

Hui Zheng, Juan Liu, and Pengfei Zhang*

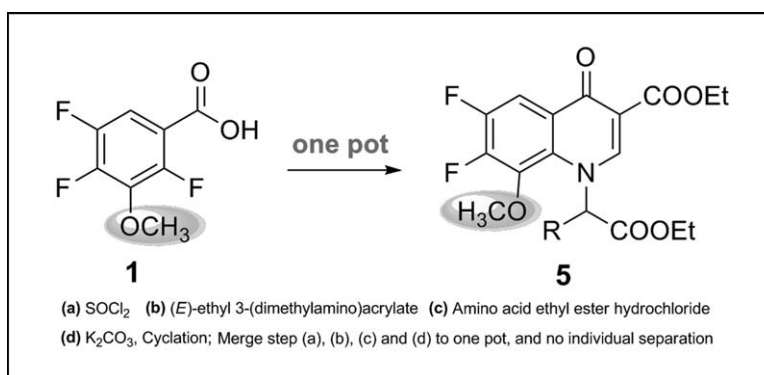
College of Material, Chemistry and Chemical Engineering, Hangzhou Normal University,
Hangzhou, 310036, China

*E-mail: chxyzpf@hotmail.com

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A series of novel quinolone heterocyclic derivatives from natural amino acid were synthesized in one-pot method, which is very beneficial for the industrial operation to save manufacturing costs. These new compounds were characterized by ^1H NMR, ^{13}C NMR, infrared spectrum, mass spectrum, and elemental analysis. The preliminary bioassays results revealed that they had certain antimicrobial activity against *Bacillus subtilis* and *Staphylococcus aureus*.

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INTRODUCTION

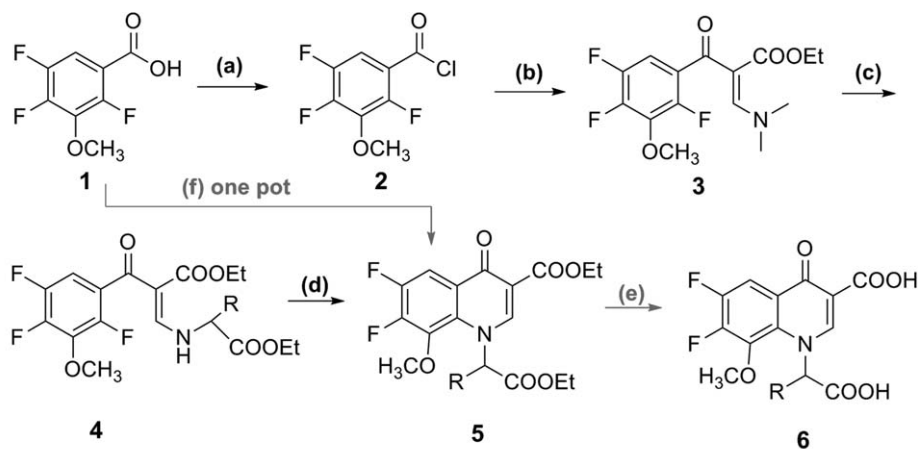
Interests in heterocycle compounds [1], especially quinolone heterocyclic compounds, have flourished for many years, largely as a result of the wide spread use on anti-asthmatic, anti-bacterial, anti-malarial, tyrosine-kinase PDGF-RTK inhibitor agent, etc. [2]. Because of such a wide range of applicability in medicine and bioorganic chemistry, there has been increasing interests in exploring new quinolone heterocyclic compounds to enrich this domain [3]. Enlightened and encouraged by the bioactivity of quinolone heterocyclic compounds and the abundant existence of amino acid in nature, we attempt to introduce amino acid piece into quinolone heterocycle and wish to discover some new bioactive compounds.

