Synthesis and Antiviral Activities of Synthetic Glutarimide Derivatives

Xing-yue Ji, Zhao-jin Zhong, Si-tu Xue, Shuai Meng, Wei-ying He, Rong-mei Gao, Yu-huan Li,* and Zhuo-rong Li*

Institute of Medicinal Biotechnology, Chinese Academy of Medical Science and Peking Union Medical College; Beijing 100050, People's Republic of China.

Received April 6, 2010; accepted August 2, 2010; published online August 4, 2010

A series of novel glutarimide compounds were synthesized and their antiviral activities were evaluated. The compounds displaying the strongest antiviral activities included 5, 6f, 7e and 9 against coxsackievirus B3 (Cox B3), 10 and 6f against influenza virus A (influenza A) and 7a against herpes simplex virus 2 (HSV-2). However, most of the synthetic glutarimides showed comparatively much weaker activity against influenza A, Cox B3 and HSV-2 than the natural glutarimide compounds tested. Based on the results, it seemed likely that a conjugated system at the β-substituted moiety provides stronger antiviral activity.

Key words glutarimide derivative; synthesis; antiviral activity; structure–activity relationship

Glutarimide antibiotics, separated from different *Streptomycete* fermentations, include more than 20 kinds of natural products. Most of those compounds possess a β-substituted glutarimide moiety in the structure, such as 9-methylstreptimidones,[1] cycloheximide (CHX)[2,3] and migrastatin[4,5] (Fig. 1). Glutarimide antibiotics have been extensively investigated because of their excellent and diverse spectrum of biological activities.[6—9] Most of the natural glutarimides possess antifungal, antibacterial, antiprotozoal, antiviral, antitumor and immuno suppression activities.

CHX, which is a well-known inhibitor of protein synthesis, shows strong inhibitory activities against plant fungi, influenza virus, reovirus and arbovirus.[10—12] Streptimidone inhibits the development of plant diseases such as phytophthora blight on pepper, gray mold on cucumber leaves, and leaf blast on rice leaves.[13] 9-Methylstreptimidone was isolated from *Streptomyces* sp., it is responsible for the high antiyeast and antifungal activity of the *Streptomycete* fermentation, and its antifungal activity is compared with that of streptimidone.[1] Migrastatin was isolated from a cultured broth of *Streptomyces* sp. as an inhibitor of tumor cell migration.[4] The 14-membered lactone core in the structure of migrastatin is the essential part for its inhibitory activity of tumor metastasis.[5] Naramycin B is a natural occurring stereoisomer of CHX, and it is active against microorganisms sensitive to CHX.[14] In our previous research, it was demonstrated that CHX and streptimidones possess potential inhibitory activities against DNA and RNA viruses, such as human immunodeficiency virus-1 (HIV-1), hepatitis B virus (HBV), herpes simplex virus (HSV), coxsackie B viruses (Cox B3, Cox B6), human enterovirus 71 (EV71) and influenza virus.[15,16] Especially, coxsackie B viruses are considered to have an important role in the etiology of viral myocarditis, and EV71 infectious disease in children became an important public health problem recently in China. In present, there are no effective drugs against these infections in clinic. Although the application of CHX was limited because of its high toxicity,[17—19] it is still a good leading compound for synthesizing and evaluating novel antiviral agents with broad spectrum activities.

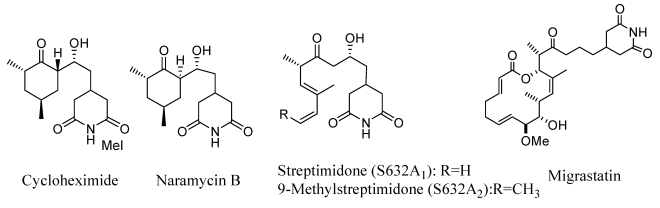


Fig. 1. The Structures of Naturally Occurring Glutarimide Compounds

In view of this, we designed and synthesized a series of analogs with glutarimide moiety in their structures, and studied their antiviral activities and toxicity. Additionally, the structure–activity relationships (SAR) of the synthesized glutarimide compounds were also investigated.

Chemistry (2,6-Dioxopiperidin-4-yl)acetaldehyde **5**, a common intermediate for the synthesis of glutarimide compounds, was synthesized according to Fig. 2.[7,20]

As shown in Fig. 2, diethyl β-oxo-glutarate was chosen as the starting material in our research. It was reacted with cyanoacetic acid under the conditions of Cope condensation to afford diethyl β-cyanomethylideneglutarate **1**, which was catalytically hydrogenated in ethanol using 5% $Pd/SrCO_3$ as catalyst to yield diethyl β-cyanomethylglutarate **2**. Then compound **2** was heated under reflux with moderately strong hydrochloric acid and the resulting homogeneous solution was evaporated to small bulk which was heated to 235 °C until a clear quiescent liquid was obtained. The melt crystallized on cooling and recrystallized from methanol to give an excellent yield of **3**. Treated **3** with $SOCl_2$ under reflux for 30 min, on cooling, compound **4** was crystallized out as a pale yellow solid. Before the Rosenmund reduction, com-

