J. Chem. Sci., Vol. 121, No. 4, July 2009, pp. 463–470.

Synthesis, antimicrobial activity of lamotrigine and its ammonium derivatives

YONG QIAN, PENG-CHENG LV, LEI SHI, RUI-QIN FANG, ZHONG-CHENG SONG and HAI-LIANG ZHU*

State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing 210093, People's Republic of China
e-mail: zhuhl@nju.edu.cn

MS received 2 December 2008; revised 6 March 2009; accepted 1 April 2009

Abstract. Antiepileptic drug lamotrigine and its thirteen ammonium salt complexes (**4a–4m**) were synthesized and characterized by IR, elemental analysis, ^{1}H-NMR, and MS spectral methods. Many of the ammonium salts (**4a–4m**) were first reported. Furthermore, the crystal structure of compound **3** was determined by single crystal X-ray diffraction analysis. All these complexes were tested *in vitro* for their antibacterial activity (*Bacillus subtilis*, *Staphylococcus aureus*, *Enterococus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterobacter cloacae*). The results indicated that most of the complexes showed good antibacterial activity against Gram-positive (*B. subtilis*, *S. aureus* and *S. faecalis*), but showed mild, even inactive against Gram-negative bacterial strains.

Keywords. Lamotrigine; ammonium; crystal structure; antibacterial activity.

1. Introduction

Lamotrigine (3,5-dimino-6-(2,3-dichlorophenyl)-1,2,4-triazine) is an antiepileptic drug, which is suitable for treating epilepsy and bipolar disorder. Recent clinical trials indicate lamotrigine is also effective in treating diabetic neuropathy, postherpetic neuralgia and trigeminal neuralgia.[1,2] For the latest years lamotrigine as an effective medicine for central nervous system disorders has been used in numerous countries.

A variety of methods for preparing this compound has been disclosed,[3–6] however, a need for a more efficient and environmentally friendly process has remained. In order to reduce the chemical reaction processing time and the number of processing operations as well as desirable yield, an improved synthetic strategy was devised based on the above viewpoints.

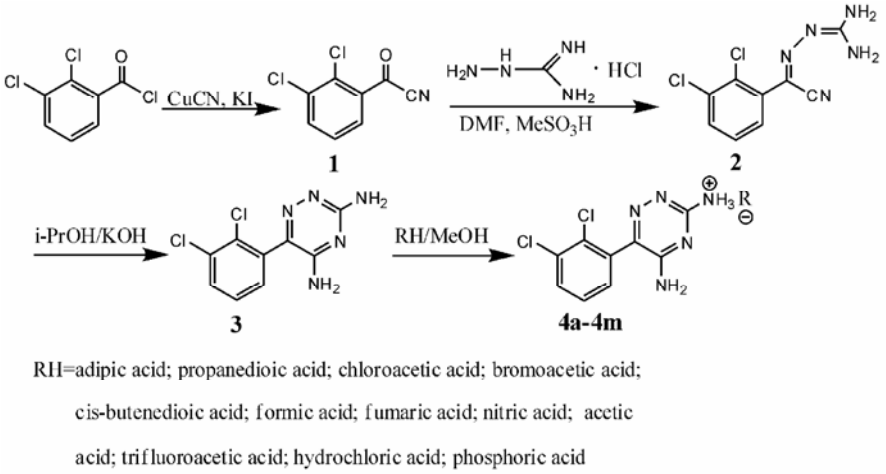
As ammonium salt compound have good water-soluble and antibacterial activity that are wildly used for control of the bacterial growth in clinical and industrial environment.[7,8] For drugs, the salts of theirs may have similar or better activity, TIAGABINE and its pharmaceutically acceptable salts, especially acid-addition salts, including salts of organic acids and mineral acids, had been found useful in the treatment of psychotic disorders, in particular psychotic affective disorders and more particular manic disorders.[9] Several salts of lamotrigine were reported.[10] So with the purpose of developing its new and different biological activities, in this paper a series of structurally similar ammonium salt complexes were prepared from lamotrigine precursor. The structural and spectroscopic characterizations of these complexes were described, and their antibacterial activities against Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus* and *Enterococus faecalis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *Enterobacter cloacae*) were evaluated. General synthetic scheme is shown as follows (scheme 1).

2. Experimental

2.1 *Materials and measurements*

All chemicals were of reagent grade and were used as received with further purification. All the ^{1}H-NMR spectra were recorded on a Bruker DRX 500 or DPX 300 model Spectrometer in DMSO-d_6. ESI-

*For correspondence



Scheme 1. Synthesis of lamotrigine and its ammonium salt.

