Synthesis of Novel Succinic Acid Derivatives as Potential Matrix Metalloproteinases Inhibitors and Anticancer Medicine

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Received November 17, 2009: Revised December 10, 2009: Accepted January 02, 2010

Abstract: In this paper we have designed different methods, according to the differences in nucleophilicity of amino, to conjugate tartaric acid or malic acid with two kinds of useful intermediates respectively *via* amide bond, envisaging they are promising matrix metalloproteinase inhibitors or anticancer medicine.

Keywords: Tartaric acid, malic acid, thiadiazole, acylation, nucleophilic substitution, matrix metalloproteinase inhibitors.

INTRODUCTION

Matrix metalloproteinases (MMPs) are a family of structurally related zinc-containing enzymes that play an important role in the breakdown of connective tissue. The balance between MMPs and its endogenous inhibitors – tissue inhibitors of metalloproteinase (TMP) maintains the stabilization of cells. The over expression of MMPs will induce excessive degradation of extracellular matrix (ECM). This is closely related with many inflammatory, malignant and degenerative diseases such as cancer, osteoarthritis, rheumatoid arthritis and so on [1-4]. Therefore, finding effective matrix metalloproteinase inhibitors (MMPIs) to cure these diseases is very important. Design and synthesis of MMPIs has always been a hot research field of medicinal chemistry since nineties of the last century. The requirement for a molecule to be an effective inhibitor of the MMPIs is a functional group (e.g., carboxylic acid, hydroxamic acid, and sulfhydryl, etc.) capable of chelating the active-site zinc (II) ion, that may be at least one functional group which provides a hydrogen bond interaction with the enzyme backbone, and one or more side chains which undergo effective van der Waals interactions with the enzyme subsites [5]. Tartaric acid and malic acids, however, are often used to chelate many metal ions.

2-Amino-1,3,4-thiadiazole derivatives are well known as compounds of a wide range of anticancer activity, including *in vivo* conditions [6]. They are also applied in antibacterial activity, and used as MMPIs, pesticide and so on [7,8]. Hydrazides are widely applied in bioassays including matrix metalloproteinase inhibitors [9]. Therefore, we design to conjugate tartaric acid and malic acid respectively *via* amide bond, expecting the designed new compounds to be good MMPIs.

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RESULTS AND DISCUSSION

Firstly four kinds of intermediates, hydrazides, 2-amino-1,3,4-thiadiazole derivatives, 2-acetyl-malic anhydride and diacetyl-tartaric anhydride are prepared as described in the literature [10-13]. According to the differences in nucleophilicity of amino, we take different acylation reagents, anhydride or acyl chloride. The hydrazides amino are comparatively active, so we can take anhydride as acylation reagent. But the activity of 2-amino-1,3,4-thiadiazoles amino proved extremely weak (δ_{NH2} = 7.4). In order to get high yield we transform tartaric acid from anhydride into acyl chloride as acylation reagent. Final deprotection of hydroxyl groups is achieved by a solution of K_2CO_3 in methanol [14] (Scheme **1**).

As for 2-acetyl-malic anhydride, though there is a reactivity difference between the two carbonyl groups, there will still be two kinds of compounds in the course of transforming anhydride into mono-methyl ester as Scheme **2** shown. But the product will be mainly the former when 2-acetyl-malic anhydride reacts with 2-amino-1,3,4-thiadiazole derivatives. Because the stereospecific blockade of 2-amino-1,3,4-thiadiazoles is more larger than methanol and the carbonyl group near the hydroxyl group is more active than the other, therefore we choose 2-acetyl-malic anhydride as acylation reagent. Further as Davenport and Watson's procedure may be followed [15]. But it will give little amount of product if integration is done with 2-amino-1,3,4-thiadiazole derivatives.

In addition, we identified the structure of compounds (**6**) that the hydroxyl group is attached to C(3) by comparing the ^{1}H NMR of compound (**6a**) and compound (**5a**) (Table **1**). This result accords with our prediction.