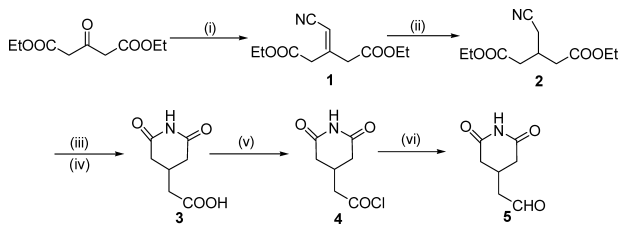
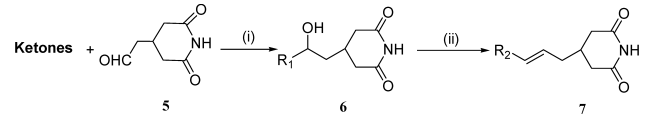


Fig. 2. The Synthesis of the Common Intermediate 3-Glutarimidylacetaldehyde

Reagents and conditions: (i) cyanoacetic acid, β-anline, HOAc, dry benzene, reflux; (ii) 5% $Pd/SrCO_3$, EtOH, H_2, 1 atm; (iii) 15% aq HCl, reflux 30 min; (iv) 235 °C, 2 h, (v) $SOCl_2$, reflux 0.5 h; (vi) 10% $Pd/BaSO_4$, dry toluene, H_2, 1 atm, reflux, 3 h.

* To whom correspondence should be addressed. e-mail: yuhuanlibj@126.com; l-z-r@263.net



	R_1		R_2
6a		**7a**	
6b		**7b**	
6c		**7c**	
6d		**7d**	
6e		**7e**	
6f		**7f**	

Fig. 3. The Synthesis of Glutarimide Derivatives

Reagents and conditions (i): LDA, tetramethylethylenediamine (TMEDA), THF, −70 °C, 0.5 h, (ii): $BF_3 \cdot Et_2O$, CH_2Cl_2, reflux, 2 h.

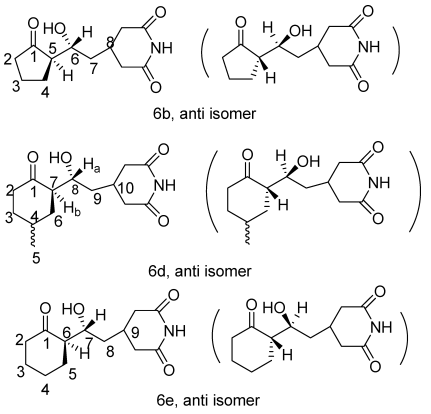


Fig. 4. The Stereo Configurations of Compounds **6b**, **6d** and **6e**

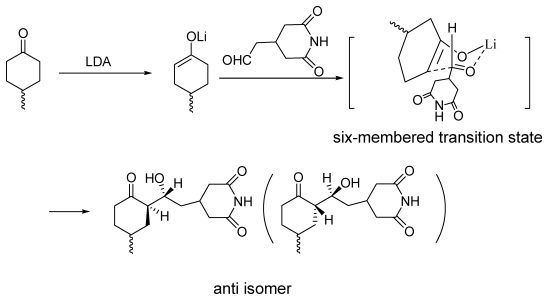


Fig. 5. A Proposed Mechanism for the Formation of *anti* Isomer

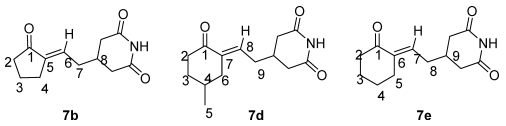


Fig. 6. The Configurations of **7b**, **7d** and **7e**

pound **4** must be recrystallized once from dry toluene and the desired key intermediate (2,6-dioxopiperidin-4-yl)acetaldehyde **5** was obtained under the conditions of Rosenmund reduction. Compounds **1**—**5** are known structures,[7,20] but only the ^{1}H-MNR spectrum data of compounds **1** and **2** was reported.[21,22] We report here for the first time the ^{1}H-NMR spectrum data of compounds **3** and **5**.

As shown in Fig. 3, a series of synthetic glutarimide derivatives were obtained by condensing compound **5** with different types of ketones, to yield novel compounds **6b**, **7b**, **6c**, **7c**, **6f**, and **7f**. Not all the alkalis used in the common aldol condensation, such as NaOH, NaH and NaOEt, were suitable for the aldol reaction because the glutarimide moiety is sensitive to base.[3,23—25] Johnson *et al.*,[3] and Egawa *et al.*[20] reported that this type of condensation can be carried out under the conditions of the Nielsen condensation, but the yield was disappointing (4—12%). In this paper, we employed the method of Kudo *et al.*[26] with a small modification: Lithium diisopropylamide (LDA) was used as the alkali for enolizing the ketone. In addition, 2 eq of LDA was used because of the acidity of (2,6-dioxopiperidin-4-yl)acetaldehyde. Consequently, 60% was achieved. The resulting compounds were dehydrated easily by treatment with $BF_3 \cdot Et_2O$ in dichloromethane to afford the dehydration products.