Generally, there are several steps and much complicated post-treatment and purification process to synthesize the quinolone and fluoroquinolone derivatives, such as 4-oxoquinoline, 8-nitrofluoro-quinolone, and other quinolone derivatives [4–7]. Therefore, we wish to develop a concise route with simple post-treatment to synthesize a series of novel quinolone heterocyclic compounds. Fortunately, when we use one-pot method to synthesize compounds **5** from compound **1**, the whole reaction goes well and smoothly. This method is very beneficial for the industrial operation to save time and manufacturing costs. Herein a series of novel quinolone heterocyclic derivatives from

natural amino acid salts were designed and prepared according to this method. The title compounds were characterized by ^1H NMR, ^{13}C NMR, infrared spectrum, mass spectrum, and elemental analysis. To investigate their preliminary antimicrobial bioactivities, these compounds were screened by *Bacillus subtilis*, *Staphylococcus aureus*, *Aspergillus fumigatus*, and *Candida albicans*. The preliminary bioassays results revealed that they had certain bactericidal activity against *Bacillus subtilis* and *Staphylococcus aureus*. The synthetic routes are shown in Figure 1.

RESULTS AND DISCUSSION

Chemistry. As part of our ongoing interests to find new quinolone heterocyclic derivatives, we try to obtain the quinolone compounds *via* intramolecular cyclization reaction, which contain natural amino acid piece at N1-position of quinolone heterocyclic ring. We selected the 2,4,5-trifluoro-3-methoxybenzoic acid **1** as started materials to synthesize quinolone derivatives **6**. At first, we synthesized compounds **6** step by step from **1** to **5** with complicated post-treatment and purification in each step. Interestingly, when we attempt to synthesize compounds **5** from compound **1** in one-pot to cut off much troublesome separation and purification in the middle process we found the whole reaction goes well. So a facile and simple route



(a) SOCl_2 ; (b) (E)-ethyl 3-(dimethylamino)acrylate; (c) Amino acid ethyl ester hydrochloride;

(d) K_2CO_3 ; (e) Hydrolysis; (f) Merge step (a), (b) and (c) to one pot, and no individual separation

Figure 1. (a) SOCl_2 ; (b) (E)-ethyl 3-(dimethylamino)acrylate; (c) Amino acid ethyl ester hydrochloride; (d) K_2CO_3 ; (e) Hydrolysis; (f) Merge step (a), (b), and (c) to open-pot, and no individual separation.

for the synthesis of compound **5** in one-pot way without any separation was developed, which is very beneficial for the industrial operation to save manufacturing costs.

The structures of quinolone heterocyclic compounds of **6a–6f** were confirmed by IR, ^1H NMR, ^{13}C NMR, MS, and elemental analysis. The infrared spectra of all compounds showed easily distinguishable carboxylic group stretching at $1789\text{--}1799\text{ cm}^{-1}$ and $1721\text{--}1747\text{ cm}^{-1}$ because of the two kind of carboxylic groups. In ^1H NMR spectrum, two kind of carboxylic group signals appear between $13.42\text{--}13.73\text{ ppm}$ and $14.42\text{--}14.58\text{ ppm}$, which demonstrate the existence of amino acids and quinolone cycle. In ^{13}C NMR spectrum, the typical signals of carboxylic group and aromatic ring appear between $164.47\text{--}176.71\text{ ppm}$ and $102.85\text{--}158.97\text{ ppm}$, which reveal the existence of the quinolone cycle.

Biological activities. All the title compounds were tested preliminarily for their antibacterial and antifungal activity. From the screening results, all the title compounds have certain activity against *Bacillus subtilis* and

Staphylococcus aureus and nearly have no activity against *Aspergillus fumigatus* and *Candida albicans*. The detail results are listed in Table 1.

From the preliminary biological results, all the new compounds have certain antibacterial and antifungal bioactivity, which intrigued us to study its QSAR further and synthesize higher activity compound, the in-depth research work is undergoing.

In conclusion, we have developed a simple one-pot way of preparing quinolone heterocyclic compounds and synthesized a series of novel quinolone heterocyclic derivatives from natural amino acid. This method facilitates the industrial operation to save manufacturing costs. The preliminary bioassays results reveal that they have certain antimicrobial activity against *Bacillus subtilis* and *Staphylococcus aureus*.

EXPERIMENTAL

All the reagents were purchased commercially and used without further purification. The melting point was determined

Table 1

The preliminary antimicrobial activity of title compounds.

