Enantioselective Synthesis and Antimicrobial Activities of Tetrahydro-*B*-Carboline Diketopiperazines

YANGMIN MA,[1]* HAO WU,[1] JIN ZHANG,[1] AND YANCHAO LI[2]

[1]*Key Laboratory of Auxiliary Chemistry and Technology for Chemical Industry, Ministry of Education, Shaanxi University of Science & Technology, Xi'an, China*

[2]*Northwest Institute of Nonferrous Metals, Xi'an, China*

ABSTRACT A series of single isomers tetrahydro-β-carboline diketopiperazines were stereoselectively synthesized starting from L-tryptophan methyl ester hydrochloride and six aldehydes through a four-step reaction including Pictet-Spengler reaction, crystallization-induced asymmetric transformations (CIAT), Schotten-Baumann reaction, and intramolecular ester amidation. The chemical structures were characterized by nuclear magnetic resonance (NMR) and elemental analysis, among which two compounds were determined by x-ray single crystal diffraction. Moreover, antimicrobial activities of all the compounds were also tested. *Chirality 00:000–000, 2013.*

KEY WORDS: antimicrobial activity; configuration; crystallization-induced asymmetric transformations; tetrahydro-β-carboline diketopiperazine; x-ray single crystal diffraction

INTRODUCTION

A series of indole alkaloids have been used on the treatment of many diseases such as cancer and AIDS.[1] Diketopiperazines as the smallest cyclopeptides have two hydrogen-bond donors and two receptors; the hydrogen bonds are the main way for coactions with receptors.[2] Tetrahydro-β-carboline diketopiperazines, which block cell cycle progression, are attracting much attention as potential therapeutic agents.[3,4] Tetrahydro-β-carboline diketopiperazines are of wide interest because they exhibit a wide range of biological activities.[5–7] Verruculogen TR-2A and fumitremorgin C, for example, are selective inhibitors of the breast cancer resistance protein (BCRP/ABCG2).[3,8,9] In addition, fumitremorgin C can also induce sustained tremors in animals.[10–16] We have found that they have a tetrahydro-β-carboline diketopiperazines skeleton by comparing the structures, as shown in Fig. 1.

Mostly, a series of tetrahydro-β-carboline diketopiperazines were extracted as secondary metabolites of fungi.[17] For example, fumitremorgin C was isolated from metabolites of *Alternaria* sp. FL25.[4] Because of the unique structures and important activities, a lot of natural tetrahydro-β-carboline diketopiperazines have been prepared through total synthesis. However, the traditional methods gave lower yield and *cis* and *trans* diastereomeric mixtures. The tetrahydro-β-carboline skeleton is the basic structure unit of tetrahydro-β-carboline diketopiperazines. The first problem to be addressed was the construction of the 1,3-disubstituted-1,2,3,4-tetrahydro-β- carboline framework (shown in Fig. 2) with establishment of the C-1 stereocenter. In 1997, Wang and Ganesan and Hino[18] and Nakagawa[19] used a two-step reaction to synthesize demethoxyfumitremorgin C. In 2001, Bailey et al.[20] reported a three-step method of total synthesis of fumitremorgins with yields between 21% and 38% to get diastereomeric mixtures. Both methods were of low yield. One of the most straightforward ways for the construction of the key skeleton was based on the Pictet-Spengler reaction,[21–24] of which the main advantage was the formation of a product with a stable C–C bond in a single step. However, almost all the approaches inevitably led to the diastereomeric mixtures of tetrahydro-β-carboline (*cis*- and *trans*-) which are difficult to separate[25–28] and may accordingly make the reaction hard to scale up. What's more, crystallization-induced asymmetric transformation (CIAT) has been reported in the purification of chiral compounds[29–31] in in recent years. The combination of an asymmetric Pictet-Spengler reaction and CIAT was a very important and useful tool for constructing synthons containing tetrahydro-β-carbolines structural moieties and able to obtain single enantiomers.[32–37]

In this study, a series of tetrahydro-β-carboline diketopiperazines were synthesized starting from L-tryptophan methyl ester hydrochloride and a series of aldehydes. Instead of the low-productive reaction by preparing Schiff base from L–tryptophan methyl ester first and then ring-closing via protonic acid,[38] L-tryptophan methyl ester hydrochloride and aldehydes were directly refluxed in isopropanol. The new one-step reaction had much higher yields. In order to obtain the single chiral isomers, CIAT was used after Pictet-Spengler reaction with high enantiomeric excess (*ee)* values. We synthesized six new tetrahydro–β–carboline diketopiperazines for the first time, and two of them were confirmed by single-crystal x-ray diffraction analysis. Moreover, the antibacterial and antifungal activities of all six final compounds were also determined.

MATERIALS AND METHODS

L-Tryptophan, benzaldehyde, vanillin, 4-hydroxy- benzaldehyde, *i*-butyraldehyde, 4-nitrobenzaldehyde, anisaldehyde, and Fmoc-L-proline were

Additional Supporting Information may be found in the online version of this article.

Contract grant sponsor: Scientific Research Project Item for Key Laboratory of Shaanxi Province Education Department, China; Contract grant number: No. 2010JS056.

Contract grant sponsor: Xianyang City Science and Technology Project, China; Contract grant number: No. 2010 K10-06.

*Correspondence to: Y. Ma, Key Laboratory of Auxiliary Chemistry and Technology for Chemical Industry, Ministry of Education, Shaanxi University of Science & Technology, Xi'an 710021, China. E-mail: mym63@sina.com

Received for publication 21 April 2013; Accepted 07 May 2013

DOI: 10.1002/chir.22193

Published online in Wiley Online Library (wileyonlinelibrary.com).

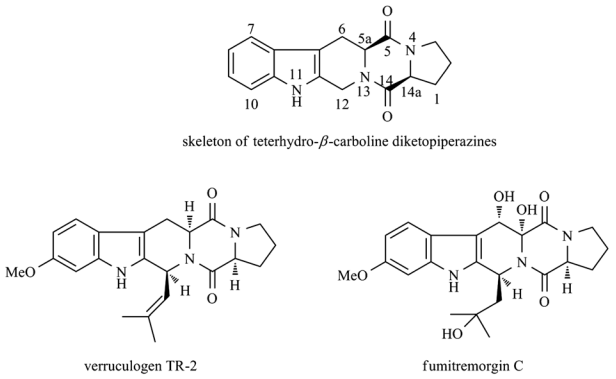


Fig. 1. Skeleton of tetrahydro-¦Â-carboline diketopiperazines and their analogues.