Didemethylcycloheximide (compound **6e**) was first synthesized by Kondo *et al.*,[27] and its stereo configuration was ascertained as an anti-configuration based on the data that the 7-H signals of **6e** were at 3.8 ppm in ^{1}H-NMR spectrum, which was in accordance with the data for naramycin B (Fig. 1), while the 7-H signals of the *syn* isomer was at about 4.2 ppm, which was in accordance with the data for natural cycloheximide (Fig. 1).[26]

As shown in Fig. 4, the configurations of the condensation products **6b** and **6d** were identified as the *anti* isomers. Compounds **6b** and **6d** showed 8-H signals at around 3.8 ppm in the ^{1}H-NMR spectrum which was consistent with that of naramycin B. The configurations of the compounds mentioned above can also be supported by the mechanism depicted in Fig. 5.

In this work, the configuration of the dehydration compounds was first determined by ^{1}H-NMR and nuclear Overhauser effect spectroscopy (NOESY) experiments. The configuration of the double bond in the dehydration products **7a**, **7c** and **7f** are the *E* configurations which were confirmed by the coupling constant (15.6, 16.0, 15.0 Hz) between the alkene double bond hydrogen. The configuration of compounds **7b**, **7d** and **7e** was determined by NOESY experiments.

Using **7b** (Fig. 6) as an example, irradiation of 6-H enhanced the intensity of 7-H, and no enhancement of intensity of 4-H was observed. Moreover, irradiation of 7-H enhanced the intensity of 4-H. Such data indicated that the configuration of the **7b** is *E* configuration, similar to **7d** and **7e**.

Compound **9**, a known natural antibiotic Actiphenol, was synthesized according to a literature method.[28] Its analog **10** was first synthesized according to Fig. 7. 2,4-Dimethylphenyl(2,6-dioxopiperidin-4-yl)acetate **8** can be obtained by mixing (2,6-dioxopiperidin-4-yl)acetyl chloride **4** and 2,4-dimethylphenol in dry pyridine and refluxed for 2 h.

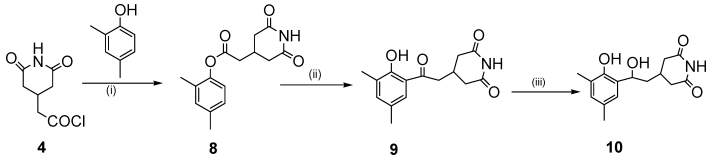


Fig. 7. Synthesis Route for Actiphenol and Its Hydrolyzed Derivatives

Reagents and conditions: (i): dry pyridine, reflux 2 h (ii): anhydrous $AlCl_3$, 155 °C, (iii): $ZnBH_4$/DME, room temperature, 2 h

Table 1. The Antiviral Activities of the Synthesized Glutarimide Derivatives

Compounds	Cox B3			Influenza virus A			HSV-2		
	TC_{50} μg/ml	IC_{50} μg/ml	SI	TC_{50} μg/ml	IC_{50} μg/ml	SI	TC_{50} μg/ml	IC_{50} μg/ml	SI
5	46.22	17.25	2.68	>500	>200	—	200	123.68	1.62
9	80.12	14.37	5.58	122.8	34.4	3.57	71.59	>18.52	—
10	38.52	>18.52	—	80.1	8.90	9.00	38.52	>18.52	—
6a	>500	129.34	>3.87	>500	>167.0	—	>500	>166.67	—
7a	4.28	>2.06	—	288.7	>167.0	—	6.17	2.06	3.00
6b	>500	>166.67	—	>500	>500	—	96.23	>18.52	—
7b	32.08	>6.67	—	>500	388.0	>1.29	32.08	>6.67	—
6c	240.37	55.56	4.33	>500	>167.0	—	240.37	43.11	5.58
7c	1.19	>0.69	—	96.2	43.11	2.2	1.71	>0.69	—
6d	>500	>166.67	—	>500	>500	—	>500	>388.03	—
7d	32.08	>18.52	—	240.4	>55.6	—	96.23	>18.52	—
6e	500	>166.67	—	>500	>500	—	>500	>166.6	—
7e	32.08	18.52	1.73	288.7	166.7	1.7	32.08	>6.67	—
6f	96.23	3.33	28.90	309.2	18.5	16.7	96.23	>18.52	—
7f	1.89	>0.23	—	96.2	32.1	3.00	3.56	>0.69	—
CHX	21.3	0.09	236.7	267.50	0.50	535	334.1	1.63	205.0
$S632A_2$	83.99	0.76	110.5	5.77	1.6	3.7	83.99	3.17	26.5
Oseltamivir	/	/	/	1260	0.29	4344.8	/	/	/
Ribavirin	2000	271.4	7.37	1278	2.13	600.1	/	/	/
Aciclovir	/	/	/	/	/	/	500	4.84	103.3