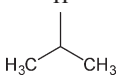
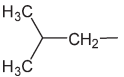
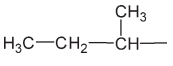

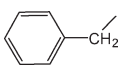
	Conc. (mg/mL)	6a	6b	6c	6d	6e	6f
<i>Bacillus subtilis</i>	1	0.95 ^a	0.75	0.85	0.80	0.90	1.25
	0.2	0.60	0.65	0.65	0.65	0.75	0.60
	0.2	– ^b	–	–	–	0.60	–
<i>Staphylococcus aureus</i>	1	0.85	0.70	0.70	1.20	1.10	0.95
	0.2	0.60	0.60	–	0.75	1.00	0.65
	0.1	–	–	–	–	0.85	–

^a Diameter of inhibition zone (cm).

^b No inhibition.

Table 2

Chemical structure, yields, and melt point of the synthesized compounds.

Compound	R	Yield (%)	M. P (°C)
6a	H	52	258–260
6b		52	251–253
6c		50	310–312
6d		49	284–286
6e		52	162–164
6f		48	195–197

using a XT-4 melting-point apparatus and uncorrected. IR spectra were recorded on a Bruker Equinox-55 spectrophotometer using KBr discs in the 4000–400 cm^{-1} region. The ^1H NMR and ^{13}C NMR data were obtained on a Bruker AC-400 (400 MHz) instrument in $\text{DMSO}-d_6$ using TMS as internal standard. Chemical shifts (δ) are expressed in ppm and coupling constants J are given in Hz. Mass spectra were obtained on a Agilent 5973N mass spectrometer operating at 70 eV by electron ionization technique (EI/MS). Elemental analyses were performed on an EA-1110 instrument.

General synthesis procedure of compound **6a** is described below and other compounds were synthesized in similar way. The chemical structure, yields, and melt points of the synthesized compounds are listed in Table 2.

1-(Carboxymethyl)-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6a). 2,4,5-trifluoro-3-methoxybenzoic acid 4.12 g (0.02 mol) was dissolved in thionyl chloride 22 mL in a 50 mL round-bottomed flask, stirring and reflux for 3 h, removing the superfluous SOCl_2 on a rotary evaporator. (E)-ethyl-3-(dimethylamino) acrylate 2.86 g (0.02 mol) was added dropwise to the toluene solution of above leavings with stirring at 40–50°C, monitoring with TLC [PE/EA = 1/1 (v/v)] to the end of reactants, adding amino acid ethyl ester hydrochloride (0.02 mol) directly and stirring for about 5–8 h at room temperature, checking the reaction *via* TLC, then potassium carbonate 4.14 g (0.03 mol) was added and stirring for 8–10 h at 100°C. When the reaction was complete (determined by TLC), the mixture was acidified with diluted hydrochloride acid (5%) to $\text{pH} = 2-3$. The reaction mixture was poured into separatory funnel and separated. The organic layer was concentrated under reduced pressure and the residual was chromatographed on silica gel (PE/EA=2/1 (v/v)) to give compound **5**, which was added to the 20% sulfuric acid solution, refluxing for about 6–8 h, cooled to room temperature and filter. The filter cake was crystallized using 20% alcohol aqueous solution to afford compounds **6a**. IR (KBr) ν : 3493, 2981, 2809, 1799, 1746, 1632, 1581, 1484, 1348, 1215, 1062 cm^{-1} ; ^1H NMR ($\text{DMSO}-d_6$, 400 MHz): δ 4.02 (s, 3H,

OCH_3), 5.37 (s, 2H, CH_2), 8.03–8.07 (m, 1H, ArH), 9.01 (s, 1H, $\text{C}=\text{CH}$), 13.42 (br s, 1H, COOH), 14.55 (br s, 1H, COOH); ^{13}C NMR ($\text{DMSO}-d_6$, 100 MHz): δ 59.33, 63.59, 107.56, 107.21 (d, $^2J_{\text{C-F}} = 17.8$ Hz), 122.90 (d, $^3J_{\text{C-F}} = 7.20$ Hz), 131.86 (d, $^3J_{\text{C-F}} = 3.39$ Hz), 140.64 (d, $^2J_{\text{C-F}} = 11.7$ Hz), 147.45 (dd, $^1J_{\text{C-F}} = 107.2$ Hz, $^2J_{\text{C-F}} = 15.4$ Hz), 149.96 (dd, $^1J_{\text{C-F}} = 104.1$ Hz, $^2J_{\text{C-F}} = 15.6$ Hz), 153.57, 165.50, 169.37, 176.43; MS (70 eV): m/z (%) = 313 (M^+); Anal. Calcd. for $\text{C}_{13}\text{H}_9\text{F}_2\text{NO}_6$: C, 49.85; H, 2.90; N, 4.47; Found: C, 50.01; H, 4.45; N, 4.51.