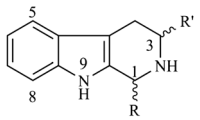


Fig. 2. Basic skeleton of 1,3-disubstituted-1,2,3,4-tetrahydro-¦Â-carboline framework.

purchased from Aladdin Chemical (Shanghai, China). Sodium carbonate, anhydrous calcium chloride and anhydrous magnesium sulfate were purchased from Tianjin Baishi Chemical Engineering (China). Morpholine, dichlorosulfane, and all the solvents were purchased from Tianjin Hongyan Chemical Reagents Factory (China). Silica gel for column chromatography (200–300 mesh) was purchased from Tsingtao Haiyang Silica Gel Desiccant Factory (China). The IR chromatography was recorded with a Bruker VECTOR–22 FT-IR Spectrometer (Billerica, MA). The optical rotations were measured through Wuhan Gelai WZZ-1S polarimeters. 1D and 2D nuclear magnetic resonance (NMR) spectra were recorded on Bruker AVANCE 400 MHz spectrometer with tetramethylsilane (TMS) as internal standard. Deuterated chloroform and deuterated dimethylsulfoxide were purchased from Beijing Boya Dabei Technological Development (China). The elemental analysis was carried out with an Elemeraor Vario EL III elemental analyzer. The x-ray single crystal diffraction analysis was carried out with a Bruker BRUKERSMARTAPEXIICCD diffractometer; the data were restored by TEXSAN program, analyzed on an Lx97 system. The bacteria *E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis*, and fungi *C. gloeosporioides*, *V. mali*, *A. alternata*, *A. brassicae* were provided from the College of Life Science & Engineering, Shaanxi University of Science & Technology (Xi'an, P.R. China). Both the antibacterial and antifungal activities were determined by the minimum inhibitory concentration (MIC) method on 96-well cell culture cluster, which was made by Costar (Cambridge, MA).

EXPERIMENTAL

Preparation of Basic Materials

L-Tryptophan methyl ester hydrochlorid. Fifty mL of methanol in a dried 3-neck flask with a condenser added and a dry pipe with anhydrous $CaCl_2$ solid, an isobarical dropping funnel, and a stirrer. An ice bath no more than 5 °C, 15 mL $SOCl_2$ was slowly dropped in the flask at the rate of 10–15 drops per minute from the isobarical dropping funnel. After droppimg, it was stirred for 30 minutes and then withdrawn in an ice-bath and heated to reflux. TLC (ethyl acetate as the developer) should be used to track the reaction process. After that, the solvent was cooled to room temperature, L-tryptophan added (3.06 g, 15 mmol), and then refluxed for 4 h, tracked by TLC (ethyl acetate as the developer), after the reaction, the solvents were removed, recrystallized from methanol-ethyl ether (5:1, v/v) to give 3.55 g (yield 93%) of **1**. White solid, mp. 217–218 °C. ^{1}H NMR (400 MHz, DMSO-d_6) (ppm, from TMS): 11.15 (s, 1H, NH on the indole ring), 8.65 (s, 3H, -NH_3^+), 7.51 (d, J = 7.96 Hz, 1H, 4-H), 7.38 (d, J = 8.04 Hz, 1H, 7-H), 7.26 (s, 1H, 2-H), 7.10 (m, 1H, 6-H), 7.01 (m, 1H, 5-H), 4.22 (t, J = 4.06 Hz, 1H, -CH_2-CH-$COOCH_3$), 3.65 (s, 3H, -$COOCH_3$), 3.30 (m, 2H, -CH_2-CH$COOCH_3$); ^{13}C NMR (100 MHz, DMSO-d_6) (ppm, from TMS): 170.23, 136.67, 127.35, 122.45, 121.62, 119.07, 118.45, 112.03, 106.77, 53.10, 26.55. IR (KBr) ν/cm^{-1}: 3256, 2955, 1746, 1230. Anal. Calcd. for $C_{12}H_{15}N_2O_2Cl$: C, 56.59; H, 6.00; N, 11.07. Found: C, 56.58; H, 5.98; N, 11.10%.

Fmoc-L-prolyl chloride (Fmoc-L-Pro-Cl, 6). Fifty mL of CH_2Cl_2 in a dried 3-neck flask with a condenser added and a dry pipe with anhydrous $CaCl_2$ solid, an isobarical dropping funnel, and a stirrer. Five mL $SOCl_2$ was slowly dropped in the flask at the rate of 10–15 drops per minute from the isobarical dropping funnel. After that, the solvent was heated and refluxed for about 4 h, tracked by TLC, and reduced pressure distillation to obtain the orange liquid **6**. **6** was dissolved in 50 mL CH_2Cl_2 and sealed to preserve.

One of the six aldehydes (10 mmol) was added with **1** (2.62 g, 12 mmol) into isopropanol (50 mL). The reaction was refluxed for 4 h and then the solvent was removed, washed with toluene, and dried. This was pushed into a toluene-nitromethane mixture, refluxed for 10–22 h, and then filtered with decompression, washed by the mixed solvent, and dried, to afford products **3a–3f**. **3** was added into saturated sodium carbonate aqueous solution (50 mL), stirred for full reaction, extracted by ethyl acetate (30 mL × 3), dried by anhydrous $MgSO_4$, solvent removed, and recrystallized from methanol to afford crystals **3a–3f**. (The yields for reaction are shown in Table 1.)

(1*S*,3*S*)-methyl 1-phenyl-2,3,4,9-tetrahydro-1*H*–pyrido [3,4–*b*]indole–3–carboxylate (*cis*-3a). Yield 86.5%, white solid, mp. 223–224 °C, $[\alpha]_D^{20}$ + 14.2° (*c* 1.5, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$) (ppm, from TMS): 7.56–7.52 (m, 1H, Ar-H), 7.45 (s, 1H, NH on the indole ring), 7.40–7.34 (m, 5H, Ar-H), 7.22–7.18 (m, 1H, Ar-H), 7.17–7.10 (m, 2H, Ar-H), 5.23 (s, 1H, 1-H), 3.98 (dd, J = 11.2, 4.2 Hz, 1H, 3-H), 3.81 (s, 3H, -$COOCH_3$), 3.23 (m, 1H, 4-Hβ), 3.05–2.97 (m, 1H, 4-Hα), 2.45 (br s, 1H, 2-H). ^{13}C NMR (100 MHz, $CDCl_3$) (ppm, from TMS): 173.23, 140.72, 136.14, 134.70, 129.01, 128.66, 127.11, 121.99, 119.66, 118.24, 110.96, 108.93, 58.71, 56.91, 52.32, 25.73. IR(KBr) ν/cm^{-1}: 3394, 3332, 2952, 2785, 1740, 1452, 1437, 1357, 1325, 1205, 746, 690. Anal. calcd. for $C_{19}H_{18}N_2O_2$: C, 74.56; H, 6.03; N, 9.15. Found: C, 74.49; H, 6.02; N, 9.15%.

(1*S*,3*S*)-methyl 1-(4-hydroxy-3-methoxyphenyl)-2,3,4,9- tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (*cis*-3b). Yield 95.7%, white solid, mp. 177–178 °C, $[\alpha]_D^{20}$ - 41.0° (*c* 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$) (ppm, from TMS): 7.58 (s, 1H, 2′-H), 7.56 (s, 1H, NH on the indole ring), 7.28–7.22 (m, 1H, Ar-H), 7.20–7.12 (m, 2H, Ar-H), 6.92–6.87 (m, 3H, Ar-H), 5.18 (s, 1H, 1-H), 3.99 (dd, J =11.1, 4.1 Hz, 1H, H-3), 3.84 (s, 3H, -$COOCH_3$), 3.80 (s, 3H, Ar-OCH_3), 3.25 (dd, J =15.1, 2.5 Hz, 1H, 4-Hβ), 3.08–2.97 (m, 1H, 4-Hα). ^{13}C NMR (100 MHz, $CDCl_3$) (ppm, from TMS): 173.27, 147.07, 145.96, 136.09, 135.02, 132.52, 127.19, 121.91, 121.55, 119.62, 118.20, 114.31, 110.99, 110.64, 108.69, 58.69, 56.98, 56.02, 52.30, 25.63. IR(KBr) ν/cm^{-1}: 3405, 3263, 2931, 1741, 1518, 1449 , 1269, 1223, 744. Anal. calcd. for $C_{20}H_{20}N_2O_4$: C, 68.17; H, 5.72; N, 7.95. Found: C, 68.43; H, 5.74; N, 7.94%.

