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SYNTHESIS OF DIHYDROTHIOPHENE DERIVATIVES AS METABOLITES OF ESONARIMOD

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Abstract – Three compounds with a dihydrothiophene ring were synthesized as authentic samples to analyze human metabolites of Esonarimod, which has been developed as a new antirheumatic drug.

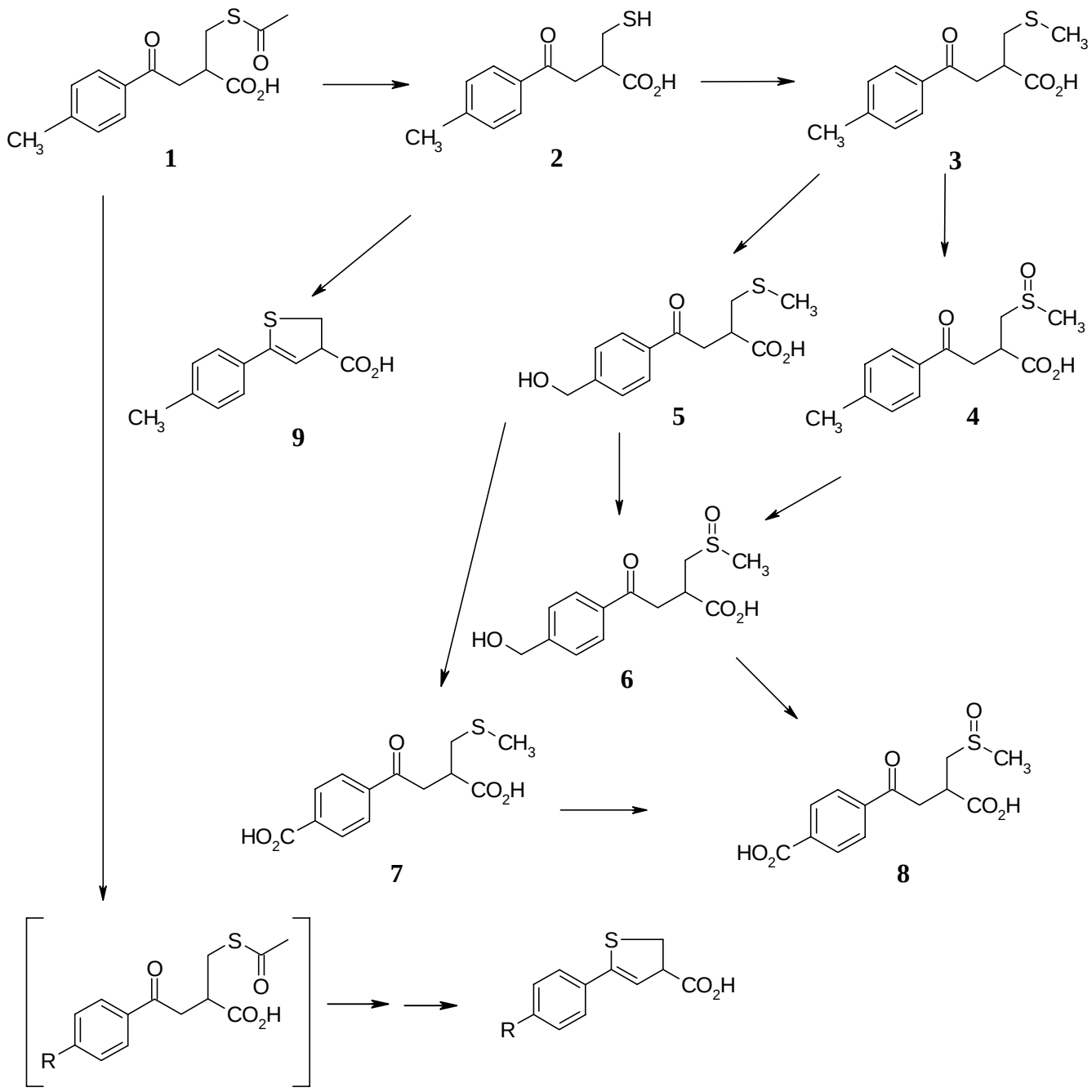
INTRODUCTION

Esonarimod, (*R,S*)-2-acetylthiomethyl-4-(4-methylphenyl)-4-oxobutanoic acid (**1**), was originally developed as a new disease-modifying antirheumatic drug (DMARD), and has been shown to have beneficial effects in rheumatoid arthritis patients in clinical studies.[1-3] In pre-clinical studies, many metabolites of **1** have been detected, and some have been synthesized. A possible metabolic pathway of **1** has been suggested, as shown in Scheme 1.[4-7]

