	>4.31	RV	CoxB-3	125.27	>300	>3.89
	VSV	22.174	>100.0	>3.68		VSV	13.77	>300	>26.70
	HSV-1	>9.9	9.9	<1	ACV	HSV-1	0.8333	>202.0	>250.0

EC_{50}: 50% effective concentration (μg/ml), CC_{50}: 50% cytotoxic concentration (μg/ml), SI: selectivity index, HIV-1: human immunodeficiency virus type 1, PV-1: poliovirus type 1, CoxB-3: coxsackie B virus type 3, VSV: vesicular stomatitis virus, HSV-1: herpes simplex virus type 1, AZT: 3'-azido-2',3'-dideoxythymidine, RV: ribavirin, ACV: acyclovir

16, which existed in the closed form **20** by the ^{1}H NMR spectroscopy. Dehydration of **20** in the presence of an acid catalyst (PTSA) in refluxing benzene with Dean-Stark water separator by the application of the synthesis of dihydro-1,4-oxathiin **3** (see Scheme 1) was not successful, probably due to the strong electron withdrawing character of trifluoromethyl moiety. Substitution of hydroxy at C-2 to the better leaving group, chlorine by the treatment of thionyl chloride and then dehydrochlorination in the presence of triethylamine in refluxing benzene gave the trifluoromethylated dihydro-1,4-oxathiin **18a** and trifluoromethylated dihydro-1,4-dithiin **18b**, respectively, in moderate yield. The structure of **18** was confirmed by ^{1}H NMR, ^{13}C NMR, IR and MS spectrometries. Hydrolysis of the esters **18** gave corresponding carboxylic acids **17**.

The next step was a construction of carboxanilide moiety by the coupling of the carboxylic acid **4** and aniline. Typically, Scheme 3 represents a synthetic procedure of the preparation of dihydro-1,4-oxathiin-3-carboxanilide **24**. Reaction of the dihydro-1,4-oxathiin-3-carboxylic acid **4** with thionyl chloride in refluxing benzene followed by treatment of aniline **22** which was prepared from an alkylation of commercially available aminobenzoic acid **21**.

Transformation of carboxanilide to thiocarboxanilides was carried out by using Lawesson's reagent [2,4-bis(p-methoxyphenyl)-1,3,2,4-dithiadiphosphetane-2,4-disulfide] (Cava *et al.*, 1985) in the presence of large excess amount of sodium hydrogen carbonate in refluxing toluene.

Table I shows the structures of product prepared, the yields and the melting points.

The inhibitory activities (*in vitro*) of synthesized compounds against five kinds of human immunodeficiency virus type 1 (HIV-1), poliovirus type 1 (PV-1), coxsackie B virus type 3 (CoxB-3), vesicular stomatitis virus (VSV) and, herpes simplex virus type 1 (HSV-1) were compared with those of commercial antiviral agents, 3'-azido-2',3'-dideoxy thymidine (AZT), ribavirin (RV) and acyclovir (ACV) (Table II).

The antiviral activity of dithiin carboxanilide (**24e**) against HIV-1 was similar with that of **UC84** (**24a**). The thiocarboxanilides (**24g**~**24k**) showed higher inhibitory activity against HIV-1 than that of the corresponding carboxanilides (**24a**, **24b**, **24d**, **24e**). No significant increased inhibitory activity against the HIV-1 was shown in the compounds (**24d**, **24f**, **24i**, **24k**) in which present trifluoromethyl group, which suggests that the lipophilicity of the compound is not important to enhance the antiviral activity. An interesting result was that trifluoromethylated dithiin (**24f**) showed higher inhibitory activity against the PV-1 and CoxB-3 virus than that of the RV. The fact that the lower activity of **24b** against all of the strains suggested that the larger group at C-2 in dihydro-1,4-oxathiin moiety was disadvantage. These results would be contributed to the study of structure-activity relationship (Esnouf *et al.*, 1997; Ren *et al.*, 1998) in development of antiviral agents of dihydro-1,4-oxathiin carboxanilides.

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