MS spectra were recorded on a Mariner System 5304 Mass spectrometer. Elemental analyses were performed on a CHN-O-Rapid instrument. Infrared spectra were recorded on a Nexus 870 FT-IR spectrometer in KBr disks at room temperature.

2.2 *Crystal structure determination*

Crystal structure determination of complexes **3** were carried out on a Nonius CAD4 diffractometer equipped with graphite-monochromated MoKα (λ = 0·71073 Å) radiation. The structure was solved by direct methods and refined on F^2 by full-matrix least-squares methods using SHELX-97.[11] All the non-hydrogen atoms were refined anisotropically. All the hydrogen atoms were placed in calculated positions and were assigned fixed isotropic thermal parameters at 1·2 times the equivalent isotropic U of the atoms to which they are attached and allowed to ride on their respective parent atoms. The contributions of these hydrogen atoms were included in the structure-factors calculations.

2.3 *Preparation of lamotrigine*

2.3a *2,3-Dichlorobenzoyl cyanide* (**1**): Copper cyanide (32·0 g, 358·0 mmol), potassium iodide (39·6 g, 238·7 mmol) and monochlorobenzene (100 mL) were added to a 500 mL three-necked round bottom flask containing 2,3-dichlorobenzoyl chloride (50·0 g, 238·7 mmol). The mixture was stirred and heated to reflux under nitrogen blanket and maintained at 150°C for 10 h. The resulting mixture was distilled to remove monochlorobenzene under reduced pressure. Purification of the crude product by crystallization from petroleum ether to obtain the pale yellow solid **1** (47·8 g, yield 70%), mp: 52–53°C. IR: 2226·3; 1698·0; 1415·0 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 8·08 (*d*, *J* = 7·86 Hz, 1H); 7·84 (*d*, *J* = 7·68 Hz); 7·50 (*t*, *J* = 7·68; 7·86 Hz); ESI-MS: 201·1 ($C_8H_4Cl_2NO^+$, $[M + H]^+$); Anal. Calcd for $C_8H_3Cl_2NO$: C, 48·04%; H, 1·51%; N, 7·00%; Found: C, 48·23%; H, 1·50%; N, 7·02%.

2.3b *2-(2,3-Dichlorophenyl)-2-(aminoguanidine)-acetonitrile* (**2**): Aminoguanidine hydrochlorate (19·4 g, 175·4 mmol) was added into 250 mL round bottom flask, then DMF (24·0 mL) and methanesulphonic acid (52·5 mL) was poured into and stirred 1 h. To this mixture was added a solution of 2,3-dichlorobenzoyl cyanide (14·0 g, 70·0 mmol) in 40·0 mL of DMF. The mixture was heated at 60°C for 2·5 h, cooled in ice bath, and 30·0 mL of water added slowly. The mixture was adjusted to PH 12 with a 50% NaOH solution, filtered, the solid washed with water and dried at vacuum desiccator to get **2** (7·0 g, yield 39·1%), mp: 176–178°C. IR: 3491·7; 2207·8; 1682·1; 1055·7 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 7·64 (*m*, 2H, CH); 7·40 (*t*, *J* = 8·1 Hz, 1H, CH); 6·67 (*br s*, 4H, NH_2); ESI-MS: 257·1 ($C_9H_8Cl_2N_5^+$, $[M + H]^+$); Anal. Calcd for $C_9H_7Cl_2N_5$: C, 42·21%; H, 2·76%; N, 27·35%; Found: C, 42·09%; H, 2·77%; N, 27·43%.

2.3c *3,5-Diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine* (**3**): **2** (6·5 g, 25·3 mmol), isopropanol (80·0 mL) and KOH (55%, 6·7 mL) were added into

a round bottomed flask. The mixture was refluxed at 105°C for 6 h. The mixture was adjusted to PH 7·0 with 5% HCl solution, then much water was poured into until solid was precipitated, this suspension was treated by ultrasound about 5 min, white precipitate was obtained which was treated by recrystallization in isopropanol, then crystal was separated out which was filtered, washed with isopropanol and dried under reduced pressure in a vacuum desiccator containing molecular sieve. (5·2 g, yield 80%), mp: 216–218°C. IR: 3494·2; 3451·1; 3326·5; 3157·2; 1666·5; 1620·0; 1555·7; 1432·5; 1155·6; 1056·0 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 7·7 (*dd*, J = 1·65; 7·95 Hz, 1H), 7·44 (*t*, J = 7·68; 7·86 Hz, 1H), 7·35 (*dd*, J = 1·65; 7·5 Hz, 1H), 6·68 (*br s*, NH_2), 6·41 (*br s*, NH_2); ESI-MS: 257·1 ($C_9H_8Cl_2N_5^+$, $[M + H]^+$); Anal. Calcd for $C_9H_7Cl_2N_5$: C, 42·21%; H, 2·76%; N, 27·35%; Found: C, 42·01%; H, 2·75%; N, 27·42%.