Actiphenol **9** was obtained using the Fries rearrangement. The system of $NaBH_4$/MeOH failed to reduce the carbonyl of Actiphenol **9** because of its sensitivity to base. Therefore, $ZnBH_4$/1,2-dimethoxyethane (DME) was explored to reduce the carbonyl to give compound **10** in good yield. $ZnBH_4$/DME was prepared by adding $NaBH_4$/DME to the suspension of anhydrous $ZnCl_2$ in DME, and the mixture was heated under reflux for 3 h, and then was filtered to remove the insoluble solid.

Results and Discussion

The antiviral activities of the synthesized glutarimide compounds against Cox B3, HSV-2 (SAV strain) and influenza A (A/Jifang/15/90, H1N1) were evaluated and the results are summarized in Table 1. The activities against Cox B3, and HSV-2 virus were tested by using African green monkey kidney cells (Vero cell) as the virus host, and the anti-influenza A activity was determined in Madin-Darby Canine Kidney (MDCK).

As shown in Table 1, compound **6f** was active against Cox B3, and influenza virus A. Although its IC_{50} was much higher than that of $S632A_2$, it presented much lower toxicity to MCDK in comparison with $S632A_2$. Compound **6c**, however, showed no activities against any virus, although it possessed a structure similar to **6f** except for the additional methyl group at the benzylic position. Consequently, it can be concluded that the methyl group at the benzylic position is imperative to the antiviral activities. Compound **6a** was also inactive. However, compound **9** and **10** showed low activity against Cox B3 virus and influenza virus A respectively. Therefore, it is possible that a conjugated system at the β-substituted moiety favors the antiviral activities. Compounds **6a**, **6b**, **6e**, **6d** were also inactive against the viral panel however cycloheximide possessed potent antiviral activities. It can be concluded that the *syn* isomer rather than the *anti* isomer of the aldol condensation compounds was necessary for antiviral activity, but it is also possible that it is the methyl group at the α position of the ketonic carbonyl of cycloheximide that dominated the antiviral activity.

Among the dehydration compounds, compound **7e** showed certain activity against the Cox B3 virus, but it still presented high toxicity to the Vero cell (IC_{50}=32.08 mg/ml). It is worthy of our attention that the dehydration compound **7a** showed potent activity against HSV-2 virus, whose IC_{50} value (2.06 μg/ml) was even lower than that of $S632A_2$ and Aciclovir. Consequently, it can be concluded that the dehydration compounds which contain an α, β unsaturated ketone at the β-substituted moiety favor more potent antiviral activities, especially **7a**, which contains a saturated branched-chain at the ketone moiety. However, most of the dehydration compounds showed high toxicity to the Vero cell.

As shown in the Table 1, the same glutarimide compound shows antiviral activities against different kinds of viruses, such as **6f**, CHX and $S632A_2$. It is well known that CHX is a inhibitor of protein synthesis, and inhibits virus replication mostly by directly inhibiting virus protein synthesis and/or

inhibiting host cell protein synthesis indirectly interfere virus replication. And it is deduced that the glutarimide derivatives synthesized in this paper share the same mechanism. Consequently, there is no doubt that the glutarimide antibiotics present broad spectrum antiviral activities. However, the precise molecule mechanism of glutarimide compounds against virus replication needs to be illustrated.

Conclusion

In summary, a series of novel glutarimide compounds were synthesized and the configuration of the aldol condensation products and the dehydration products was ascertained along with their antiviral activities. Some compounds presented potent antiviral activity, especially **6f**. Although its IC_{50} value was higher than $S632A_2$, it was less toxic to the MDCK. The methyl group at the benzylic position was imperative to the antiviral activity; accordingly, we can deduce that the methyl at α position of the ketonic carbonyl of $S632A_2$ may also be essential to the antiviral activity. A conjugated system in the β-substituted moiety particularly at the β position of the ketonic carbonyl also favored the antiviral activity. Because compounds **6b**, **6d** and **6e** showed no antiviral activity, it can be deduced that the *syn* isomer rather than the *anti* isomer was important to the antiviral activity. However, just like the importance of the methyl to the antiviral activity of **6f** and $S632A_2$, it is possible that the methyl at the α position of the ketonic carbonyl of CHX also determined the antiviral activity. Meanwhile, the dehydration compounds which contained an α, β unsaturated ketone at the β-substituted moiety favored the antiviral activities, in particular **7a**. However, most of the dehydration compounds showed high toxicity to the Vero cell. Therefore, our future work will be focused on the synthesis and evaluation of the antiviral activities of the syn isomer of the aldol condensation and the dehydration compounds, in order to further investigate the SAR of the glutarimide antibiotics.