CONCLUSION

In conclusion, several proposals to synthesize asymmetry compounds and regional selectivity in chemical reactions are described in this paper. Two kinds of scaffolds which have

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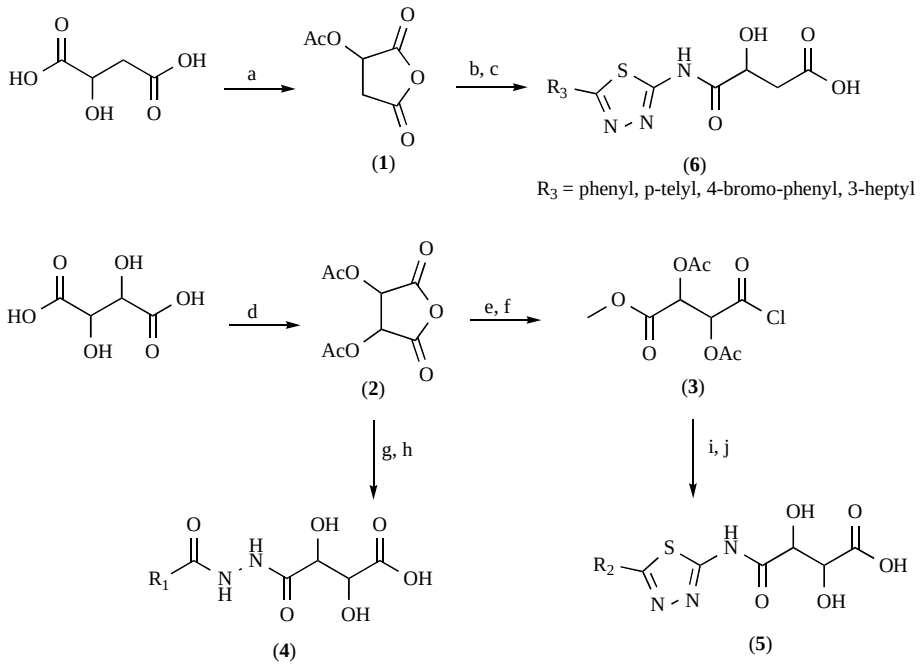
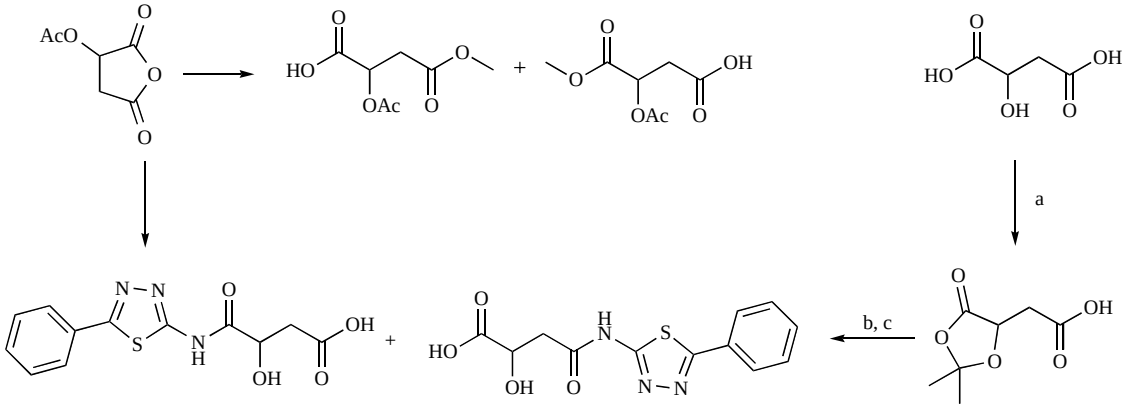
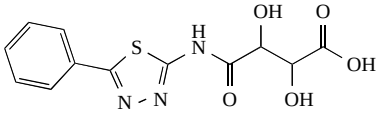
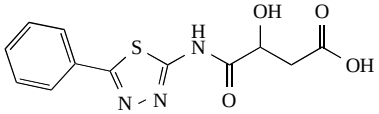


Scheme 1. (**a**) Acetyl chloride, 50 ˚C, 2 h, 90%; (**b**) 2-amino-1,3,4-thiadiazoles, THF, 0 ˚C, 48 h; (**c**) 1 M NaOH, 4 h; (**d**) acetic anhydride, sulfuric acid, 120 ˚C, 10 min, 78% (**e**) dry MeOH, 0 ˚C, 15 min, 100%; (**f**) $SOCl_2$, 75 ˚C, 2 h, 97%; (**g**) hydrazides, THF, 30 min; (**h**) 1 M NaOH, 4 h; (**i**) 2-amino-1,3,4-thiadiazoles, triethylamine, THF, 24 h; (**j**) K_2CO_3, MeOH, H_2O, 65 ˚C, 12 h.