2.4 *Preparation of the ammonium salt of lamotrigine*

Methanol solution (5 mL) of lamotrigine (25·0 mg, 0·098 mmol) was added to methanol solutions (10 mL) of adipic acid (14·3 mg, 0·098 mmol), propanedioic acid (10·3 mg, 0·098 mmol), chloroacetic acid (9·2 mg, 0·098 mmol), bromoacetic acid (13·6 mg, 0·098 mmol), oxalic acid (8·8 mg, 0·098 mmol), *cis*-Butene dioic acid (11·3 mg, 0·098 mmol), formic acid (4·5 mg, 0·098 mmol), fumaric acid (11·3 mg, 0·098 mmol), nitric acid (6·2 mg, 0·098 mmol), acetic acid (5·9 mg, 0·098 mmol), trifluoroacetic acid (11·1 mg, 0·098 mmol), hydrochloric acid (3·6 mg, 0·098 mmol), and phosphoric acid (9·6 mg, 0·098 mmol) respectively. The mixture was stirred for 30 min and standing until the precipitates were obtained, then precipitates were separated by filtration, washed by methanol and dried under reduced pressure in a vacuum desiccator to get the respective complex **(4a–4m)**.

2.4a *3,5-Diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine adipic acid salt* (**4a**): mp: 165–168°C. IR: 3398·6; 3254·2; 2994·8; 2835·2; 1653·1; 1583·7; 1577·4; 1498·1; 1414·5; 1413·6; 1239·5; 1167·8; 1059·6 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 7·76 (*dd*, J = 7·87; 1·65 Hz, 1H), 7·65 (*dd*, J = 8·04; 1·65 Hz, 1H), 7·40 (*t*, J = 8·04 Hz, 1H), 6·08 (*br s*, 2H), 2·23 (*s*, 6H), 1·52 (*s*, 5H); ESI-MS: 402·2 ($C_{15}H_{17}Cl_2N_5O_4^+$, $[M + H]^+$); Anal. Calcd for $C_{15}H_{16}Cl_2N_5O_4$: C, 44·90%; H, 4·02%; N, 17·45%; Found: C, 44·77%; H, 4·03%; N, 17·38%. **3**·1HOOC$(CH_2)_4$COOH.

2.4b *3,5-Diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine propanedioic acid salt* (**4b**): mp: 152–153°C. IR: 3386·5; 3277·7; 3089·6; 2825·4; 1641·3; 1595·6; 1587·5; 1489·6; 1434·4; 1411·3; 1289·7; 1158·9; 1052·3 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 7·74 (*dd*, J = 1·62; 7·77 Hz, 1H), 7·65 (*dd*, J = 1·65; 7·95 Hz, 1H), 7·40 (*t*, J = 8·04 Hz, 1H), 6·08 (*br s*, 2H), 3·35 (*br s*, 2H), 12·4 (*br s*, 3H); ESI-MS: 360·1 ($C_{12}H_{11}Cl_2N_5O_4^+$, $[M + H]^+$); Anal. Calcd for $C_{12}H_{10}Cl_2N_5O_4$: C, 40·13%; H, 2·81%; N, 19·50%; Found: C, 40·28%; H, 2·79%; N, 19·45%. **3**·1HOOCCH_2COOH.

2.4c *3,5-Diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine chloroacetic acid salt* (**4c**): mp: 209–213°C. IR: 3359·8; 3116·2; 3094·6; 2835·1; 2733·4; 1689·8; 1638·4; 1593·1; 1579·7; 1411·2; 1408·6; 1501·5; 1299·8; 1187·7; 1149·1 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 7·75 (*dd*, J = 1·62; 7·77 Hz, 1H), 7·65 (*dd*, J = 1·65; 7·96 Hz, 1H), 7·40 (*t*, J = 7·86, 1H), 6·08 (*br s*, 2H), 4·26 (*br s*, 2H); ESI-MS: 351·6 ($C_{11}H_{11}Cl_3N_5O_2^+$, $[M + H]^+$); Anal. Calcd for $C_{11}H_{10}Cl_3N_5O_2$: C, 37·68%; H, 2·87%; N, 19·98%; Found: C, 37·55%; H, 2·88%; N, 19·91%. **3**·1ClCH_2COOH.