TABLE 1. Preparation of single enantiomer tetrahydro-β-carboline diketopiperazines from L-tryptophan methyl ester hydrochloride and aldehydes by a four-step synthesis

			Step 2 (CIAT)							
Entry	R	Step 1 time (h)	Solvent CH_3NO_2 / Toluene[a]	Time[b] (h)	Configuration (*cis*-/*trans*-)[c]	*ee* (%)	Yield (%)	Step 3 time (h)	Step 4 time (min)	Yield[d] (%)
5a	Ph	4	1:10	22	*cis*-**3a**	98	86.5	3	30	92.1
5b	3-OMe-4-OH-C_6H_3	4	1:1	10	*cis*-**3b**	98	95.7	3	30	97.7
5c	4-OH-C_6H_4	4	2:3	10	*cis*-**3c**	99	97.2	3	30	96.5
5d	*i*-Pr	4	1:1	20	*trans*-**3d**	98	94.6	3	30	95.6
5e	4-NO_2-C_6H_4	4	2:3	20	*cis*-**3e**	99	93.5	3	30	91.4
5f	4-OCH_3-C_6H_4	4	4:5	12	*trans*-**3f**	99	87.4	3	30	93.8

[a]Volume ratio.
[b]Under reflux.
[c]Determined by ^{1}H-^{1}H NOESY.
[d]Total yield of Steps 3 and 4.

(1*S*,3*S*)-methyl 1-(4-hydroxyphenyl)-2,3,4,9-tetrahydro- 1*H*-pyrido [3,4-*b*]indole-3-carboxylate (*cis*-3c). Yield 97.2%, white solid, mp. 227–228 °C, $[\alpha]_D^{20}$ - 33.2° (*c* 1.2, acetone), ^{1}H NMR (400 MHz, DMSO-d_6) (ppm, from TMS): 10.31 (s, 1H, NH on the indole ring), 9.42 (s, 1H, ArOH), 7.42 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.21 (d, *J* = 7.9 Hz, 1H, Ar-H), 7.14 (d, *J* = 8.4 Hz, 2H, Ar-H), 6.97 (dt, *J* = 14.7, 7.1 Hz, 2H, Ar-H), 6.75 (d, *J* = 8.3 Hz, 2H, Ar-H), 5.11 (s, 1H, 1-H), 3.86 (dd, *J* = 11.0, 4.0 Hz, 1H, 3-H), 3.71 (s, 3H, -OCH_3), 3.02 (dd, *J* = 14.6, 3.1 Hz, 1H, 4-H*β*), 2.81 (t, *J* = 13.8 Hz, 1H, 4-H*α*). ^{13}C NMR (100 MHz, DMSO-d_6) (ppm, from TMS): 173.45, 157.53, 136.74, 136.36, 132.70, 130.17, 127.03, 121.06, 118.80, 117.97, 115.51, 111.66, 107.20, 57.70, 56.74, 52.24, 25.93. IR(KBr) ν/cm^{-1}: 3365, 3300, 3269, 2981, 1730, 1615, 1522, 1458, 1437, 1325, 1278, 1219, 1178, 835, 752, 740. Anal. calcd. for $C_{19}H_{18}N_2O_3$: C, 70.70; H, 5.62; N, 8.70. Found: C, 70.79; H, 5.63; N, 8.69%.

(1*R*,3*S*)-methyl 1-isopropyl-2,3,4,9-tetrahydro-1*H*-pyrido [3,4-*b*] indole-3-carboxylate (*trans*-3d). Yield 94.6%, white solid, mp. 146–147 °C, $[\alpha]_D^{20}$ + 53.4° (*c* 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, DMSO-d_6) (ppm, from TMS): 10.65 (s, 1H, NH on the indole ring), 7.38 (d, *J* = 7.7 Hz, 1H, Ar-H), 7.28 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.01 (t, *J* = 7.3 Hz, 1H, Ar-H), 6.94 (t, *J* = 7.3 Hz, 1H, Ar-H), 4.12 (d, *J* = 2.7 Hz, 1H, 1-H), 3.97 (t, *J* = 5.0 Hz, 1H, 3-H), 3.59 (s, 3H, -OCH_3), 2.91 (d, *J* = 4.8 Hz, 2H, 4-H), 2.70 (br s, 1H, 2-H), 2.33–2.11 (m, 1H, -C<u>H</u>$(CH_3)_2$), 1.05 (d, *J* = 6.9 Hz, 3H, -CH-C<u>H_3</u>), 0.73 (d, *J* = 6.7 Hz, 3H, -CH-C<u>H_3</u>). ^{13}C NMR (100 MHz, DMSO-d_6) (ppm, from TMS): 175.03, 136.40, 136.06, 127.13, 120.78, 118.62, 117.79, 111.30, 106.40, 54.48, 53.67, 51.99, 32.19, 24.76, 20.04, 17.24. IR(KBr) ν/cm^{-1}: 3330, 2965, 2880, 1730, 1459, 1457, 1425, 1338, 1271, 1225, 1196, 1135, 1003, 836, 742, 630. Anal. calcd for $C_{16}H_{20}N_2O_2$: C, 70.56; H, 7.40; N, 10.29. Found: C, 70.47; H, 7.42; N, 10.32%.

(1*S*,3*S*)-methyl 1-(4-nitrophenyl)-2,3,4,9-tetrahydro-1*H*- pyrido [3,4-*b*]indole-3-carboxylate (*cis*-3e). Yield 93.5%, yellow solid, mp. 171–172 °C, $[\alpha]_D^{20}$ - 5.4° (*c* 2.5, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$) (ppm, from TMS): 8.21 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.59 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.56 (d, *J* = 7.2 Hz, 1H, Ar-H), 7.46 (s, 1H, NH on the indole ring), 7.22 (t, *J* = 6.2 Hz, 1H, Ar-H), 7.19–7.11 (m, 2H, Ar-H), 5.38 (s, 1H, 1-H), 3.98 (dd, *J* = 11.1, 4.1 Hz, 1H, 3-H), 3.83 (s, 3H, -OCH_3), 3.26 (dd, *J* = 14.7, 3.1 Hz, 1H, 4-H*β*), 3.03 (ddd, *J* = 15.12, 11.24, 2.16 Hz, 1H, 4-H*α*), 2.60 (br s, 1H, 2-H). ^{13}C NMR (100 MHz, $CDCl_3$) (ppm, from TMS): 172.92, 148.24, 148.05, 136.36, 132.98, 129.62, 126.87, 124.16, 122.47, 119.98, 118.43, 111.06, 109.53, 58.07, 56.58, 52.46, 25.49. IR(KBr) ν/cm^{-1}: 3397, 3338, 2950, 2789, 1452, 1440, 1357, 650. Anal. calcd. for $C_{19}H_{17}N_3O_4$: C, 64.95; H, 4.88; N, 11.96. Found: C, 64.86; H, 4.87; N, 12.00%.