Experimental

Chemistry All reagents and solvent were used without purification except for the benzene and toluene which were dried with sodium before usage, and tetrahydrofuran (THF), pyridine which were redistilled from sodium and benzophenone. ^{1}H-NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Varian Inova 400/500 MHz spectrometer (Varian, San Francisco, CA, U.S.A.). Chemical shift was reported in parts per million relative to tetramethylsilane as the internal standard. Melting points were determined with a X6 microscope melting point apparatus and were uncorrected.

Diethyl β-Cyanomethylideneglutarate (1) A mixture of diethyl β-oxoglutarate (50 g, 0.25 mol), cyanoacetic acid (23.3 g, 0.27 mol), β-alanine (1.74 g) and glac HOAc (7.5 ml) in dry benzene (50 ml) were heated under reflux and H_2O was liberated. After 10 h, β-alanine (0.75 g) and glc HOAc (2.5 ml) were added, and the reaction mixture was heated under reflux until no more H_2O was separated out (*ca.* 18 h). The mixture was cooled and EtOAc (50 ml) was added, the organic layer was washed successively with water, a saturated solution of $NaHCO_3$, water and brine. The organic layer was separated out and dried over anhydrous $MgSO_4$. The solvent was removed and the residue was purified by column chromatography on silica gel to give the title compound (41.8 g, yield 75%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.26 (6H, t, J=6.8 Hz), 3.36 (2H, s), 3.6 (2H, s), 4.15 (4H, q, J=6.8 Hz), 5.50 (1H, s).

Diethyl β-Cyanomethylglutarate (2) Catalytic hydrogenation of diethyl β-cyanomethylideneglutarate (40 g, 0.18 mol) in absolute EtOH (300 ml) was carried out at atmospheric pressure using 5% Pd/$SrCO_3$ (4.0 g) as the catalyst for 24 h. The catalyst was filtered away, and the filtrate was concentrated to give a colorless oil which was used directly in the next step (yield 98%). ^{1}H-NMR ($CDCl_3$) δ: 1.26 (6H, t, J=6.8 Hz), 2.49 (4H, m), 2.69 (3H, m), 4.15 (4H, q, J=6.8 Hz).

(2,6-Dioxopiperidin-4-yl)acetic Acid (3) A mixture of diethyl β-cyanomethylglutarate (15 g, 0.07 mol) and 15% HCl (50 ml) solution was heated under reflux for 30 min to give a homogenous solution, and the reaction mixture was concentrated *in vacuo* to afford a pale yellow oil which was then heated to 235 °C until a clear quiescent liquid was obtained. The liquid was cooled and the product was crystallized out, after recrystallization from absolute MeOH, the title compound was obtained (9.4 g, yield 78%) as white solid. mp 172—173 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.29 (3H, m), 2.38 (2H, m), 2.53 (2H, dd, J=3.2, 16 Hz), 10.71 (1H, s), 12.29 (1H, s).

(2,6-Dioxopiperidin-4-yl)acetyl Chloride (4) A slurry of the acid (8.6 g, 0.05 mol) and thionyl chloride (40 ml) was heated under reflux for 25 min. The orange reaction mixture was cooled and the crystalline solid that precipitated was filtered rapidly, washed with dry toluene. A colorless prism was obtained after recrystallization from dry toluene (yield 70%). mp 130—132 °C.

(2,6-Dioxopiperidin-4-yl)acetaldehyde (5) To a flask with a gas inlet tube, stirrer and condenser were added dry toluene (150 ml), once-recrystallized (2,6-dioxopiperidin-4-yl)acetyl chloride (3.4 g, 0.018 mol) and 10% palladiumon–barium sulfate catalyst (0.3 g). The mixture was heated under reflux and hydrogen was passed through the system. The hydrochloric acid formed was neutralized with KOH (0.94 g, 0.016 mol) in water (10 ml). When the solution of KOH became neutral, the reaction was stopped and the hot toluene solution was filtered free of catalyst. On cooling, long colorless needles of the aldehyde were deposited. These were removed and a second crop was obtained by concentrating the filtrate. In total, 1.8 g (yield 64%) of the aldehyde was obtained. mp 121—123 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.38 (2H, dd, J=10.8, 18 Hz), 2.61 (2H, d, J=6.0 Hz), 2.71 (3H, m), 7.92 (1H, br), 9.78 (1H, s).