Scheme 2. (**a**) 2,2-dimethoxypropane, tosic acid, 100%; (**b**) 2-amino-1,3,4-thiadiazole, DCC, THF; (**c**) THF, 2 M HCl.

Table 1. Structure Identification by Comparing the ^{1}H NMR of Compound (6a) and (5a)

Compound	δ (ppm) -NH	δ (ppm) 3-OH	δ (ppm) 3-CH	δ (ppm) 2-OH	δ (ppm) 2-CH	δ (ppm) -COOH
 **5a**	12.18	5.89	4.63	5.37	4.47	12.78
 **6a**	12.40	6.00	4.60	\	2.61 2.76	12.40

extensive biological activity have been successfully conjugated with tartaric acid and malic acid. We expect these new compounds have better biological activity.

EXPERIMENTAL

All reagents were obtained from commercial suppliers and used without further purification except $SOCl_2$, methanol and triethylamine. All the solvents were purified before use. All reactions were monitored by thin-layer chromatography on 0.25 mm silica gel plates (GF-254). Melting points were determined on an electrothermal melting point apparatus and were uncorrected. ESI mass spectra were recorded using a *Waters ZQ4000/2695* LC–MS spectrometer. Infrared spectra were obtained as KBr pellets on a Shimadzu FTIR-8000 spectrometer. ^{1}H NMR spectra were obtained in DMSO on a Bruker 600 MHz spectrometer. The chemical shifts were reported in δ values (ppm) relative to tetramethylsilane (TMS) as internal standard.

Synthesis of (2R, 3R)-2,3-dihydroxy-4-(N'-Benzoyl-hydrazino)-4-oxo-butyric acid (4a)

The product (**4a**) was prepared from the reaction of diacetyl-l-tartaric anhydride with benzoic acid hydrazide in THF for 30 minutes. Finally, acetyls were removed by 1 M NaOH. Purification was by recrystallization from chloroform if necessary. White solid (yield 92%); Mp 128-130°C; IR (KBr, cm^{-1}): 3533, 3476, 3360, 3213, 3028, 1744, 1693, 1651,1578, 1528, 1273, 1123, 1072, 694; ESI-MS m/z: $[M+Na]^+$ 291.10; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 4.39 (s, 2H), 5.04 (s, 1H), 5.76 (d, *J* = 6.0 Hz, 1H), 7.47-7.50 (q, 2H), 7.55-7.58 (m, 1H), 7.89-7.90 (q, 2H), 9.74 (d, *J* = 1.2 Hz, 1H), 10.44 (d, *J* = 1.2 Hz, 1H), 12.68 (s, 1H).

(2R, 3R)-2,3-dihydroxy-4-(N'-phenylacetyl- hydrazino)-4-oxo-butyric acid (4b)

White solid (yield 81%); Mp 120-122°C; IR (KBr, cm^{-1}): 3530, 3476, 3387, 3206, 3028, 1740, 1697, 1659, 1570, 1520, 1420, 1238, 1111, 1072, 721, 694; ESI-MS m/z: $[M+Na]^+$ 305.13; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 3.48 (s, 2H), 4.33 (s, 2H), 5.02 (s, 1H), 5.71 (s, 1H), 7.23 (t, *J* = 3.0 Hz, 1H), 7.28-7.30 (q, 4H), 9.62 (d, *J* = 2.4 Hz, 1H), 10.25 (d, *J* = 2.4 Hz, 1H), 12.64 (s, 1H).

(2R, 3R)-2,3-dihydroxy-4-[N'-(4-Fluoro-benzoyl)-hydrazino]-4-oxo-butyric acid (4c)

White solid (yield 84%); Mp 165-166°C; IR (KBr, cm^{-1}): 3533, 3479, 3340, 3209, 3017, 1740, 1693, 1651, 1605, 1508, 1273, 1246, 1123, 1072, 853; ESI-MS m/z: $[M+Na]^+$ 309.10; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 4.39 (s, 2H), 5.04 (s, 1H), 5.77 (d, *J* = 6.0 Hz, 1H), 7.33 (t, *J* = 9.0 Hz, 2H), 7.97 (dd, *J* = 5.4 Hz, 9.0 Hz, 2H), 9.75 (d, *J* = 1.8 Hz, 1H), 10.48 (d, *J* = 1.8 Hz, 1H), 12.69 (s, 1H).