(1*R*,3*S*)-methyl 1-(4-methoxyphenyl)-2,3,4,9-tetrahydro- 1*H*-pyrido [3,4-*b*]indole-3-carboxylate (*trans*-3f).. Yield 87.4%, white solid, mp. 195–197 °C, $[\alpha]_D^{20}$ - 44.0° (*c* 2.5, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$) (ppm, from TMS): 7.73 (br s, 1H, on the indole ring NH), 7.54 (d, *J* = 6.96 Hz, 1H, Ar-H), 7.22–7.18 (m, 1H, Ar-H), 7.17–7.09 (m, 4H, Ar-H), 6.82 (d, *J* = 8.52 Hz. 2H, Ar-H), 5.30 (s, 1H, 1-H), 3.93 (t, *J* = 6.10 Hz, 1H, 3-H), 3.76 (s, 3H, -COOC<u>H_3</u>), 3.69 (s, 3H, -ArOC<u>H_3</u>), 3.24 (dd, *J* = 15.36 Hz, 5.24 Hz, 1H, 4-H*α*), 3.09 (dd, *J* = 15.28 Hz, 6.88 Hz, 1H, 4-H*β*), 2.46 (br s, 1H, 2-H) ^{13}C NMR (100 MHz, $CDCl_3$) (ppm, from TMS): 174.22, 159.43, 136.13, 134.14, 133.63, 129.60, 127.02(2), 121.91, 119.49, 118.24, 114.05(2), 110.93, 108.34, 55.35, 54.31, 52.52, 52.16, 24.68. IR(KBr) ν/cm^{-1}: 3400, 3343, 2791, 1740, 1442, 1205, 710. Anal. calcd. for $C_{20}H_{20}N_2O_3$: C, 71.41; H, 5.99; N, 8.33. Found: C, 71.35; H, 5.98; N, 8.35%.

Synthesis of Tetrahydro-β-carboline Diketopiperazines (cis- or trans-5a–5f)

Compound **6** (1.01 g, 2.84 mmol) was dissolved in CH_2Cl_2, then compound **3** added (2.4 mmol), which was also dissolved in CH_2Cl_2 under stirring. After that, it was put into saturated sodium carbonate aqueous solution (50 mL) to make a biphasic system. It was stirred for 4 h and then stilled, separated from the CH_2Cl_2 phase, the water phase extracted by CH_2Cl_2 (30 mL × 3), the organic phases gathered and dried by anhydrous magnesium sulfate, and the solvent removed to give **4**. **4** was resolved in CH_2Cl_2, morpholine added (5 mL), and after stirring for 40 min at room temperature, the solvent removed to obtain the final product. The product was purified by column chromatography with the eluents of petroleum ether, ethyl acetate, and methanol to afford compounds **5a–5f**. (The yields for reaction were shown in Table 1.)

(5a*S*,12*S*,14a*S*)-12-phenyl-1,2,3,5a,6,14a-hexahydropyrrolo [1",2":4',5']pyrazino[1',2':1,6]pyrido[3,4-*b*]indole-5,14 (11*H*,12*H*)-dione (*cis*-5a). Yield 92.1%, white solid, mp. 329–330 °C, ^{1}H NMR (400 MHz, DMSO-d_6) (ppm, from TMS): 11.25 (s, 1H, NH on the indole ring), 7.58 (d, *J* = 7.7 Hz, 1H, Ar-H), 7.35 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.29 (m, 4H, Ar-H), 7.17 (t, *J* = 7.0 Hz, 1H, Ar-H), 7.08 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.02 (t, *J* = 7.4 Hz, 1H, Ar-H), 6.36 (s, 1H, 12-H), 4.56 (dd, *J* = 11.5, 5.0 Hz, 1H, 5a-H), 4.36 (t, *J* = 7.8 Hz, 1H, 14a-H), 3.59–3.48 (m, 2H, 3-H), 3.46 (d, *J* = 5.4 Hz, 1H, 6-H*β*), 3.03 (dd, *J* =15.7,

11.8 Hz, 1H ,6-Hα), 2.26–2.14 (m, 1H, 1-H), 2.00–1.90 (m, 1H, 1-H), 1.90–1.79 (m, 2H, 2-H). ^{13}C NMR (100 MHz, DMSO-d_6) (ppm, from TMS): 170.40, 165.86, 143.25, 136.52, 134.51, 128.94, 127.41, 126.22, 126.19, 121.72, 119.40, 118.62, 111.85, 104.96, 58.88, 56.74, 55.46, 45.35, 28.50, 23.11, 22.04. IR(KBr) ν/cm^{-1}: 3285, 1663,1456, 1396. Anal. calcd. for $C_{23}H_{21}N_3O_2$: C, 74.37; H, 5.70; N, 11.31. Found: C, 74.44; H, 5.69; N, 11.28%.

(5a*S*,12*S*,14a*S*)-12-(4-hydroxy-3-methoxyphenyl)-1,2,3,5a,6,14a-hexahydropyrrolo[1'',2'':4',5']pyrazino[1',2':1,6]pyrido[3,4-*b*] indole-5,14 (11*H*,12*H*)-dione (*cis*-5b). Yield 97.7%, white solid, mp. 254–255 °C, ^{1}H NMR (400 MHz, DMSO-d_6) (ppm, from TMS): 11.22 (s, 1H, NH on the indole ring), 8.90 (s, 1H, Ar-OH), 7.55 (d, J = 7.8 Hz, 1H, Ar-H), 7.33 (d, J = 8.0 Hz, 1H, Ar-H), 7.06 (t, J = 7.5 Hz, 1H, Ar-H), 7.00 (t, J = 7.4 Hz, 1H, Ar-H), 6.89 (s, 1H, Ar-H), 6.62 (d, J = 8.2 Hz, 1H, Ar-H), 6.55 (d, J = 8.1 Hz, 1H, Ar-H), 6.29 (s, 1H, 12-H), 4.52 (dd, J = 11.6, 5.3 Hz, 1H, 5a-H), 4.36 (t, J = 7.9 Hz, 1H, 14a-H), 3.70 (s, 3H, Ar-OCH$_3$), 3.55-3.44 (m, 2H, 3-H), 3.41 (dd, J = 15.8, 5.4 Hz, 1H, 6-Hβ), 2.97 (dd, J = 15.6, 11.8 Hz, 1H, 6-Hα), 2.21 (td, J = 12.0, 5.2 Hz, 1H, 1-H), 1.97 (dt, J = 11.7, 8.7 Hz, 1H, 1-H), 1.87 (dd, J = 12.5, 6.1 Hz, 2H, 2-H). ^{13}C NMR (100 MHz, DMSO-d_6) (ppm, from TMS): 170.20, 166.13, 147.73, 145.95, 136.33, 134.93, 134.01, 126.18, 121.56, 119.32, 118.54, 117.93, 115.77, 111.82, 110.79, 104.61, 58.93, 56.60, 55.95, 54.56, 45.37, 28.33, 23.27, 21.68. Anal. calcd. for $C_{24}H_{23}N_3O_4$: C, 69.05; H, 5.55; N, 10.07. Found: C, 69.10; H, 5.54; N, 10.04%.