1-(1-Carboxy-2-methylpropyl)-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6b). IR (KBr) ν : 3488, 2981, 2898, 1795, 1726, 1631, 1573, 1499, 1378, 1216, 1058 cm^{-1} ; ^1H NMR ($\text{DMSO}-d_6$, 400 MHz): δ 0.82 (d, $J = 7.20$ Hz, 6H, CH_3), 2.11–2.14 (m, 1H, CH), 3.68–3.70 (m, 1H, CH), 4.21 (s, 3H, OCH_3), 8.01–8.04 (m, 1H, ArH), 9.12 (s, 1H, $\text{C}=\text{CH}$), 13.73 (br s, 1H, COOH), 14.52 (br s, 1H, COOH); ^{13}C NMR ($\text{DMSO}-d_6$, 100 MHz): δ 21.71, 22.18, 29.42, 61.56, 68.51, 108.22, 109.43 (d, $^2J_{\text{C-F}} = 15.6$ Hz), 121.75 (d, $^3J_{\text{C-F}} = 5.62$ Hz), 132.13 (d, $^3J_{\text{C-F}} = 4.2$ Hz), 141.21 (d, $^2J_{\text{C-F}} = 14.6$ Hz), 146.13 (dd, $^1J_{\text{C-F}} = 108.3$ Hz, $^2J_{\text{C-F}} = 14.3$ Hz), 148.68 (dd, $^1J_{\text{C-F}} = 107.5$ Hz, $^2J_{\text{C-F}} = 15.1$ Hz), 152.98, 164.67, 169.66, 175.13; MS (70 eV): m/z (%) = 355 (M^+); Anal. Calcd. for $\text{C}_{16}\text{H}_{15}\text{F}_2\text{NO}_6$: C, 54.09; H, 4.26; N, 3.94; Found: C, 54.12; H, 4.19; N, 4.01.

1-(1-Carboxy-3-methylbutyl)-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6c). IR (KBr) ν : 3482, 2959, 2852, 1789, 1721, 1631, 1557, 1499, 1348, 1210, 1078 cm^{-1} ; ^1H NMR ($\text{DMSO}-d_6$, 400 MHz): δ 0.87 (d, $J = 7.20$ Hz, 6H, CH_3), 1.36–1.42 (m, 1H, CH), 2.11–2.15 (m, 1H, CH_2), 3.81–3.83 (m, 1H, CH), 4.36 (s, 3H, OCH_3), 8.63–8.67 (m, 1H, ArH), 9.07 (s, 1H, $\text{C}=\text{CH}$), 13.62 (br s, 1H, COOH), 14.58 (br s, 1H, COOH); ^{13}C NMR ($\text{DMSO}-d_6$, 100 MHz): δ 22.25, 23.19, 26.22, 29.57, 62.96, 71.55, 102.73, 107.60 (d, $^2J_{\text{C-F}} = 15.8$ Hz), 128.08 (d, $^3J_{\text{C-F}} = 4.2$ Hz), 139.11 (d, $^3J_{\text{C-F}} = 4.8$ Hz), 142.94 (d, $^2J_{\text{C-F}} = 15.2$ Hz), 145.39 (dd, $^1J_{\text{C-F}} = 108.3$ Hz, $^2J_{\text{C-F}} = 14.6$ Hz), 148.84 (dd, $^1J_{\text{C-F}} = 108.1$ Hz, $^2J_{\text{C-F}} = 14.2$ Hz), 153.29, 165.82, 171.79, 176.71; MS (70 eV): m/z (%) = 369 (M^+); Anal. Calcd. for $\text{C}_{17}\text{H}_{17}\text{F}_2\text{NO}_6$: C, 55.29; H, 4.64; N, 3.79; Found: C, 55.38; H, 4.52; N, 3.92.