(2R, 3R)-2,3-dihydroxy-4-[N'-(4-methoxy-benzoyl)-hydrazino]-4-oxo-butyric acid (4d)

White solid (yield 87%); Mp 121-122°C; IR (KBr, cm^{-1}): 3533, 3479, 3340, 3206, 3016, 1740, 1690, 1647, 1608, 1578, 1528, 1254, 1123, 1072, 1026, 845; ESI-MS m/z: $[M+Na]^+$ 321.12; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 3.82 (s, 3H), 4.39 (s, 2H), 5.05 (s, 1H), 5.74 (s, 1H), 7.01 (d, *J* = 9.0 Hz, 2H), 7.88 (d, *J* = 9.0 Hz, 2H), 9.67 (s, 1H), 10.30 (s, 1H), 12.66 (s, 1H).

Synthesis of 2,3-dihydroxy-N-(5-phenyl- [1,3,4]thiadiazol-2-yl)-succinamic acid (5a)

To the solution of 2-amino-5-phenyl-1,3,4- thiadiazole (3.54 g, 0.020 mol) in THF and excess triethylamine dropwise added the solution of compound (**3**) (6.40 g, 0.024 mol) in THF which was derived from diacetyl-tartaric anhydride reacted with anhydrous methanol and then $SOCl_2$. The solvent was removed with a rotary evaporator after 24 hours and the residue was hydrolyzed in a solution of K_2CO_3 in methanol at 65°C for 12 hours to afford compound (**5a**) (5.31 g, 86%); Mp 210°C (decomposition); IR (KBr, cm^{-1}): 3242, 1736, 1693, 1533, 1466, 1304, 1269, 1146, 1082, 762, 683; ESI-MS m/z: $[M+H]^+$ 310.08; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 4.47 (d, *J* = 1.8 Hz, 1H), 4.63 (s, 1H), 5.37 (s, 1H), 5.89 (s, 1H), 7.53-7.55 (m, 3H), 7.95-7.96 (q, 2H), 12.18 (s, 1H), 12.78 (s, 1H).

2,3-dihydroxy-N-(5-Benzyl-[1,3,4]thiadiazol-2-yl)-succinamic acid (5b)

White solid (4.39 g, 68%); Mp 162-163°C; IR (KBr, cm^{-1}): 3256, 1736, 1693, 1533, 1308, 1269, 1150, 1082, 700, 669; ESI-MS m/z: $[M+H]^+$ 324.02; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 4.36 (s, 2H), 4.40 (d, *J* = 1.8 Hz, 1H), 4.55 (s, 1H), 5.30 (s, 1H), 5.83 (s, 1H), 7.26-7.29 (m, 1H), 7.32-7.36 (m, 4H), 11.89 (s, 1H), 12.74 (s, 1H).

2,3-dihydroxy-N-[5-(4-methoxy-phenyl)-[1,3,4]thiadiazol-2-yl]-succinamic acid (5c)

White solid (6.03 g, 89%); Mp 193-194°C; IR (KBr, cm^{-1}): 3240, 1740, 1693, 1609, 1535, 1462, 1308, 1258, 1142, 1084, 1034, 833; ESI-MS m/z: $[M+H]^+$ 340.07; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 3.83 (s, 3H), 4.47 (s, 1H), 4.61 (d, *J* = 3.6 Hz, 1H), 5.36 (s, 1H), 5.88 (d, *J*= 6.0 Hz, 1H), 7.09 (dd, *J*= 1.8 Hz, 7.2 Hz, 2H), 7.89 (dd, *J* = 1.8 Hz, 6.6 Hz, 2H), 12.07(s, 1H), 12.78 (s, 1H).

2,3-dihydroxy-N-[5-(1-Ethyl-pentyl)-[1,3,4]thiadiazol-2-yl]-succinamic acid (5d)

White solid (4.30 g, 65%); Mp 130-132°C; IR (KBr, cm^{-1}): 3233, 2959, 2932, 2858, 1697, 1535, 1458, 1312, 1142, 1080; ESI-MS m/z: $[M+H]^+$ 332.09; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 0.80-0.84 (m, 6H), 1.20-1.30 (m, 4H), 1.61-1.65 (m, 2H), 1.70-1.75 (m, 2H), 2.99-3.04 (m, 1H), 4.43 (d, *J* = 2.4 Hz, 1H), 4.57 (d, *J* = 2.4 Hz, 1H), 5.34 (s, 1H), 5.84 (s, 1H), 11.86 (s, 1H), 12.76 (s, 1H).