(5a*S*,12*S*,14a*S*)-12-(4-hydroxyphenyl)-1,2,3,5a,6,14a-hexahydropyrrolo[1'',2'':4',5']pyrazino[1',2':1,6]pyrido[3,4-*b*] indole-5,14 (11*H*,12*H*)-dione (*cis*-5c). Yield 96.5%, white solid, mp. 278–279 °C, ^{1}H NMR (400 MHz, DMSO-d_6) (ppm, from TMS): 11.13 (s, 1H, NH on the indole ring), 9.30 (s, 1H, Ar-OH), 7.56 (d, J = 7.7 Hz, 1H, Ar-H), 7.31 (d, J = 8.0 Hz, 1H, Ar-H), 7.08–6.97 (m, 4H, Ar-H), 6.62 (d, J = 8.5 Hz, 2H, Ar-H), 6.25 (s, 1H, 12-H), 4.50 (dd, J = 11.6, 5.3 Hz, 1H, 5a-H), 4.33 (t, J = 7.8 Hz, 1H, 14a-H), 3.54–3.44 (m, 2H, 3-H), 3.41 (dd, J = 16.0, 5.3 Hz, 1H, 6-Hβ), 2.97 (dd, J = 15.6, J = 11.8 Hz, 1H, 6-Hα), 2.25–2.16 (m, 1H, 1-H), 1.95 (dd, J = 17.3, 9.1Hz, 1H, 1-H), 1.86 (dt, J = 15.1, 7.6 Hz, 2H, 2-H). ^{13}C NMR (100 MHz, DMSO-d_6) (ppm, from TMS): 170.33, 165.95, 156.81, 136.43, 135.14, 133.39, 127.73, 126.23, 121.56, 119.30, 118.53, 115.48, 111.79, 104.77, 58.91, 56.75, 54.70, 45.29, 28.41, 23.15, 21.88. Anal. calcd. for $C_{23}H_{21}N_3O_3$: C, 71.30; H, 5.46; N, 10.85. Found: C, 71.39; H, 5.43; N, 10.82%.

(5a*S*,12*R*,14a*S*)-12-isopropyl-1,2,3,5a,6,14a-hexahydro-pyrrolo[1'',2'':4',5']pyrazino[1',2':1,6]pyrido[3,4-*b*]indole-5,14(11*H*,12*H*)-dione (*tras*-5d). Yield 95.6%, white solid, mp. 254–255 °C, ^{1}H NMR (400 MHz, DMSO-d_6) (ppm, from TMS): 10.93 (s, 1H, NH on the indole ring), 7.43 (d, J = 7.8 Hz, 1H, Ar-H), 7.34 (d, J = 8.0 Hz, 1H, Ar-H), 7.07 (t, J = 7.3 Hz, 1H, Ar-H), 6.98 (t, J = 7.3 Hz, 1H, Ar-H), 5.45 (d, J = 8.0 Hz, 1H, 12-H), 4.59 (dd, J = 8.7, 6.2 Hz, 1H, 5a-H), 4.37–4.30 (m, 1H, 14a-H), 3.65 (dt, J = 11.5, 7.8 Hz, 1H, 3-H), 3.33–3.23 (m, 2H, 3-H, 6-Hα), 2.97 (dd, J = 15.7, 9.2 Hz, 1H, 6-Hβ), 2.25–2.14 (m, 2H, 1-H, -C$\underline{\text{H}}$(CH$_3$)$_2$), 1.93–1.74 (m, 3H, 1-H, 2-H), 1.03 (d, J = 6.7 Hz, 3$\underline{\text{H}}$, -CH-CH$\underline{_3}$), 0.94 (d, J = 6.8 Hz, 3H, -CH-C$\underline{\text{H}_3}$). ^{13}C NMR (100 MHz, DMSO-d_6) (ppm, from TMS): 165.96, 164.80, 136.37, 133.24, 126.44, 121.57, 119.14, 118.19, 111.62, 105.91, 58.77, 54.35, 54.17, 45.17, 33.53, 29.46, 26.73, 21.84, 20.14, 20.07. Anal. calcd. for $C_{20}H_{23}N_3O_2$: C, 71.19; H, 6.87; N, 12.45. Found: C, 71.28; H, 6.85; N, 12.11%.

(5a*S*,12*S*,14a*S*)-12-(4-nitrophenyl)-1,2,3,5a,6,14a-hexa-hydro-pyrrolo[1'',2'':4',5']pyrazino[1',2':1,6]pyrido[3,4-*b*] indole-5,14 (11*H*,12*H*)-dione (*cis*-5e). Yield 91.4%, yellow solid, mp. 262–264 °C, ^{1}H NMR (400 MHz, DMSO-d_6) (ppm, from TMS): 11.21 (s, 1H, NH on the indole ring), 8.14 (d, J = 8.7 Hz, 2H, Ar-H), 7.59 (d, J = 8.8 Hz, 3H, Ar-H), 7.33 (d, J = 8.0 Hz, 1H, Ar-H), 7.08 (t, J = 7.3 Hz, 1H, Ar-H), 7.01 (t, J = 7.3 Hz, 1H, Ar-H), 6.35 (s, 1H, 12-H), 4.58 (dd, J = 11.5, 4.7 Hz, 1H, 5a-H), 4.37 (t, J = 7.3 Hz, 1H, 14a-H), 3.60–3.44 (m, 3H, 6-Hβ, 3-H), 3.05 (dd, J = 15.9, 11.7 Hz, 1H, 6-Hα), 2.25–2.14 (m, 1H, 1-H), 1.94–1.82 (m, 3H, 1-H, 2-H). ^{13}C NMR (100 MHz, DMSO-d_6) (ppm, from TMS): 170.73, 165.40, 150.82, 146.88, 136.79, 132.98, 127.64, 126.13, 124.31, 122.05, 119.52, 118.81, 111.92, 105.65, 58.76, 56.80, 55.82, 45.40, 28.56, 22.90, 22.48. Anal. calcd. for $C_{23}H_{20}N_4O_4$: C, 66.34; H, 4.84; N, 13.45. Found: C, 66.42; H, 4.85; N, 13.40%.