1-(1-Carboxy-2-methylbutyl)-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6d). IR (KBr) ν : 3491, 2965, 2851, 1798, 1742, 1656, 1581, 1498, 1346, 1213, 1078 cm^{-1} ; ^1H NMR ($\text{DMSO}-d_6$, 400 MHz): δ 0.74–0.79 (m, 6H, CH_3), 0.88–0.95 (m, 2H, CH_2), 2.01–2.05 (m, 1H, CH), 3.89–3.92 (m, 1H, CH), 4.23 (s, 3H, OCH_3), 8.00–8.05 (m, 1H, ArH), 9.18 (s, 1H, $\text{C}=\text{CH}$), 13.53 (br s, 1H, COOH), 14.49 (br s, 1H, COOH); ^{13}C NMR ($\text{DMSO}-d_6$, 100 MHz): δ 11.74, 13.77, 25.55, 38.01, 65.82, 67.97, 102.85 (d, $^2J_{\text{C-F}} = 19.1$ Hz), 109.68, 129.76 (d, $^3J_{\text{C-F}} = 5.6$ Hz), 139.05 (d, $^3J_{\text{C-F}} = 6.4$ Hz), 142.12 (d, $^2J_{\text{C-F}} = 17.1$ Hz), 147.92 (dd, $^1J_{\text{C-F}} = 108.2$ Hz, $^2J_{\text{C-F}} = 12.2$ Hz), 149.15 (dd, $^1J_{\text{C-F}} = 109.1$ Hz, $^2J_{\text{C-F}} = 14.8$ Hz), 158.97, 165.62, 171.12, 176.55; MS (70 eV): m/z (%) = 369 (M^+); Anal. Calcd. for $\text{C}_{17}\text{H}_{17}\text{F}_2\text{NO}_6$: C, 55.29; H, 4.64; N, 3.79; Found: C, 55.31; H, 4.59; N, 3.77.

1-(Carboxy(phenyl)methyl)-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6e). IR (KBr) ν : 3495, 2926, 2809, 1796, 1746, 1632, 1581, 1484, 1348, 1259, 1105 cm^{-1} ; ^1H NMR ($\text{DMSO}-d_6$, 400 MHz): δ 4.11 (s, 3H, OCH_3), 4.33 (s, 1H, CH), 7.49–7.54 (m, 5H, ArH), 8.06–8.08 (m, 1H, ArH), 9.05 (s, 1H, $\text{C}=\text{CH}$), 13.56 (br s, 1H, COOH),

14.42 (br s, 1H, COOH); ^{13}C NMR (DMSO- d_6 , 100 MHz): δ 62.75, 69.83, 107.31 (d, $^2J_{\text{C-F}} = 18.2$ Hz), 109.78, 123.34, 129.39, 130.21 (d, $^3J_{\text{C-F}} = 5.7$ Hz), 131.22, 132.44, 133.03, 133.41, 140.78 (d, $^3J_{\text{C-F}} = 5.2$ Hz), 141.06 (d, $^2J_{\text{C-F}} = 16.4$ Hz), 148.12 (dd, $^1J_{\text{C-F}} = 108.8$ Hz, $^2J_{\text{C-F}} = 13.6$ Hz), 149.47 (dd, $^1J_{\text{C-F}} = 107.6$ Hz, $^2J_{\text{C-F}} = 12.2$ Hz), 150.69, 165.14, 168.89, 176.32; MS (70 eV): m/z (%) = 389 (M^+); Anal. Calcd. for $\text{C}_{19}\text{H}_{13}\text{F}_2\text{NO}_6$: C, 58.62; H, 3.37; N, 3.60; Found: C, 58.71; H, 3.39; N, 3.71.