2.4d *3,5-Diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine bromoacetic acid salt* (**4d**): mp: 264°C. IR: 3358·8; 3117·1; 3093·8; 2834·7; 2734·1; 1687·5; 1637·9; 1593·4; 1578·8; 1410·2; 1407·4; 1501·7; 1299·3; 1187·7; 1149·0 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 7·73 (*m*, 2H), 7·44 (*m*, 1H), 4·8 (*s*, 1H), 3·7 (*br s*, 2H), 3·17 (*s*, 5H); ESI-MS: 396·1 ($C_{11}H_{11}BrCl_2N_5O_2^+$, $[M + H]^+$); Anal. Calcd for $C_{11}H_{10}BrCl_2N_5O_2$: C, 33·44%; H, 2·55%; N, 17·73%; Found: C, 33·32%; H, 2·54%; N, 17·65%. **3**·1BrCH_2COOH.

2.4e *3,5-Diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine oxalic acid salt* (**4e**): mp: 265–266°C. IR: 3390·5; 3290·2; 3094·6; 2805·0; 1638·5; 1593·2; 1573·7; 1494·3; 1453·7; 1411·2; 1299·8; 1157·7; 1049·1 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 7·80 (*m*, 1H), 7·52 (*m*, 2H), 8·25 (*br s*, 3H); ESI-MS: 346·1 ($C_{11}H_9Cl_2N_5O_4^+$, $[M + H]^+$); Anal. Calcd for $C_{11}H_8Cl_2N_5O_4$: C, 38·28%; H, 2·34%; N, 20·29%; Found: C, 38·14%; H, 2·33%; N, 20·33%. **3**·1HOOCCOOH.

2.4f *3,5-Diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine cis-Butene dioic acid salt* (**4f**): mp: 185°C. IR: 3393·4; 3289·7; 3093·4; 2806·1; 1649·8; 1638·6; 1593·7; 1574·1; 1493·9; 1453·7; 1410·3; 1297·8; 1157·5; 1050·2 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 7·75 (*dd*, *J* = 1·47; 7·77 Hz, 1H), 7·67 (*dd*, *J* = 1·65; 8·04 Hz, 1H), 7·41 (*t*, *J* = 8·07 Hz, 1H), 6·24 (*s*, 3H), 3·17 (*s*, 2H); ESI-MS: 373·2 ($C_{13}H_{12}Cl_2N_5O_4^+$, $[M + H]^+$); Anal. Calcd for $C_{13}H_{11}Cl_2N_5O_4$: C, 41·95%; H, 2·98%; N, 18·82%; Found: C, 41·84%; H, 2·97%; N, 19·42%· **3**·1*cis*-HOOCCHCHCOOH.

2.4g *3,5-Diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine formic acid salt* (**4g**): mp: 223°C. IR: 3443·1; 3317·4; 2993·9; 2821·9; 2713·4; 1667·3; 1580·6; 1557·6; 1494·3; 1442·6; 1413·9; 1364·3; 1337·0; 1190·4; 996·5 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 8·16 (*s*, 1H), 7·71 (*dd*, *J* = 1·65; 7·95 Hz, 1H), 7·45 (*t*, *J* = 7·86 Hz, 1H), 7·38 (*dd*, *J* = 1·65; 7·41 Hz, 1H), 6·81 (*br s*, 2H), 6·56 (*br s*, 2H); ESI-MS: 303·1 ($C_{10}H_{10}Cl_2N_5O_2^+$, $[M + H]^+$); Anal. Calcd for $C_{10}H_9Cl_2N_5O_2$: C, 39·76%; H, 3·00%; N, 23·18%; Found: C, 39·68%; H, 3·01%; N, 23·22%. **3**·1HCOOH.

2.4h *3,5-Diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine fumaric acid salt* (**4h**): mp: 217–218°C. IR: 3391·8; 3288·9; 3093·4; 2806·3; 1668·1; 1637·8; 1592·9; 1574·1; 1493·3; 1453·7; 1410·3; 1297·6; 1156·8; 1049·5 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 7·70 (*dd*, *J* = 1·65; 8·04 Hz, 1H), 7·45 (*t*, *J* = 7·68 Hz, 1H), 7·36 (*dd*, *J* = 1·65 Hz; 7·68, 1H), 6·77 (*br s*, 2H), 6·61 (*s*, 1H), 6·54 (*br s*, 2H); ESI-MS: 373·1 ($C_{13}H_{12}Cl_2N_5O_4^+$, $[M + H]^+$); Anal. Calcd for $C_{13}H_{11}Cl_2N_5O_4$: C, 41·95%; H, 2·98%; N, 18·82%; Found: C, 41·83%; H, 2·89%; N, 19·37%. **3**·1*trans*-HOOCCHCHCOOH.