(5a*S*,12*R*,14a*S*)-12-(4-methoxyphenyl)-1,2,3,5a,6,14a-hexahydropyrrolo[1'',2'':4',5']pyrazino[1',2':1,6]pyrido[3,4-*b*] indole-5,14(11*H*,12*H*)-dione (*trans*-5f). Yield 93.8%, white solid, mp. 262–264 °C, ^{1}H NMR (400 MHz, DMSO-d_6) (ppm, from TMS): 10.96 (s, 1H, NH on the indole ring), 7.52 (d, J = 7.7 Hz, 1H, Ar-H), 7.30 (d, J = 8.0 Hz, 1H, Ar-H), 7.19 (d, J = 8.6 Hz, 2H, Ar-H), 7.09 (t, J = 7.5 Hz, 1H, Ar-H), 7.02 (t, J = 7.4 Hz, 1H, Ar-H), 6.90 (d, J = 8.6 Hz, 2H, Ar-H), 6.85 (s, 1H, 12-H), 4.35 (dd, J = 10.6, 4.5 Hz, 1H, 5a-H), 4.15 (dd, J = 8.5, 6.6 Hz, 1H, 14a-H), 3.72 (s, 3H, Ar-OCH$_3$), 3.67 (dd, J = 13.6, 6.2 Hz, 1H, 3-H), 3.45 (dd, J = 15.8, 4.8 Hz, 1H, 2-H), 3.32–3.22 (m, 1H, 3-H), 2.89 (dd, J = 15.6, 10.9 Hz, 1H, 2-H), 2.24 (dd, J = 9.4, 6.1 Hz, 1H, 1-H), 1.89 (dd, J = 11.1, 7.4 Hz, 1H, 1-H), 1.85–1.72 (m, 2H, 6-H). ^{13}C NMR (100 MHz, DMSO-d_6) (ppm, from TMS): 165.30, 164.00, 159.53, 136.87, 132.38, 131.38, 129.76, 126.33, 121.95, 119.25, 118.52, 114.32, 111.71, 107.11, 59.10, 55.60, 53.25, 51.62, 45.04, 29.77, 27.68, 21.53. Anal. calcd. for $C_{24}H_{23}N_3O_3$: C, 71.80; H, 5.77; N, 10.47. Found: C, 71.85; H, 5.76; N, 10.45%.

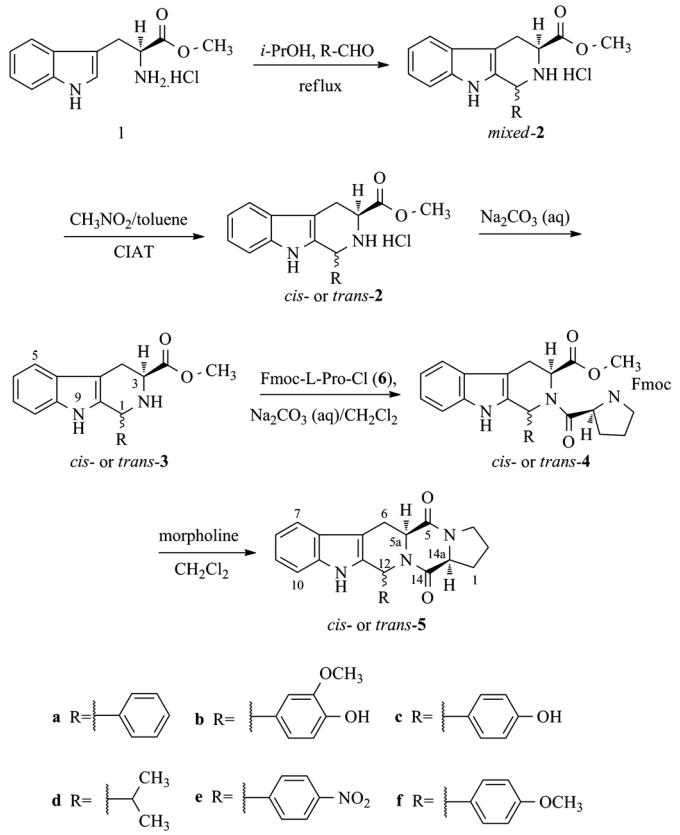
Biological Assay

Both antibacterial and antifungal activities were assayed by the minimum inhibitory concentrations (MICs) method. Each compound was set at 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, and 0.39 μg/mL by the continuous dilution method[44] while the tested strains were incubated in the liquid mediums at the set temperatures. For bacteria, the beef extract peptone medium was made up of beef extract 3.0 g/L, peptone 10 g/L, NaCl 5 g/L, pH 7.2–7.4. The culture temperature was 37 °C. For fungi, the potato-glucose medium was made up of percolate of 200 g potato under boiling for 30 min, glucose 20 g, constant volume to be 1 L by water. The culture temperature was 28 °C. The positive control of Gram-positive bacteria (*S. aureus* and *B. subtilis*) was penicillin sodium, while Gram-negative bacteria (*E. coli* and *P. aeruginosa*) streptomycin sulfate, and fungi ketoconazole. The minimum inhibitory concentrations were defined as the lowest concentration at which no microbial growth could be observed.

RESULTS AND DISCUSSION

Synthesis and Characterization

The four-step synthetic route for tetrahydro-β-carboline diketopiperazines 5 including Pictet-Spengler, CIAT, Schotten-Baumann reactions, and intramolecular ester amidation, starting with L-tryptophan methyl ester hydrochloride and aldehydes, as shown in Scheme 1.



Scheme 1. Synthesis of tetrahydro-¦Â-carboline diketopiperazines.

In this work, we have reported a concise and efficient synthetic route to generate a series of single enantiomers. Six tetrahydro-*β*-carboline diketopiperazines (**5a–5f**) were synthesized in this way for the first time. Starting from optically pure L-tryptophan methyl ester hydrochloride with various aldehydes in isopropanol, a mixture of the hydrochloride salts of *cis*- and *trans*-tetrahydro-*β*-carbolines were obtained, and then a process of CIAT was performed in a mixed solvent with different ratios of nitromethane and toluene (Table 1). After dehydrochlorination, the ^{1}H NMR analysis showed the rings were formed (a single-peak with C-1 proton at between δ 5.11–5.38 ppm except **3d** showed a double-peak at δ 4.12 ppm). The assignment of *cis*-/*trans*-stereochemistry in these tetrahydro-*β*-carbolines relied on the NMR methods established by Cook and colleagues.[38] The proton at C-3 position has an obvious correlation with the proton at C-1 position in the ^{1}H-^{1}H NOESY (nuclear Overhauser enhancement spectroscopy) spectra of the *cis*-isomers, while the proton at C-3 position correlates with the neighboring protons but does not correlate with the proton at C-1 position in the *trans*-isomers. The NMR signals of protons for C-1 and C-3 were assigned by ^{1}H-^{1}H COSY and HSQC experiments.

According to Table 1, there were four *cis*- and two *trans*-compounds (**3a ~ 3f**) after CIAT. The product, whether *cis*- or *trans*-, depended on the solubility between the two configurations in the nitromethane-toluene solvent. The precipitation of the isomer with lower solubility destroyed the balance between the *cis*- and *trans*-isomers, leading to the higher solubility of one transfer into the other. In the end, a single one was acquired as a solid, but the specific of the isomer was uncertain.

Single-crystal structures of compounds **5c** and **5e** were obtained by recrystallizing from methanol, and the further structural information of **5c** and **5e** was confirmed by single-crystal x-ray diffraction analysis (Fig. 3). The structures were solved by direct methods and refined by full-matrix least squares analysis on F2 using SHELXL.[39–43] Hydrogen atoms were refined on the riding model with isotropic thermal parameters set 20% greater than those of their bonding partners. All other atoms were refined anisotropically.