Recently, three dihydrothiophene compounds, which are metabolites of **1**, were isolated from rat urine. These metabolites are presumed to be 5-(4-methylphenyl)-2,3-dihydrothiophene-3-carboxylic acid (**9**), 5-(4-hydroxymethylphenyl)-2,3-dihydrothiophene-3-carboxylic acid (**10**), and 5-(4-carboxyphenyl)-2,3-dihydrothiophene-3-carboxylic acid (**11**). The metabolic pathway of **1** to **9**—**11** has been suggested to be as shown in Scheme 1.[8] To identify the structures of these metabolites, it is important to obtain these compounds. However, the synthesis of these compounds has not yet been reported.
In this article, we report the synthesis of postulated metabolites (**9**—**11**).

RESULTS and DISCUSSION

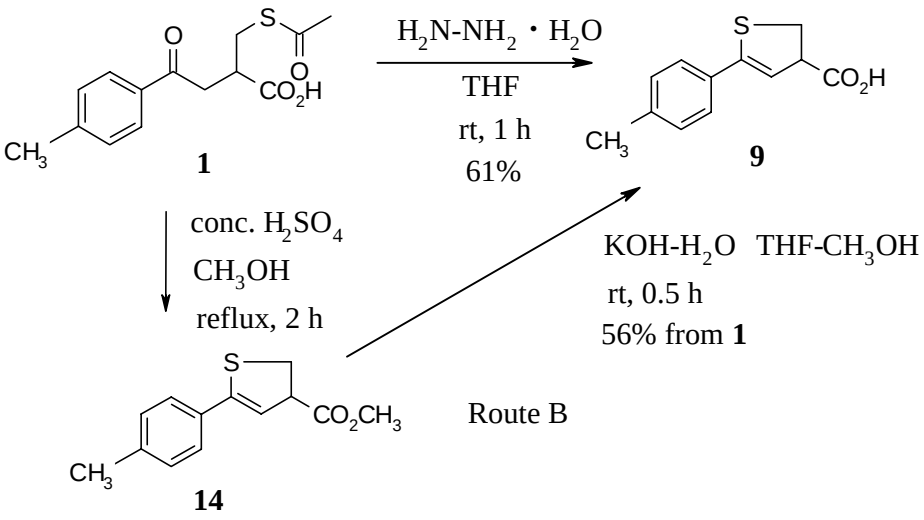
When **1** was treated with hydrazine hydrate (Scheme 2, route A), **9** was obtained in 61% yield. Compound (**14**), methyl ester of **9**, was obtained by refluxing with **1** and methanolic sulfuric acid. The hydrolysis of **14** gave **9** in 56% yield (Scheme 2, Route B).
Compound (**10**) was obtained from 4-(4-acetyloxymethylphenyl)-2-acetylthiomethyl-4-oxobutanoic acid (**15**) [6] through methyl ester (**16**) (Scheme 3). The acetyl group was removed