2.4i *3,5-Diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine nitric acid salt* (**4i**): mp: 227–230°C. IR: 3316·5; 3073·7; 3011·2; 2752·4; 1709·7; 1557·8; 1518·8; 1418·7; 1035·7 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 7·88 (*m*, 1H), 7·69 (*m*, 1H), 7·57 (*m*, 1H); ESI-MS: 382·1 ($C_9H_{10}Cl_2N_7O_6^+$, $[M + H]^+$); Anal. Calcd for $C_9H_9Cl_2N_7O_6$: C, 28·29%; H, 2·37%; N, 25·66%; Found: C, 28·19%; H, 2·36%; N, 25·75%. **3**·2HNO_3.

2.4j *3,5-Diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine acetic acid salt* (**4j**): mp: 225–228°C. IR: 3318·1; 3307·9; 2994·3; 2818·5; 2713·1; 1667·3; 1580·6; 1557·6; 1495·2; 1441·4; 1413·5; 1363·7; 1336·6; 1189·1 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 7·75 (*d*, *J* = 7·68 Hz, 1H), 7·64 (*d*, *J* = 8·67 Hz, 1H), 7·39 (*t*, *J* = 7·86 Hz, 1H), 6·11 (*br s*, 2H); ESI-MS: 331·1 ($C_{12}H_{14}Cl_2N_5O_2^+$, $[M + H]^+$); Anal. Calcd for $C_{12}H_{13}Cl_2N_5O_2$: C, 43·65%; H, 3·97%; N, 21·21%; Found: C, 43·71%; H, 3·99%; N, 21·27%. **3**·1CH_3COOH.

2.4k *3,5-Diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine trifluoroacetic acid salt* (**4k**): mp: 233–235°C. IR: 3364·7; 3114·8; 2830·8; 2720·3; 1698·5; 1650·7; 1596·9; 1581·0; 1506·8; 1439·4; 1304·4; 1190·2; 1143·81; 1058·7 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 9·11 (*br s*, 1H), 8·35 (*br s*, 2H), 8·13 (*br s*, 1H), 7·84 (*t*, *J* = 4·95 Hz, 1H), 7·53 (*dd*, *J* = 8·43; 12·4 Hz, 2H); ESI-MS: 485·1 ($C_{13}H_{10}Cl_2F_6N_5O_4^+$, $[M + H]^+$); Anal. Calcd for $C_{13}H_9Cl_2F_6N_5O_4$: C, 32·25%; H, 1·87%; N, 14·47%; Found: C, 32·34%; H, 1·87%; N, 14·42%. **3**·2CF_3COOH.

2.4l *3,5-Diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine hydrochloric acid salt* (**4l**): mp: 227–228°C. IR: 3286·7; 3064·1; 2998·7; 2752·8; 1709·3; 1554·7; 1518·7; 1419·1; 1036·7 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 7·85 (*d*, *J* = 8·07 Hz, 1H), 7·73 (*d*, *J* = 7·68 Hz, 1H), 7·53 (*t*, *J* = 8·07 Hz, 1H); ESI-MS: 329·1 ($C_9H_{10}Cl_4N_5^+$, $[M + H]^+$); Anal. Calcd for $C_9H_9Cl_4N_5$: C, 32·85%; H, 2·76%; N, 21·29%; Found: C, 32·92%; H, 2·75%; N, 21·24%. **3**·2HCl.

2.4m *3,5-Diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine phosphoric acid salt* (**4m**): mp: 239°C. IR: 3316·9; 3074·5; 3010·7; 2752·2; 1710·0; 1419·0; 1035·8; 974·9 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 7·75 (*d*, *J* = 7·89 Hz, 1H), 7·65 (*d*, *J* = 8·07 Hz, 1H), 7·40 (*t*, *J* = 7·86 Hz, 1H), 6·09 (*s*, 2H); ESI-MS: 355·1 ($C_9H_{11}Cl_2N_5O_4P^+$, $[M + H]^+$); Anal. Calcd for $C_9H_{10}Cl_2N_5O_4$ P: C, 30·53%; H, 2·85%; N, 19·78%; Found: C, 30·61%; H, 2·84%; N, 19·83%. **3**·1HPO_4.

2.5 *Antibacterial activity*

The antibacterial activity of the synthesized compounds was tested against Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus* and *Enterococus faecalis*), Gram-negative (*Escherichia coli*, *Pseu-*

Table 1. MICs (minimum inhibitory concentrations) (μg/mL) of the synthetic compounds.