Antimicrobial Activities

The in vitro antimicrobial activities were tested against two Gram-positive (G^+) bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), two Gram-negative (G^-) bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), and four plant pathogenic fungi (*Colletotirchum gloeosporioides*, *Valsa mali*, *Alternaria alternata*, *Alternaria brassicae*). The activities were determined at different concentrations of the compounds **5a–5f** (0.39–50 μg/mL) in DMSO and the results were compared with lower concentrations of known antibiotics including penicillin sodium against G^+ bacteria, streptomycin sulfate against G^- bacteria, and ketoconazole against fungi. The microdilution method for estimation of minimum inhibitory concentration (MIC) values was carried out to evaluate the antimicrobial activity. The MIC values were determined on 96-well microdilution plates.

The screening data of the antifungal activity of these series of compounds showed a wide range of antifungal activity with MIC values 6.25–12.5 μg/mL (ketoconazole showed MIC 12.5 μg/mL). It is interesting that **5b** and **5c** exhibited the most potent in vitro antifungal activity with MIC 6.25 μg/mL against *A. brassicae*, while **5e** and **5f** exhibited the same MIC value against *C. gloeosporioides* (6.25 μg/mL).

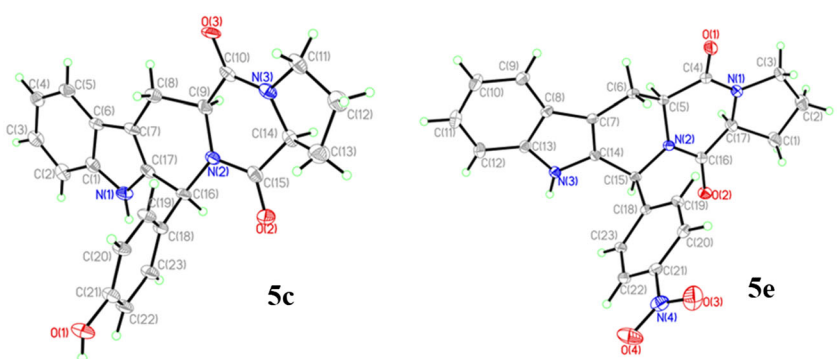


Fig. 3. ORTEP Drawing of Compounds 5c and 5e.

TABLE 2. Results of antibacterial and antifungal test of compounds 5a-5f

Compound	MIC (μg/mL)							
	S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*	*C. gloeosporioides*	*V. mali*	*A. alternata*	*A. brassicae*
5a	0.78	1.56	1.56	0.78	12.5	12.5	12.5	12.5
5b	0.78	1.56	1.56	0.78	12.5	6.25	12.5	6.25
5c	0.78	1.56	1.56	0.78	12.5	12.5	12.5	6.25
5d	0.39	1.56	1.56	0.78	12.5	12.5	12.5	12.5
5e	0.78	1.56	1.56	0.78	6.25	12.5	12.5	12.5
5f	0.78	1.56	1.56	0.78	6.25	12.5	6.25	12.5
Penicillin sodium	0.78	1.56	—	—	—	—	—	—
Streptomycin sulfate	—	—	1.56	0.78	—	—	—	—
Ketoconazole	—	—	—	—	12.5	12.5	12.5	12.5

Meanwhile, **5b** and **5f** showed pronounced antifungal activity against *V. mali* and *A. alternata* with MIC 6.25 μg/mL. Nevertheless, compounds **5a–5f** showed certain satisfactory results as antibacterials with MIC 0.78 ∼ 1.56 μg/mL against the four bacteria, similar to that of penicillin sodium and streptomycin sulfate, except **5d** against *S. aureus* (0.39 μg/mL). These results compared with known antibiotics as standards are shown in Table 2.

CONCLUSIONS

Six novel tetrahydro-*β*-carboline diketopiperazines were highly enantioselective synthesized as single enantiomers from L-tryptophan methyl ester hydrochloride and six aldehydes. The single enantiomers were obtained in high yields and two x-ray crystallographic structures were successfully confirmed for the first time to determine the configurations. Their antibacterial and antifungal activities were tested. Particularly the test showed that all activities of the six compounds were similar or a little better than the traditional drugs, and antifungal activities were shown to be better than antibacterial activities. In a further study the present route will be employed to synthesize the derivatives of tetrahydro-*β*-carboline diketopiperazines.

ACKNOWLEDGMENTS

We thank the Scientific Research Project Item for Key Laboratory of Shaanxi Province Education Department, China (grant No. 2010JS056) and the Xianyang City Science and Technology Project, China (grant No. 2010 K10-06).