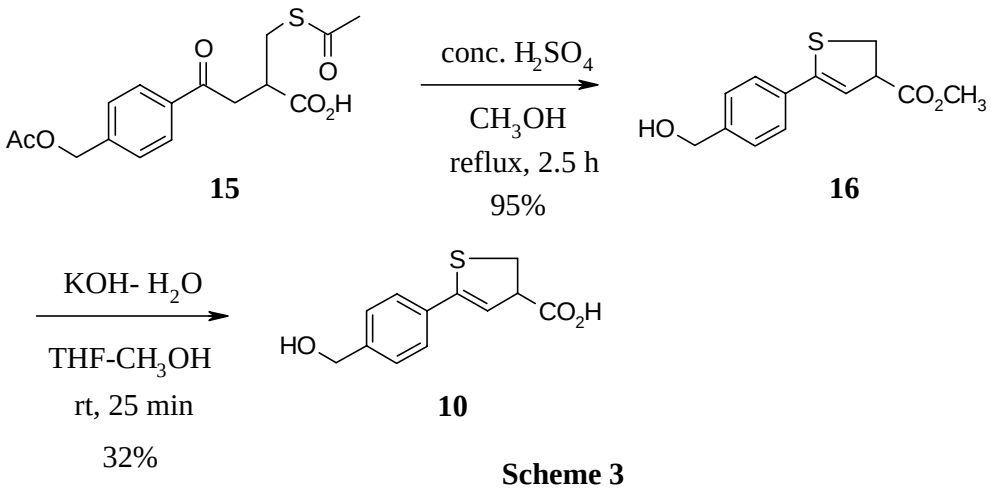
Scheme 1

under the methanolic sulfuric acid.

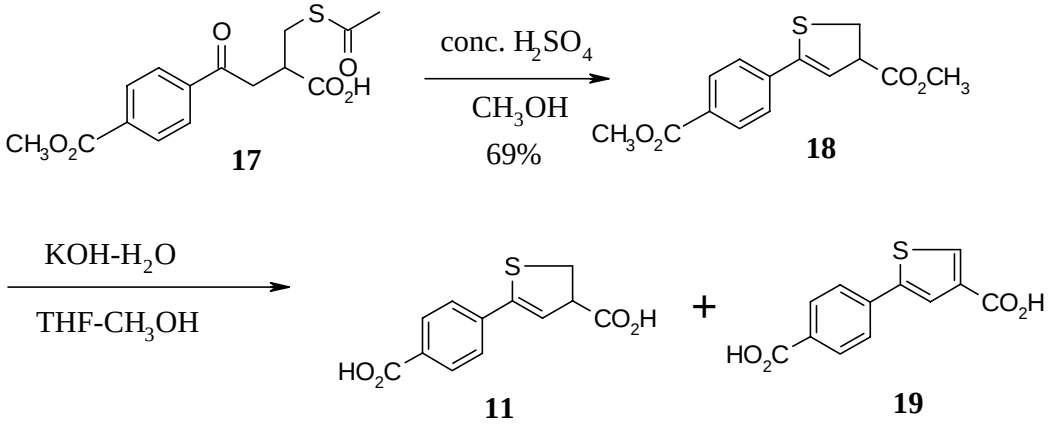
On the other hand, **11** was not obtained from 2-acetylthiomethyl-4-(4-methoxycarbonyl-phenyl)-4-oxobutanoic acid (**17**)[6] in the same way as for **9** and **10** by Route B. Treatment of **17** with methanolic sulfuric acid gave **18**. However, hydrolysis of **18** with potassium hydroxide gave the mixture of **11** and thiophene (**19**), which was the oxidized product of dihydrothiophene (**11**) (Scheme 4). The separation of **11** and **19** was not succeeded by silica