	Microorganisms					
	Gram-positive			Gram-negative		
Compounds	*B. subtilis*	*S. aureus*	*S. faecalis*	*P. aeruginosa*	*E. coli*	*E. cloacae*
1	6·25	25	12·5	25	50	50
2	6·25	25	25	25	50	50
3	6·25	25	12·5	25	50	50
4a	6·25	12·5	25	25	>50	50
4b	6·25	25	12·5	25	50	50
4c	12·5	25	25	25	50	50
4d	25	25	25	25	50	50
4e	6·25	25	12·5	25	50	50
4f	25	12·5	25	25	50	50
4g	6·25	25	12·5	25	50	50
4h	6·25	25	12·5	25	50	50
4i	6·25	25	12·5	25	>50	50
4j	25	25	25	12·5	>50	50
4k	25	25	25	25	50	50
4l	25	25	25	25	>50	50
4m	25	25	25	25	>50	50
Penicillin	1·562	1·562	1·562	6·25	6·25	3·125
Kanamycin	0·39	1·562	3·125	3·125	3·125	1·562

domonas aeruginosa and *Enterobacter cloacae*) using MH medium (Mueller–Hinton medium: casein hydrolysate 17·5 g, soluble starch 1·5 g, beef extract 1000 mL), The MICs (minimum inhibitory concentrations) of the test compounds were determined by a colorimetric method using the dye MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl trtrazolium bromide).[12] A stock solution of the synthesized compound (50 μg/mL) in DMSO was prepared and graded quantities of the test compounds were incorporated in specified quantity of sterilized liquid MH medium. A specified quantity of the medium containing the compound was poured into microtitration plates. Suspension of the microorganism was prepared to contain approximately 10^5 cfu/mL and applied to microtitration plates with serially diluted compounds in DMSO to be tested and incubated at 37°C for 24 h. After the MICs were visually determined on each of the microtitration plates, 50 μL of PBS (Phosphate Buffered Saline 0·01 mol/L, pH 7·4: $Na_2HPO_4{\cdot}12H_2O$ 2·9 g, KH_2PO_4 0·2 g, NaCl 8·0 g, KCl 0·2 g, distilled water 1000 mL) containing 2 mg of MTT/mL was added to each well. Incubation was continued at room temperature for 4–5 h. The content of each well was removed, and 100 μL of isopropanol containing 5% 1 mol/L HCl was added to extract the dye. After 12 h of incubation at room temperature, the optical density (OD) was measured with a microplate reader at 550 nm. The observed MICs were presented in table 1.

3. Results and discussion

3.1 *Syntheses*

Condensation of the 2,3-dichlorobenzoyl chloride with copper (I) cyanide was useful for the preparation of 2,3-dichlorobenzoyl cyanide.[13] Comparing with the work of Baxter *et al*[14] which 2,3-dichlorobenzoyl cyanide **1** (scheme 1) was an oily chemical, much solvent xylene and longer reaction time (72 h) were needed, this method was a short and efficient synthetic strategy. Meanwhile, in this study, we found the oily product wound become the solid after put it into the vacuum desiccator to stay overnight, which was treated by recrystallization in petroleum ether to obtain the pale yellow solid with desirable purity. Direct condensation of **1** with aminoguanidine hydrochlorate at methanesulphonic acid afforded 2-(2,3-dichlorophenyl)-2-(aminoguanidine)-acetonitrile **2** (scheme 1) in 39·1% yield, yet in the work of Gunntoori *et al*[15] give 39·2% yield of iminoguanidine derivative, which aminoguanidine hydrochloride in DMF was treated with $MeSO_3H$ and stirred for 1 h, then $SOCl_2$ was added, and stirred for