1-(1-Carboxy-2-phenylethyl)-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6f). IR (KBr) ν : 3495, 2958, 2868, 1798, 1747, 1617, 1568, 1474, 1353, 1279, 1110 cm^{-1} ; ^1H NMR (DMSO- d_6 , 400 MHz): δ 3.23–3.27 (m, 2H, CH_2), 3.89–3.92 (m, 1H, CH), 4.21 (s, 3H, OCH_3), 7.31–7.42 (m, 5H, ArH), 8.11–8.14 (m, 1H, ArH), 9.02 (s, 1H, $\text{C}=\text{CH}$), 13.42 (br s, 1H, COOH), 14.58 (br s, 1H, COOH); ^{13}C NMR (DMSO- d_6 , 100 MHz): δ 36.89, 74.09, 106.78 (d, $^2J_{\text{C-F}} = 16.8$ Hz), 107.94, 123.14, 127.08, 127.24, 128.74, 129.33, 129.71, 129.89 (d, $^3J_{\text{C-F}} = 5.2$ Hz), 136.91, 140.05 (d, $^3J_{\text{C-F}} = 6.1$ Hz), 145.32 (d, $^2J_{\text{C-F}} = 15.6$ Hz), 148.16 (dd, $^1J_{\text{C-F}} = 107.4$ Hz, $^2J_{\text{C-F}} = 15.6$ Hz), 150.41 (dd, $^1J_{\text{C-F}} = 108.1$ Hz, $^2J_{\text{C-F}} = 14.7$ Hz), 154.03, 165.16, 172.41, 176.15; MS (70 eV): m/z (%) = 403 (M^+); Anal. Calcd. for $\text{C}_{20}\text{H}_{15}\text{F}_2\text{NO}_6$: C, 59.56; H, 3.75; N, 3.47; Found: C, 59.62; H, 3.69; N, 3.51.

All of the synthesized compounds were tested for their antibacterial and antifungal activity *in vitro* by broth dilution method with some bacteria *Bacillus subtilis*, *Staphylococcus aureus*, *Aspergillus fumigatus*, and *Candida albicans* according to the literature [8]. The antimicrobial discs (diameter, 0.55 cm) were prepared at concentrations of 1, 0.2, and 0.1 mg/mL and applied to each of the culture plates previously seeded with the test bacteria. These culture plates were then incubated at 37°C for 24 h. The preliminary antimicrobial activity was determined by the diameter of inhibition zone. For each com-

pound, three replicate trials were conducted against each organism.

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REFERENCES AND NOTES

- [1] (a) Enguehard, C. G.; Gueiffier, A. *Mini-Rev Med Chem* 2007, 7, 888; (b) Chrisman, W.; Knize, M. G.; Tanga, M. J. *J Heterocycl Chem* 2008, 45, 1641; (c) Abdelhamid, A. O. *J Heterocycl Chem* 2009, 46, 680.
- [2] (a) Chen, Y. L.; Fang, K. C.; Sheu, J. Y.; Hsu, S. L.; Tzeng, C. C. *J Med Chem* 2001, 44, 2374; (b) Silva, A. D.; Almeida, M. V.; Souza, M. V. N.; Couri, M. R. C. *Curr Med Chem* 2003, 10, 21; (c) Beek, D. V.; Wijdicks, E. F. M.; Vermeij, F. H.; Haan, R. J.; Prins, J. M.; Spanjaard, L.; Dippel, D. W. J.; Nederkoorn, P. J. *Arch Neuro* 2009, 66, 1076.
- [3] (a) Murugesan, D.; Palaniappan, S.; Perumal, Y.; Arnab, C.; Valakunja, N.; Dharmarajan, S. *Bioorg Med Chem Lett* 2008, 18, 1229; (b) Chai, Y.; Wan, Z. L.; Wang, B.; Guo, H. Y.; Liu, M. L. *Eur J Med Chem* 2009, 44, 4063.
- [4] Harbeson, S. L. PCT WO 2009/035662, 19 March 2009.
- [5] Al-Hiari, Y. M.; Al-Mazari, I. S.; Shakya, A. K.; Darwish, R. M.; Abu-Dahab, R. *Molecules* 2007, 12, 1240.
- [6] Okada, T.; Tsuji, T.; Tsushima, T.; Yoshida, T.; Matsuura, S. *J Heterocycl Chem* 1991, 28, 1061.
- [7] Kimura, Y.; Atarashi, S.; Kawakami, K.; Sato, K.; Haya-kawa, I. *J Med Chem* 1994, 37, 3344.
- [8] (a) Kong, W. J.; Zhao, Y. L.; Xiao, X. H.; Li, Z. L.; Jin, C.; Li, H. B. *J Appl Microbiol* 2009, 107, 1072; (b) Andre, S.; Patrick, J. K. *Plant Cell Tissue Organ Culture* 1984, 3, 111.