Scheme 2



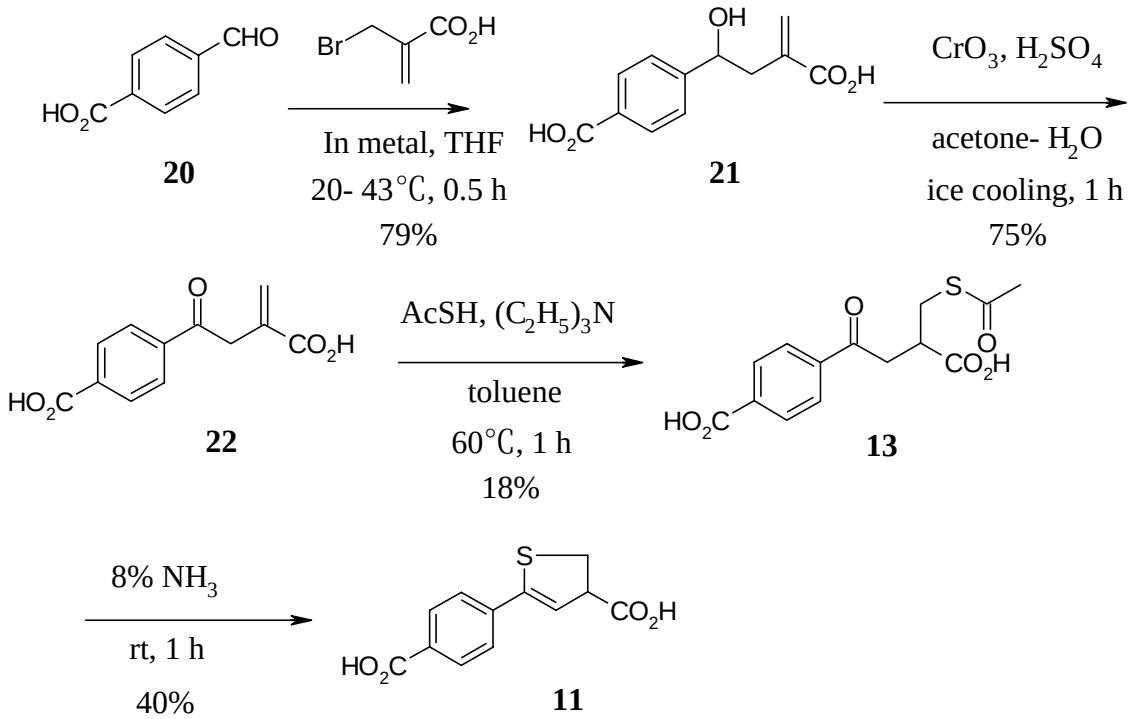
Scheme 3



Scheme 4

gel column chromatography. The ratio of **11** and **19** were determined by ^{1}H-NMR spectroscopy (δ 6.35 and 8.34).

Consequently, Route A to obtain **11** was attempted, but this required the carboxylic acid (**13**). However, **13** was not obtained by the usual hydrolysis of methyl ester (**17**). When the methyl ester was hydrolyzed, the acetyl group was removed at once to give dihydrothiophene, which was oxidized to thiophene (**19**) as in Route B. Therefore, a synthetic route for **13** was devised, based on the preparation of **16**.[6] Terephthalaldehydic acid (**20**) was used as a atarting material. The key step was the indium-promoted coupling of **20** and 2-(bromomethyl)acrylic acid to give 4-(4-carboxyphenyl)-4-hydroxy-2-methylidene- butanoic acid (**21**).[9] In the preparation of **17**, the carboxylic group of **20** had to be protected to avoid s side reaction.[6] However, the coupling of carboxylic acid (**20**) with 2-(bromomethyl)acrylic acid proceeded without any problems. The benzylalcohol (**21**) was oxidized to a ketone (**22**). The Michael addition of **22** with thioacetic acid gave **13**. Incidentally, the treatment of **13** in aqueous ammonia readily gave **11** (Scheme 5).



Scheme 5

CONCLUSION

Three compounds with a dihydrothiophene ring were synthesized as authentic samples to identify the structures of metabolites of Esonarimod (**1**). 5-(4-Methylphenyl)-thiophene-3-carboxylic acid (**9**) and 5-(4-hydroxymethylphenyl)-2,3-dihydrothiophene-3-carboxylic acid (**10**) were easily prepared from **1** or 4-(4-acetyloxymethylphenyl)-2-acetylthiomethyl-4-oxobutanoic acid (**15**). In preparing 5-(4-carboxyphenyl)-2,3-dihydrothiophene-3-carboxylic acid (**11**) from 2-acetylthiomethyl-4-(4-methoxycarbonylphenyl)-4-oxobutanoic acid (**17**), dihydrothiophene was oxidized to thiophene during hydrolysis of the methyl ester. However, **11** was obtained from 2-acetylthiomethyl-4-(4-carboxylphenyl)-4-oxobutanoic acid (**13**),

which was carboxylic acid of **17**.