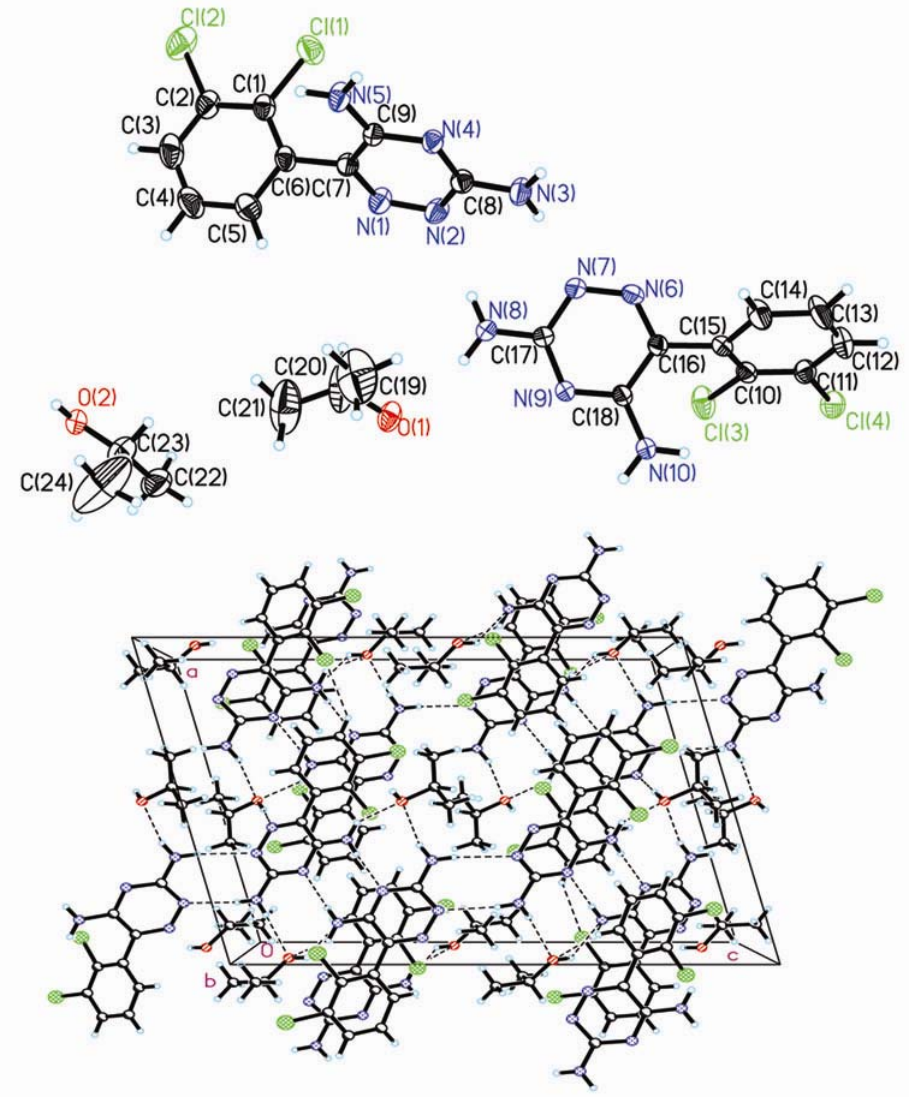


Figure 1. Molecular structures of complexes **3**.

another 1 h. Incidentally, we found similar result was obtained without $SOCl_2$. We studied the relationship between the yield of **2** and the reactant ratio, the results showed that the mole ratio between aminoguanidine hydrochlorate, 2,3-dichlorobenoyl chloride and methanesulphonic acid from 2·5 : 1 : 12 to 3·5 : 1 : 12 was feasible. 3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine **3** (scheme 1) was obtained on refluxing **2** with KOH in isopropanol. This cyclisation step was easily and preferably carried out in isopropanol and at 105°C, a clear solution was obtained at the end of the reaction, then adjusted the mixture PH to 7·0. According to our study, the precipitation with residue formed after adding water, we discovered accidentally that this suspension was treated by ultrasound about 5 min, white precipitate was obtained, so this finding was an important method to eliminate impurity and improve the quality of the compound. The pharmaceutical lamotrigine could obtain by recrystallization of the above crude product in isopropanol. Complexes **4a–4m** (scheme 1) was prepared by slow evaporation of the resulting methanol solution until the precipitation was formed. All of them were easily afforded and with excellent yield.

3.2 *Molecular structure of complexes* **3**

Compound **3** was successfully crystallized and its structure was determined by single-crystal X-ray diffraction analysis. Figure 1 gives a perspective view of this compound together with the atomic labelling system. The collection data details are condensed in table 1. The X-ray crystal structures of

lamotrigine and many of its salts had been reported, but the results between the previous experiment and ours were not identical. The crystal habit of the previously reported crystal structures were classified as orthorhombic ($Pna2_1$),[16] orthorhombic ($Pbca$)[17] and monoclinic (C2/c),[17] whereas the X-ray structure reported here was on a molecule crystallizing from isopropanol in the monoclinic system space group ($P2_1/c$). An obvious difference between the crystal habits was attributed to the contents of each unit cell. In the latter, two molecular of solvent isopropanol were found coassociated with two lamotrigine molecules. The difference was clearly the result of our growing the lamotrigine **3** crystal from isopropanol.