4-(2-Hydroxy-5-methyl-4-oxohexyl)piperidin-2,6-dione (6a) To a solution of 2 N LDA in hexane (3 ml) in dry THF was added 3-methylbutan-2-one (516 mg, 6 mmol) and tetramethylethylenediamine (TMEDA) (697 mg, 6 mmol) at −70 °C under N_2 atmosphere. The mixture was stirred 30 min at this temperature, then a solution of (2,6-dioxopiperidin-4-yl)acetaldehyde (465 mg, 3 mmol) in dry THF (10 ml) was added at −70 °C, and stirring was continued for another 30 min. The reaction was quenched with 10% of HOAc aqueous solution (30 ml), and extracted with CH_2C1_2 (3×10 ml). The extract was washed with aq. $NaHCO_3$ and brine, and dried over anhydrous $MgSO_4$. Evaporation of the solvent left a pale yellow oil, which was triturated with petroleum ether to wash away the unreacted ketone, and the residue was recrystallized form CH_2Cl_2/petroleum ether to give a white solid of the title compound (yield 60%). mp 94—96 °C ^{1}H-NMR (DMSO-d_6) δ: 0.97 (6H, d, J=6.8 Hz), 1.33 (2H, m), 2.24 (3H, m), 2.45 (2H, m), 2.58 (3H, m), 3.94 (1H, m), 4.67 (1H, d, J=5.6 Hz), 10.64 (1H, s). Electrospray ionization (ESI)-MS m/z: 242.13911 $[M+H]^+$ (Calcd for $C_{12}H_{20}NO_4$: 242.13923).

(*E*)-4-(5-Methyl-4-oxohex-2-enyl)piperidin-2,6-dione (7a) A solution of **6a** (200 mg, 0.83 mmol) and 46% $BF_3 \cdot Et_2O$ (1 ml) in CH_2Cl_2 (15 ml) was heated under reflux for 2 h. The reaction mixture was poured into saturated aq. $NaHCO_3$ (20 ml), the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2×10 ml). The organic layer was combined and washed with saturated aqueous solution of $NaHCO_3$, water, and brine successively. Dried over anhydrous $MgSO_4$, and removed of the solvent to give a pale yellow oil which was recrystallized from CH_2Cl_2/petroleum ether to afford the title compound as a white solid (yield 78%). mp 62—64 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.12 (6H, d, J=6.8 Hz), 2.32 (5H, m), 2.74 (3H, m), 6.26 (1H, d, J=15.6 Hz), 6.75 (1H, m), 7.80 (1H, s). Electron ionization (EI)-MS m/z: 223.1198 $[M]^+$ (Calcd for $C_{12}H_{17}NO_3$: 223.1208).

4-[2-Hydroxy-2-(2-oxocyclopentyl)ethyl]piperidin-2,6-dione (6b) By a similar method to **6a**, compound **6b** was synthesized (yield 56%) as a white solid. mp 120—122 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.15 (1H, m), 1.62 (3H, m), 1.76 (1H, m), 1.88 (2H, m), 2.16 (5H, m), 2.51 (2H, m), 3.79 (1H, m), 4.77 (1H, d, J=5.2 Hz), 10.63 (1H, s). ESI-MS m/z: 240.12557 $[M+H]^+$ (Calcd for $C_{12}H_{18}NO_4$: 240.12358).

(*E*)-4-[2-(2-Oxocyclopentylidene)ethyl]piperidin-2,6-dione (7b) By a similar method to **7a**, compound **7b** was obtained (yield 76%) as a white solid. mp 90—92 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.85 (1H, m), 1.97 (2H, m), 2.26 (2H, t, J=6.8 Hz), 2.35 (4H, m), 2.57 (2H, t, J=6.8 Hz), 2.72 (2H, d, J=13.2 Hz), 6.45 (1H, t, J=6.8 Hz), 7.83 (1H, s). EI-MS m/z: 221.1035 $[M]^+$ (Calcd for $C_{12}H_{15}NO_3$: 221.1052).

4-(2-Hydroxy-4-oxo-5-*o*-tolylpentyl)piperidin-2,6-dione (6c) By a similar method to **6a**, compound **6c** was synthesized (yield 54%) as a white solid. mp 65—66 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.28 (3H, m), 2.06 (1H, d, J=6.8 Hz), 2.12 (3H, s), 2.26 (5H, m), 3.79 (2H, s), 3.93 (1H, m), 4.82 (1H, d, J=5.6 Hz), 7.10 (4H, m), 10.64 (1H, s). ESI-MS m/z: 304.15541 $[M+H]^+$ (Calcd for $C_{17}H_{22}NO_4$: 304.15488).

(*E*)-4-(4-Oxo-5-*o*-tolylpent-2-enyl)piperidin-2,6-dione (7c) By a similar method to **7a**, compound **7c** was obtained (yield 78%) as a white solid. mp 152—154 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.22 (3H, s), 2.28 (5H, m), 2.70 (2H, d, J=17.0 Hz), 3.82 (2H, s), 6.21 (1H, d, J=16.0 Hz), 6.78 (1H, m), 7.11 (1H, d, J=6.5 Hz), 7.18 (3H, m), 7.73 (1H, s). EI-MS m/z: 285.1351 $[M]^+$ (Calcd for $C_{17}H_{19}NO_3$: 285.1365).