EXPERIMENTAL

Melting points were determined using a Buchi 535 melting point apparatus and are uncorrected. IR spectra were obtained on a Perkin-Elmer 1760 spectrometer. ^{1}H-NMR spectra were recorded on a Varian VXL-200 (200 MHz) spectrometer. Chemical shifts are reported in ppm(δ) value from tetramethylsilane as an internal standard, as determined by a JEOL JMS-SIX102 spectrometer. Elemental analyses were performed on a Perkin-Elmer 2400. TLC was performed on silica gel pre-coated plates (Merck, Kieselgel 60F254). Column chromatograpy was performed over silica gel (Wako, Wako gel C-200). Esonarimod (**1**), 4-(4-acetyloxymethylphenyl)-2-acetylthiomethyl-4-oxobutanoic acid (**15**) and 2-acetylthiomethyl-4-(4-methoxycarbonylphenyl)-4-oxobutanoic acid (**17**) were prepared by known procedures.[6, 7]

5-(4-Methylphenyl)-2,3-dihydrothiophene-3-carboxylic Acid (9) (Route A).

To a solution of Esonarimod (**1**) (2.80 g, 9.99 mmol) and tetrahydrofuran (THF) (20 mL) was added 80% hydrazine monohydrate (8 mL, 128 mmol), and the mixture was stirred at rt for 1 h. The reaction mixture was acidified with 10% hydrochloric acid and then extracted with ether. The extract was washed with water, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography using hexane-dichloromethane (2: 1- 1: 2), and recrystallized from hexane-dichloromethane to give 1.34 g (61%) of **9 as** a pale yellow powder.

Compound (9) (Route B).

A solution of **1** (7.60 g, 27.1 mmol) in methanol (19 mL) and conc. sulfuric acid (1.9 mL) was refluxed for 2 h and then evaporated *in vacuo*. To the residue was added 150 mL of water, and the mixture was extracted with ethyl acetate. The extract was washed with water, dried over $MgSO_4$ and evaporated *in vacuo* to give 6.80 g of methyl 5-(4-methylphenyl)-2,3-dihydrothiophene-3-carboxylate (**14**) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ : 2.36 (3H, s), 3.54 (1H, dd, *J*=9.5,11.4 Hz), 3.76 (1H, dd, *J*=8.4,11.4 Hz), 3.78 (s, 3H), 4.12- 4.24 (1H, m), 5.95 (1H, d, *J*=3.1 Hz), 7.16 (2H, d, *J*=8.4 Hz), 7.39 (2H, d, *J*=8.4 Hz).

To a solution of **14** in THF (100 mL) and methanol (20 mL) was added 85% potassium hydroxide (5.90 g, 89.4 mmol)- water (10 mL). The reaction mixture was stirred at rt for 0.5 h, and then evaporated *in vacuo*. To the residue was added conc. hydrochloric acid (15 mL)-water (150 mL), and the mixture was extracted with ethyl acetate. The extract was washed with water and brine, dried over $MgSO_4$, and evaporated *in vacuo*, and the residue was recrystallized from chloroform-hexane to give 3.32 g (56% from **1**) of **9** as a pale yellow powder. mp: 110- 112℃, ^{1}H-NMR ($CDCl_3$) δ : 2.32 (3H, s), 3.53 (1H, dd, *J*=9.7,11.6 Hz), 3.74 (1H, dd, *J*=7.9,11.6 Hz), 4.15- 4.27 (1H, m), 5.94 (1H, d, *J*=3.1 Hz), 7.14 (2H, d, *J*=8.4

Hz), 7.38 (2H, d, *J*=8.4 Hz). IR (KBr) cm^{-1}: 3436, 2915, 1701, 1405, 1224, 1203, 1147, 1051, 915, 761, 626. EI-MS m/z: 220 (M^+). *Anal.* Calcd for $C_{12}H_{12}O_2S$: C, 65.43; H, 5.49; S, 14.56. Found: C, 65.60; H, 5.40; S, 14.45.

5-(4-Hydroxymethylphenyl)-2,3-dihydrothiophene-3-carboxylic Acid (10) (Route B)

A solution of 4-(4-acetyloxymethylphenyl)-2-acetylthiomethyl-4-oxobutanoic acid (**15**) (2.86 g, 8.45 mmol) in methanol (14 mL) and conc. sulfuric acid (1.4 mL) was refluxed for 2.5 h. Water (100 mL) was added, and the mixture was extracted with ethyl acetate. The extract was washed with brine, dried over $MgSO_4$ and evaporated *in vacuo* to give 2.00 g (95%) of methyl 5-(4-hydroxymethylphenyl)-2,3-dihydrothiophene-3-carboxylic acid (**16**) as a pale yellow oil. ^{1}H-NMR (DMSO-d_6) δ: 3.54 and 3.59 (2H, Abq, *J*=11.4 Hz), 3.69 (3H, s), 4.23- 4.35 (1H, m), 4.51 (2H, d, J=5.7 Hz), 5.20 (1H, t, J=5.7 Hz), 6.14 (1H, d, *J*=3.3 Hz), 7.34 (2H, d, *J*=8.6 Hz), 8.35 (2H, d, *J*=8.6 Hz).