3.3 *Biological activity*

All the synthesized compounds were screened for antimicrobial activity against three Gram-positive bacterial strains (*B. subtilis*, *S. aureus* and *S. faecalis*) and three Gram-negative bacterial strains (*E. coli* and *P. aeruginosa* and *E. cloacae*) by MTT method. Also included the activity of reference compounds kanamycin and penicillin. The results revealed that most of the synthesized compounds exhibited significant antibacterial activity. All these compounds exhibited higher antibacterial activities against Gram-positive bacterial strains than Gram-negative bacterial strains. Compound lamotrigine (**3**) and its two synthetic intermediate **1** and **2** showed significant activity against *B. subtilis* (6·25, 6·25 and 6·25 μg/mL) and *S. faecalis* (12·5, 25 and 12·5 μg/mL), exhibited mild activity against *S. aureus* (25, 25, and 25 μg/mL), while these three complexes showed mild activity against Gram-negative bacterial strains: *P. aeruginosa* (25, 25 and 25 μg/mL), *E. coli* (50, 50 and 50 μg/mL) and *E. cloacae* (50, 50 and 50 μg/mL). Dicarboxylic acid salt of lamotrigine **4a**, **4b**, **4e**, **4g**, **4i** exhibited significant inhibitory activity against *B. subtilis* (6·25, 6·25, 6·25, 6·25, 6·25 and 6·25 μg/mL), also good activity against *S. faecalis* (25, 12·5, 12·5, 12·5, 12·5 and 12·5 μg/mL) and mild activity against *S. aureus* (12·5, 25, 25, 25, 25, and 25 μg/mL). The other acid salt of lamotrigine **4c**, **4d**, **4f**, **4j**, **4k**, **4l**, **4m** show mild activity against these three Gram-positive bacterial strains (all are 25 μg/mL). It demonstrated that the ammonium salts which prepared from dicarboxylic acid showed more favourable bactericidal activities against Gram-positive bacteria than other monoacid and inorganic acid salts of lamotrigine. With the activity of parent compound at the same time, the solubility of them was also increased. All these compounds showed inactivity against *E. coli* and *E. cloacae*, although they showed mild activity against *P. aeruginosa* (almost all the results were 25 μg/mL except **4j** showed 12.5 μg/mL). This suggested that these complexes and their parent molecular were similar to inactivity against Gram-negative bacteria.

4. Conclusions

Lamotrigine and a serial of its ammonium salt complexes were synthesized. Lamotrigine crystal from isopropanol solution and its structure was determined by X-ray diffraction analysis. In this structure two molecular of solvent isopropanol were found co-associated with two lamotrigine molecules, which is different from the previous work. To study the potential antibacterial of the synthesized complexes, screening experiment was performed for six bacterial strains. The result showed that these compounds had higher activity against Gram-positive bacterial strains than Gram-negative bacterial strains. Among these compounds **1**, **2**, **3**, **4a**, **4b**, **4e**, **4g**, **4h**, **4i** showed significant activity against *Bacillus subtilis*, *Enterococus faecalis* and *Staphylococcus aureus*.

Acknowledgements

Financial support for this project from National Natural Science Foundation of China (Project 30772627) is acknowledged. We thank Modern Analysis Center, Nanjing University, Nanjing, China for providing single crystal X-ray diffraction analysis.

References

1. Zakrzewska J M, Chaudry Z, Nurmikko T J, Patton D W and Mullens E L 1997 *Pain* **73** 223
2. Rowbotham M, Harden N and Stacey B 1998 *J. Am. Med. Assoc.* **280** 1837
3. Vyas S K 2000 *US Patent* 6,111,101A
4. Nadaka V, Lexmer J and Kaspi J 2001 *US Patent* 6,329,521B2
5. Billmunt J and Barjoan P D 2007 *US Patent* 7,179,913B2
6. Ramakrishnan, A, Bhushan, V, Dabholkar D and Deore B 2007 WO 069265

7. Brannon D K 1997 *Cosmetic microbiology: A practical handbook* (CRC Press, Inc., Boca Raton, Fla)
8. Shimizu M, Okuzumi K, Yoneyama A, Kunisada T, Araake M, Ogawa H and Kimura S 2002 *Dermatology* **204** (Suppl. 1) 21
9. Anders Fink-Jensen Kbhv 1999 *US Patent* 5,914,333
10. Feng-Jing Chen and Mahesh V Patel 2002 *US Patent* 6,383,471B1
11. Sheldrick G M 1997 SHELX-97. Program for X-ray Crystal Structure Solution and Refinement, Göttingen University, Germany
12. Meletiadis J, Meis J F, Mouton J W, Donnelly J P and Verweij P E 2000 *J. Clin. Microbiol.* **38** 2949
13. Radhakrishnan T V, Sasikumar T M and Srivastava A R 2003 *US Patent* 6,639,072
14. Baxter M G, Elphick A R, Miller A A and Sawyer D A 1984 *US Patent* 4,486,354
15. Guntoori B R, Che Daqing and Murthy K S K 2003 *US Patent* 6,586,593B1
16. Kubicki M and Codding P W 2000 *J. Mol. Struct.* **570** 53
17. Rex A P, Brian S P, Michael J L and Babur Z C 2008 *J. Chem. Crystallogr.* **38** 387