4-[2-Hydroxy-2-(5-methyl-2-oxocyclohexyl)ethyl]piperidin-2,6-dione (6d) By a similar method to **6a**, compound **6d** was synthesized (yield 55%) as a white solid. mp 125—127 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.93 (3H, d, J=6.8 Hz), 1.36 (4H, m), 1.75 (1H, m), 1.90 (1H, m), 2.09 (2H, m), 2.22 (4H, m), 2.62 (3H, m), 3.82 (1H, m), 4.72 (1H, d, J=6.8 Hz), 10.65 (1H, s). ESI-MS m/z: 268.15707 $[M+H]^+$ (Calcd for $C_{14}H_{22}NO_4$: 268.15488).

(*E*)-4-[2-(5-Methyl-2-oxocyclohexylidene)ethyl]piperidin-2,6-dione (7d) By a similar method to **7a**, compound **7d** was obtained (yield 78%) as a white solid. mp 125—127 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.07 (3H, d, J=6.4 Hz), 1.53 (1H, m), 1.93 (3H, m), 2.17 (2H, m), 2.36 (4H, m), 2.64 (4H, m), 6.49 (1H, t, J=7.6 Hz), 7.78 (1H, s). EI-MS m/z: 249.1348 $[M]^+$ (Calcd for $C_{14}H_{19}NO_3$: 249.1365).

4-[2-Hydroxy-2-(2-oxocyclohexyl)ethyl]piperidin-2,6-dione (6e) By a similar method to **6a**, compound **6e** was synthesized (yield 61%) as a white solid. mp 93—95 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.29 (2H, t, J=6.0 Hz), 1.44 (1H, m), 1.59 (2H, m), 1.81 (2H, m), 1.97 (1H, m), 2.27 (5H, m,), 2.38 (1H, m), 2.56 (2H, m), 3.87 (1H, m), 4.51 (1H, d, J=5.6 Hz), 10.63 (1H, s). ESI-MS m/z: 254.14052 $[M+H]^+$ (Calcd for $C_{13}H_{20}NO_4$: 254.13923).

(*E*)-4-[2-(2-Oxocyclohexylidene)ethyl]piperidin-2,6-dione (7e) By a similar method to **7a**, compound **7e** was obtained (yield 82%) as a white solid. mp 152—154 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.78 (2H, m), 1.88 (2H, m), 2.22 (2H, t, J=6.4 Hz), 2.33 (3H, m), 2.45 (4H, m), 2.73 (2H, m), 6.50 (1H, t, J=7.6 Hz), 7.77 (1H, s). EI-MS m/z: 235.1200 $[M]^+$ (Calcd for $C_{13}H_{17}NO_3$: 235.1208).

4-(2-Hydroxy-4-oxo-5-*o*-tolylhexyl)piperidin-2,6-dione (6f) By a similar method to **6a**, compound **6f** was synthesized (yield 63%) as a white solid. mp 121—123 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.20 (3H, d, J=7.0 Hz), 1.26 (2H, m), 2.16 (4H, m), 2.31 (3H, s), 2.35 (1H, d, J=7.0 Hz), 2.44 (2H, m,), 3.84 (1H, br), 4.01 (1H, q, J=7.0 Hz), 4.65 (1H, d, J=5.5 Hz), 6.97 (1H, d, J=6.5 Hz), 7.16 (2H, m), 7.19 (1H, d, J=5.5 Hz), 10.62 (1H, s). ESI-MS m/z: 318.17107 $[M+H]^+$ (Calcd for $C_{18}H_{24}NO_4$: 318.17053).

(*E*)-4-(4-Oxo-5-*o*-tolylhex-2-enyl)piperidin-2,6-dione (7f) By a similar method to **7a**, compound **7f** was obtained (yield 81%) as a white solid. mp 149—151 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.41 (3H, d, J=6.5 Hz), 2.17 (5H, m), 2.44 (3H, s), 2.60 (2H, d, J=16.0 Hz), 4.02 (1H, q, J=6.5 Hz), 6.04 (1H, d, J=15.0 Hz), 6.68 (1H, m), 7.02 (1H, t, J=5.0 Hz), 7.22 (3H, m), 7.71 (1H, s). EI-MS m/z: 300.16142 $[M+H]^+$ (Calcd for $C_{18}H_{22}NO_3$: 300.15997).

2,4-Dimethylphenyl(2,6-dioxopiperidin-4-yl)acetate (8) The acid chloride **4** (from 5 g, of **3**) was dissolved in dry pyridine (35 ml) and 2,4-dimethylphenol (5 g, 0.041 mol) added in one portion. The mixture was heated at 90 °C for 2 h, and then poured into a mixture of water (250 ml) and methylene chloride (100 ml). Some unwanted black material was separated at this stage by filtration. The organic layer in the filtrate was washed with dilute hydrochloric acid, then water, and dried over anhydrous magnesium sulfate. Removed of the solvent to give a white solid followed by recrystallization from methylene chloride–ether to gave the title compound (yield 80%), mp 154—156 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.13 (3H, s), 2.31 (3H, s), 2.48 (2H, dd, J=10.4, 16.8 Hz), 2.69 (2H, d, J=6.8 Hz), 2.78 (1H, m), 2.87 (2H, dd, J=4.0, 16.8 Hz), 6.86 (1H, d, J=8.0 Hz), 7.01 (1H, d, J=8.0 Hz), 7.05 (1H, s), 7.90 (1H, s).