To a solution of **16** (2.00 g, 7.99 mmol) in THF (20 mL) and methanol (4 mL) was added 85% potassium hydroxide (1.32 g, 20.0 mmol)- water (2 mL). The reaction mixture was stirred at rt for 25 min. To the reaction mixture was added conc. hydrochloric acid (2.5 mL)- water (100 mL), and the mixture was extracted with ethyl acetate. The extract was washed with water and brine, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography using hexane-THF= 3: 2. Recrystallization from hexane-THF gave 599 mg (32%) of **10** as a pale yellow powder. mp: 169- 170℃, ^{1}H-NMR ($CDCl_3$) δ: 3.51 (1H, dd, *J*=9.9, 11.4 Hz), 3.61 (1H, dd, *J*=7.5, 11.4 Hz), 4.12- 4.24 (1H, m), 4.50 (2H, s), 5.24 (1H, br s), 6.14 (1H, d, *J*=3.3 Hz), 7.32 (2H, d, *J*=8.4 Hz), 7.44 (2H, d, *J*=8.4 Hz), 12.72 (1H, br s). IR (KBr) cm^{-1}: 3446, 2946, 2575, 1713, 1395, 1212, 1007, 838. EI-MS m/z:236 (M^+). *Anal.* Calcd for $C_{12}H_{12}O_3S$: C, 61.00; H, 5.12; S, 13.57. Found: C, 60.90; H, 4.99；S, 13.69.

Mixture of 5-(4-Carboxyphenyl)-2,3-dihydrothiophene-3-carboxylic Acid (11) and 5-(4-Carboxyphenyl)-thiophene-3-carboxylic Acid (19) (Route B)

A solution of 2-acetylthiomethyl-4-(4-methoxycarbonylphenyl)-4-oxobutanoic acid (**17**) (2.00 g, 6.17 mmol) in methanol (10 mL) and conc. sulfuric acid (0.5 mL) was refluxed for 2.5 h. Water (100 mL) was added, and the mixture was extracted with ethyl acetate. The extract was washed with water and brine, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography using hexane-ethyl acetate = 3: 1 to give 1.19 g (69%) of methyl 5-(4-methoxycarbonylphenyl)-2,3-dihydrothiophene-3-carboxylate (**18**) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 3.55 (1H, dd, *J*=9.7, 11.4 Hz), 3.77 (1H, dd, *J*=8.4, 11.4 Hz), 3.78 (3H, s), 3.93 (3H, s), 4.14- 4.24 (2H, m), 6.12 (1H, d, *J*=3.1 Hz), 7.54 (2H, d, *J*=8.6 Hz), 8.01 (2H, d, *J*=8.6 Hz).

To a solution of **18** (600 mg, 2.16 mmol) in THF (20 mL) and methanol (4 mL) was added 85% potassium hydroxide (471 mg, 7.14 mmol)- water (2 mL). The reaction mixture was stirred at rt for 0.5 h. To the reaction mixture was added conc. hydrochloric acid (5.0 mL)- water (100 mL), and the mixture was extracted with ethyl acetate. The extract was washed with water and brine, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was washed

with chloroform and ethyl acetate to give 137 mg of the mixture of **11** and **18** (1: 1). ^{1}H-NMR(DMSO-d_6) δ: 3.58 (0.5H, dd, *J*=9.9, 11.4 Hz), 3.60 (0.5H, dd, *J*=7.7, 11.4 Hz), 4.18—4.28 (0.5H, m), 6.35 (0.5H, d, J=3.3 Hz), 7.62 (1H, d, *J*=8.6 Hz), 7.82—8.02 (3.5H, m), 8.34 (0.5H, d, *J*=1.3 Hz),12.98 (2H, br s).