LITERATURE CITED

1. Bhatia PA, Moaddel R, Wainer IW. The synthesis and characterization of cellular membrane affinity chromatography columns for the study of human multidrug resistant proteins MRP1, MRP2 and human breast cancer resistant protein BCRP using membranes obtained from Spodoptera frugiperda (Sf9) insect cells. Talanta 2010;81:1477–1481.
2. Gisin BF, Merrifield RB. Carboxyl-catalyzed intramolecular aminolysis. A side. reaction in solid-phase synthesis. J Am Chem Soc 1972;94:3102–3106.
3. Cui CB, Kakeya H, Osada H. Novel mammalian cell cycle inhibitors, cyclotryprostatins A-D, produced by *Aspergillus fumigatus*, which inhibit mammalian cell cycle at G2/M phase. Tetrahedron 1997;53:59–72.
4. Ma YM, Feng CL, Zhang HC, Zhou XN. Two indole alkaloids produced by endophytic fungus FL25 from *Ficus carica*. Chem Res Appl 2009;21:1173–1175.
5. Shukla S, Robey RW, Bates SE, Ambudkar SV. The calcium channel blockers, 1, 4-dihydropyridines, are substrates of the multidrug resistance-linked ABC drug transporter, $ABCG_2$. Biochemistry 2006;45:8940–8951.
6. Allen JD, Loevezijn A, Lakhai JM, Valk M, Tellingen O, Reid G, Schellens JHM, Koomen GJ, Schinkel AH. Potent and specific inhibition of the breast cancer resistance protein multidrug transporter in vitro and in mouse intestine by a novel analogue of fumitremorgin C. Mol Cancer Ther 2002;6:417–425.
7. Glavinas H, Krajcsi P, Cserepes J, Sarkadi B. The role of ABC transporters in drug resistance, metabolism and toxicity. Curr Drug Del 2004;1:27–42.
8. Rabindran SK, He H, Singh M, Brown E, Collins KI, Annable T, Greenberger LM. Reversal of a novel multidrug resistance mechanism in human colon carcinoma cells by fumitremorgin C. Cancer Res 1998;58:5850–5858.
9. Hazlehurst LA, Foley NE, Gleason-Guzman MC, Hacker MP, Cress AE, Greenberger LW, DeJong MC, Dalton WS. Multiple mechanisms confer drug resistance to mitoxantrone in the human 8226 myeloma cell line. Cancer Res 1999;59:1021–1028.
10. Betina V, editor. In Mycotoxins-production, isolation, separation and purification. Amsterdam: Elsevier; 1984.
11. Cole RJ ,Cox RH. Handbook of toxic fungal metabolites. New York: Academic; 1981.
12. Cysewsk SJ. In Mycotoxic fungi, mycotoxins and mycotoxicoses. Wyllie TD, Morehouse LG, editors. New York: Marcel Dekker; 1977.
13. Cole RJ. In: Mycotoxins in human and animal health/ Rodricks JV, Hesseltine CW, Hehlman MA, editors. Park Forest South, IL: Pathotox; 1977.
14. Ciegler A, Vesonder RF, Cole RJ. In: Mycotoxins and other fungal-related food problems. Rodericks JV, editor. Advances in chemistry series 149. Washington, DC: American Chemical Society; 1976.
15. Nakatsuka S, Teranishi K, Goto T. Total synthesis of fumitremorgin B. Tetrahedron Lett 1986;27:6361–6364.
16. Kodato S, Nakagawa M, Hongu M, Kawate T, Hino T. Total synthesis of (+)-fumitremorgin B, its epimeric isomers, and demethoxy derivatives. Tehhnh 1988;44:359–377.
17. O'malley GJ, Cava MP. Tremorgenic my cotoxins: synthesis of 6-demethoxy-fumitremorgin C. Tetrahedron Lett 1987;28:1131–1134.
18. Wang H, Ganesan A. Concise synthesis of the cell cycle inhibitor demethoxy-fumitremorgin C. Tetrahedron Lett 1997;38:4327–4328.
19. Hino T, Nakagawa M. Total synthesis of fumitremorgins and verruculogens. Heterocycles 1997;46:673–704.
20. Bailey PD, Philip JC, Katrin L. Efficient route to fumitremorgins. Tetrahedron Lett 2001;42:113–115.
21. Paulvannan K, Hale R, Mesis R, Chen T. Tandem *N*-acyliminium/Pictet-Spengler/intramolecular Diels- Alder reaction: an expedient route to hexacyclic tetrahydro-*β*-carbolines. Tetrahedron Lett 2002;43:203–207.
22. Cui CB, Kakeya H, Osada H. Novel mammalian cell cycle inhibitors, spirotryprostatins A and B, produced by *Aspergillus fumigatus*, which inhibit mammalian cell cycle at G2/M phase. Tetrahedron 1996;52:12651–12666.
23. Plate R, Hermkens PHH, Behm H, Ottenheijm HCJ. Application of an isoxazolidine in a stereoselective approach to the fumitremorgin series. J Org Chem 1987;52:560–564.
24. Wang HS, Usui T, Osada H. Synthesis and evaluation of tryprostatin B and demethoxyfumitremorgin C analogues. J Med Chem 2000;43:1577–1585.
25. Tohru H, Tomohiko K, Masako N. A synthesis of so-called fumitremorgin C. Tetrahedron 1989;45:1941–1944.
26. Liu JW, Wu GF, Cui GH, Xuan W, Zhao M, Wang C, Zhang ZD, Peng SQ. A new class of anti-thrombosis hexahydropyrazino-[1',2':1,6]pyrido- [3,4-*b*]-indole-1,4-diones: Design, synthesis, log*K* determination, and QSAR analysis. Bioorg Med Chem 2007;15:5672–5693.

27. Deveau AM, Costa NE, Joshi EM, Macdonald TL. Synthesis of diketopiperazine- based carboline homodimers and in vitro growth inhibition of human carcinomas. Bioorg Med Chem Lett 2008;18:3522–3525.
28. Masako N, Kodato S, Mitsuya H, Kawate T, Hino T. Total synthesis of fumitremorgin B. Tetrahedron Lett 1986;27:6217–6220.
29. Reider PJ, Davis P, Hughes DL, Grabowski EJJ. Crystallization-induced asymmetric transformation: stereospecific synthesis of a potent peripheral CCK antagonist. J Org Chem 1987;52:955–957.
30. Brunetto G, Gori S, Fiaschi R, Napolitano E. Crystallization–induced asymmetric transformations. enantiomerically pure (−)–(*R*)– and (+)–(*S*)–2,3– dibromopropan–1–ol and epibromohydrins. A study of dynamic resolution via the formation of diastereoisomeric esters. Helv Chim Acta 2002;85:3785–3791.
31. Marchalin S, Cvopova K, Kriz M, Baran P, Oulyadi H, Daich A. New resolution of 2–formyl-1,4-DHP derivatives using CIDR methodology. Facile access to new chiral tricyclic thiolactam. J Org Chem 2004;69:4227–4237.
32. Komatsu H, Awano H. First stereoselective synthesis of 2-deoxy-α-D-ribosyl-1-phosphate: Novel application of crystallization-induced asymmetric transformation. J Org Chem 2002;67:5419—5421.
33. Vedejs E, Donde Y. Crystallization-induced asymmetric transformation of a tertiary phosphine. J Org Chem 2000;65:2337–2343.
34. Vedejs E, Chapman RW, Lin S, Muller M, Powell DR.Crystallization-induced asymmetric transformation vs quasi-racemate formation in tetravalent boron complexes J Am Chem Soc 2000;122:3047–3052.
35. Nishide K, Kajimoto T, Node M. A practical improvement of crystallization-induced asymmetric transformation of allene-1,3-dicarboxylates. Tetrahedron Asymmetry 2006;17:2943–2951.
36. Yen YH, Chu YH. Synthesis of tetrahydro-β-carbolinediketopiperazines in [bdmim] [PF_6] ionic liquid accelerated by controlled microwave heating. Tetrahedron Lett 2004;45:8137–8140.
37. Xiao S, Lu X, Shi XX, Sun Y, Liang LL. Synthesis of tadalafil (cialis) from L-tryptophan. Tetrahedron Asymmetry 2009;20:2090–2096.
38. Ungemach F, Soerens D, Weber R, DiPierro M, Campos O, Mokry P, Cook JM, Silverton JV. General method for the assignment of stereochemistry of 1,3-disubstituted 1,2,3,4-tetrahydro-β–carboline by carbon– 1,3 spectroscopy. J Am Chem Soc 1980;102:6976–6984.
39. Sheldrick GM. Phase annealing in SHELX–90: direct methods for larger structures. Acta Crystallogr Sect A 1990;46:467–473.
40. Sheldrick GM. SHELXS. University of Göttingen, Germany; 1997.
41. Madison W. Bruker APEX2 Software Bruker AXS Inc.V2.0–1, Billerica, MA; 2005.
42. Sheldrick GM. SADABS Program for Empirical Absorption Correction of Area Detector Data. University of Göttingen, Germany; 1997.
43. Sheldrick GM. SHELXL, Program for the Refinement of Crystal Structures. University of Göttingen, Germany; 1997.
44. Zhang M, Wang WL, Fang YC, Zhu TJ, Gu QQ, Zhu WM. Cytotoxic alkaloids and antibiotic nordammarane triterpenoids from the marine-derived fungus *Aspergillus sydowi*. J Nat Prod 2008;71:985–989.