Synthesis of 3-hydroxy-4-oxo-4-(5-phenyl-1,3,4-thiadiazol-2-ylamino)butanoic acid (6a)

The product was prepared from the reaction of compound (**1**) (3.79 g, 0.024 mol) with 2-amino-5-phenyl-1,3,4-thiadiazole (3.54 g, 0.020 mol) in THF at 0°C for 48 hours.

Purification is by recrystallization from water and acetic acid. Yield 52%; Mp 192-193°C; IR (KBr, cm^{-1}): 3317, 1701, 1543, 1462, 1439, 1308, 1211, 1177, 1107, 764, 687; ESI-MS m/z: $[M+H]^+$ 294.08; ^1H NMR (DMSO-d_6, 600 MHz): δ 2.61 (dd, *J* = 7.2 Hz, 15.6 Hz, 1H), 2.76 (dd, *J* = 5.4 Hz, 15.6 Hz, 1H), 4.60 (dd, *J* = 5.4 Hz, 7.2 Hz, 1H), 6.00 (s, 1H), 7.53-7.55 (m, 3H), 7.94-7.96 (m, 2H), 12.40 (s, 2H).

3-hydroxy-4-oxo-4-(5-p-tolyl-1,3,4-thiadiazol-2-ylamino) butanoic acid (6b)

White solid (3.25 g, 53%); Mp 220-221°C; IR (KBr, cm^{-1}): 3491, 3233, 1678, 1535, 1462, 1308, 1277, 1215, 1103, 814; ESI-MS m/z: $[M+H]^+$ 308.15; ^1H NMR (DMSO-d_6, 600 MHz): δ 2.37 (s, 3H), 2.60 (dd, *J* = 7.2 Hz, 15.6 Hz, 1H), 2.76 (dd, *J* = 5.4 Hz, 15.6 Hz, 1H), 4.60 (dd, *J* = 5.4 Hz, 7.2 Hz, 1H), 5.98 (s, 1H), 7.34 (d, *J* = 7.8 Hz, 2H), 7.83 (d, *J* = 7.8 Hz, 2H), 12.36 (s, 2H).

4-(5-(4-bromo-phenyl)-1,3,4-thiadiazol-2-ylamino)-3-hydroxy-4-oxobutanoic acid (6c)

White solid (3.72 g, 50%); Mp 218-219°C; IR (KBr, cm^{-1}): 3506, 3217, 1678, 1528, 1458, 1308, 1281, 1219, 1103, 837; ESI-MS m/z: $[M+2]^+$ 374.00; ^1H NMR (DMSO-d_6, 600 MHz): δ 2.61 (dd, *J* = 7.2 Hz, 16.2 Hz, 1H), 2.76 (dd, *J* = 5.4 Hz, 16.2 Hz, 1H), 4.60 (dd, *J* = 5.4 Hz, 7.2 Hz, 1H), 5.98 (s, 1H), 7.74 (dd, *J* = 2.4 Hz, 6.6 Hz, 2H), 7.91 (dd, *J* = 2.4 Hz, 6.6 Hz, 2H), 12.36 (s, 1H), 12.48 (s, 1H).

4-(5-(heptan-3-yl)-1,3,4-thiadiazol-2-ylamino)-3-hydroxy-4-oxobutanoic acid (6d)

White solid (3.72, 59%); Mp 166-167°C; IR (KBr, cm^{-1}): 3499, 3233, 2959, 2932, 2858, 1701, 1682, 1539, 1458, 1308, 1277, 1215, 1099; ESI-MS m/z: $[M+H]^+$ 316.24; ^1H NMR (DMSO-d_6, 600 MHz): δ 0.80-0.84 (m, 6H), 1.13-1.30 (m, 4H), 1.61-1.64 (m, 2H), 1.71-1.75 (q, 2H), 2.57 (dd, *J* = 7.2 Hz, 15.6 Hz, 1H), 2.73 (dd, *J* = 5.4 Hz, 15.6 Hz, 1H), 3.00-3.03 (m, 1H), 4.55 (dd, *J* = 5.4 Hz, 7.2 Hz, 1H), 5.93 (s, 1H), 12.14 (s, 1H), 12.30 (s, 1H).

ACKNOWLEDGEMENT

Thank Natural Science Foundation of JiangXi province for financial support.

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