Compound (11) (Route A)

To a solution of terephthalaldehydic acid (**20**) (5.00 g, 33.3 mmol) in THF (35 mL) was added indium metal (4.21 g, 36.7 mmol) and then 2-bromomethylacrylic acid (6.60 g, 40.0 mmol)- THF (15 mL). The reaction mixture was heated from 20℃ to 43℃ and stirred for 0.5 h. To the reaction mixture was added 2 mol/L hydrochloric acid (30 mL), and the mixture was then extracted with ethyl acetate. The extract was washed with brine and water, dried over $MgSO_4$ and evaporated *in vacuo* to give 6.22 g (79%) of 4-(4-carboxyphenyl)-4-hydroxy-2-methylenebutanoic acid (**21**).

To a solution of **21** (6.18 g, 26.2 mmol) in acetone (175 mL) was added 4 mol/L of Jones reagent (7.85 mL) under ice cooling. The reaction mixture was stirred for 1 h and then 100 mL of water was added. Crystals were collected by filtration to give 3.10 g of 4-(4-carboxylphenyl)-2-methylidene-4-oxobutanoic acid (**22**). To the mother liquor was added water, and the mixture was extracted with ethyl acetate. The extract was dried over $MgSO_4$ and evaporated *in vacuo* to give 1.51 g of **22**. Overall, 4.61 g (75%) of **22** was obtained.

To a mixture of **22** (1.00 g, 4.27 mmol) and toluene (15 mL) was added thioacetic acid (0.39 g, 5.12 mmol). Triethylamine (0.09 g, 0.89 mmol)- toluene (2.0 mL) were then added, and the mixture was stirred at 60℃ for 1 h. To the reaction mixture was added 10 mL of 0.2 mol/L hydrochloric acid, and the mixture was extracted with ethyl acetate. The extract was washed with water, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was recrystallized from acetone-ethyl acetate to give 0.61 g of 2-acetylthiomethyl-4-(4-carboxyphenyl)-4-oxobutanoic acid (**13**). The crystals were recrystallized again from ether to give 240 mg (18%) of **13** as colorless crystals. mp 161- 162℃, ^{1}H-NMR(DMSO-d_6) δ: 2.34 (3H, s), 3.03-3.56 (5H, m), 8.01- 8.12 (4H, m), 13.02 (2H, br s). IR (KBr) cm^{-1}: 2992, 2671, 1686, 1573, 1506, 1428, 1357, 1295, 1214, 1129. MS m/z: 311 (MH^+). *Anal.* Calcd for $C_{14}H_{14}O_6S$: C, 54.19; H, 4.55. Found: C, 54.27; H, 4.39.

A solution of **13** (2.00 g, 6.47 mmol) in 8% ammonia solution (21.4 mL) was stirred at rt for 1 h. The reaction mixture was acidified with 10% hydrochloric acid and then extracted with ethyl acetate. The extract was washed with brine, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was dissolved in 200 mL of ethyl acetate, and then 3.0 mL of conc. hydrochloric acid- water (200 mL) were added and the reaction mixture was stirred at rt for 3 h. The organic layer was washed with brine and evaporated *in vacuo*. The residue was purified by column chromatography using hexane-THF =3:2. Recrystallization from hexane-THF gave 637 mg (40%) of **11** as pale yellow crystals. mp >300℃, ^{1}H-NMR($CDCl_3$) δ: 3.56 (1H, dd, *J*=9.9, 11.4 Hz), 3.64 (1H, dd, *J*=7.5, 11.4 Hz), 4.18- 4.30 (1H, m), 6.35 (1H, d, J=3.3 Hz), 7.61 (2H, d, *J*=8.6 Hz), 7.94 (2H, d, *J*=8.6 Hz), 12.96 (2H, br s). IR (KBr) cm^{-1}: 2886, 1698,

1608, 1412, 1292, 1214, 920, 856, 758. EI-MS m/z: 250 (M^+). *Anal.* Calcd for $C_{12}H_{10}O_4S$: C, 57.59; H, 4.03; S, 12.81. Found: C, 57.56; H, 3.90; S, 12.84.

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