Actiphenol (9) A flask containing the finely ground ester **8** (5.0 g, 0.018 mol) mixed with pulverized anhydrous aluminum chloride (10 g, 0.075 mol) was placed in an oil bath at 100 °C, and the temperature rose to 155 °C quickly. The oil bath was held at this temperature for 2 h, and the reaction mixture then was allowed to cool. The resulting fused mass was ground in a mortar and added to a mixture of hydrochloric acid (200 ml 2 N) and methylene chloride (100 ml) and ice. After stirring for a short period, the organic layer was separated and processed as usual. The resulting solid was purified by column chromatography over silica gel to afford actiphenol (2.7 g, yield 55%) mp 193—196 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.23 (3H, s), 2.28 (3H, s), 2.42 (2H, dd, J=10.0, 16.8 Hz), 2.89 (3H, m), 3.09 (2H, d, J=6.4 Hz), 7.20 (1H, s), 7.29 (1H, s), 7.88 (1H, s), 12.17 (1H, s). EI-MS m/z: 275.1157 $[M]^+$ (Calcd for $C_{15}H_{17}NO_4$: 275.1158).(the ^{1}H-NMR data correspond well to that previously reported in the literature[29]).

4-[2-Hydroxy-2-(2-hydroxy-3,5-dimethylphenyl)ethyl]piperidin-2,6-dione (10) Actiphenol **9** (200mg, 0.73 mmol) dissolved in DME (10 ml) was added to $Zn(BH_4)_2$ in DME (2 ml) at room temperature, the stirring was continued for 2 h, the mixture was cooled to 0 °C, and hydrochloric acid was added to adjust the pH to 2. The mixture was extracted with CH_2Cl_2 (3×15 ml), the organic layer was washed with a saturated aqueous solution of $NaHCO_3$, water, and brine successively, and dried over anhydrous $MgSO_4$. The solvent was removed to afford a white solid which was purified by column chromatography over silica gel to give the title compound (yield 78%). mp 166—168 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.55 (2H, m), 2.09 (3H, s), 2.14 (3H, s), 2.26 (3H, m), 2.58 (2H, m), 4.87 (1H, m), 5.55 (1H, d, J=4.4 Hz), 6.74 (1H, s), 6.84 (1H, s), 8.27 (1H, s), 10.64 (1H, s). EI-MS m/z: 277.1301 $[M]^+$ (Calcd for $C_{15}H_{19}NO_4$: 277.1314).

Antiviral Assays African green monkey kidney cells (Vero), Madin-Darby Canine Kidney (MDCK), Coxsackie viruses (Cox B3 Nancy strain), influenza A (A/Jifang/15/90, H1N1) and HSV-2 (SAV strain) were all from the Institute of Virology, Chinese Academy of Preventive Medicine.

Cytotoxicity Determination. Cytotoxicity Assay The cytotoxicity of compounds in the presence of Vero and MDCK cells were monitored by cytopathic effect (CPE). Vero and MDCK cells (2.5×10^4/well) were plated into a 96-well plate. A total of 24 h later, the monolayer cells were incubated in the presence of various concentrations of test compounds. After 48 h of culture at 37 °C and 5% CO_2 in a carbon–dioxide incubator, the cells were monitored by CPE. Median toxic concentration (TC_{50}) was calculated by Reed and Muench analyses.

Anti Coxsackie B3 and HSV-2 Activity Assay Confluent Vero cells grown in 96-well microplates were infected respectively with 100 median tissue culture infective dose ($100TCID_{50}$) HSV-2 or Cox B3. After 1 h adsorption at 37 °C, the monolayers were washed by phosphate buffered saline (PBS) and incubated at 37 °C in the maintenance medium (MEM plus 2% fetal bovine serum (FBS)) with or without different concentrations of test compounds. Viral cytopathic effect (CPE) was observed when the viral control group reached 4 and the antiviral activity of tested compounds was determined by the Reed and Muench analyses.

Anti-influenza A Assays Confluent MDCK cells grown in 96-well microplates were infected $100TCID_{50}$ with influenza A. After 2 h of adsorption at 37 °C, the monolayers were washed by PBS and incubated at 37 °C in the maintenance medium with or without different concentrations of test compounds. Viral cytopathic effect (CPE) was observed when the viral control group reached 4 and the antiviral activity of glutarimide derivatives was determined by the Reed and Muench analyses.

Acknowledgements This work was supported by the National Natural Science Foundation of China (Grant no. 30772600) and National S&T Major Special Project on Major New Drug Innovation (Item Number: 2009ZX09103-135).

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