Advances in Structural Modifications and Biological Activities of Berberine: An Active Compound in Traditional Chinese Medicine

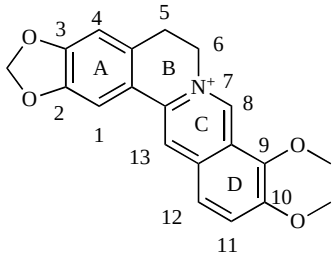
Z.-J. Huang, Y. Zeng, P. Lan, P.-H. Sun and W.-M. Chen*

Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, College of Pharmacy, Jinan University,Guangzhou 510632, P. R. China

Abstract: Berberine is an isoquinoline alkaloid isolated from Chinese herbs such as *Coptidis Rhizome*. This paper is a systematic review of the structural modifications of berberine for different biological activities such as antitumor, antimicrobial, anti-Alzheimer's disease, antihyperglycemic, anti-inflammatory and antimalaria. The current review would provide some useful information for further studies on structural modification of berberine for discovering new drug leads.

Keywords: Berberine, structural modification, biological activities.

Berberine (Fig. (**1**)), which is a major active compound in *Coptidis Rhizoma,* is commonly used in both China and other countries as antimicrobial drug. It widely exists in the plant kingdom: *Papaveraceae, Ranunculaceae* and *Berberidaceae* [1]. Due to its multiple pharmacological activities such as antimicrobial, antitumor, anti-inflammatory, antihyperglycemic and so on, berberine becomes a leading compound for new drug discovery. The structural modifications of berberine have been done in different research groups which mainly focus on its C-8, C-9, C-12, C-13 positions for different pharmaceutical purposes. However, there is no systematic review about structural modifications of berberine and associated biological activities of its derivatives up to now. This review assembles a collection of classic and current cases that illustrate and underscore the structure-activity relationship of them, which might provide useful information in the structural modification of active ingredient in traditional Chinese medicine and help for new drug discovery.



Berberine

Fig. (1). Basic structure of berberine.

*Address correspondence to this author at the Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, College of Pharmacy, Jinan University,Guangzhou 510632, P. R. China; Tel: 86-20-85224497; Fax: 86-20-85224476;
E-mail: twmchen@jnu.edu.cn

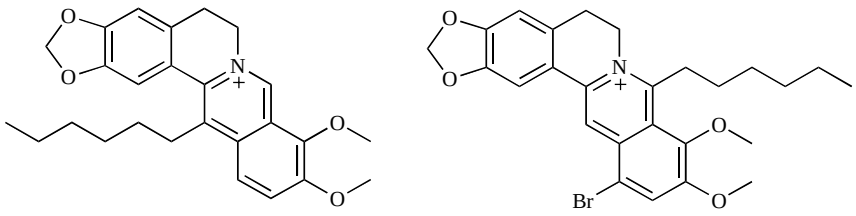
1. STRUCTURAL MODIFICATION FOR ANTI-TUMOR ACTIVITY

The pharmacological studies of berberine for anticancer have been explored since the 1990s [2]. Berberine could induce DNA topoisomerase I poisoning and apoptotic cell death [3]. Recent findings, stapled into the antineoplastic story of berberine, were based on the knowledge of cell-cycle regulation and the downstream effects or phenotypes related to cell-cycle inhibition, particularly the caspase(s)-dependent apoptosis and associated cell signaling pathways [4]. In addition, some investigations showed that mitochondria might be the target of berberine, which was correlated with the concentration of this alkaloid [5]. At low concentration, berberine was accumulated in mitochondria and DNA synthesis was not markedly affected. However, at higher doses berberine was concentrated in cytoplasmic and nuclear. Meanwhile, DNA synthesis was considerably inhibitory. Berberine, accumulated by mitochondria, leaded to mitochondrial fragmentation and dysfunction [6].

Iwasa and coauthors [7] had found that alkyl chain at position C-8 or C-13 strongly influenced the cytotoxic activity, and lipophilic function groups were preferred. Compounds **1** and **2** showed the highest cytotoxicity, with GI_{50} values of 0.83 and 0.41 μM, respectively.

It had been demonstrated that hydroxyl substituent at C-8 and concomitant saturation of double bond between N-7 and C-8 of protoberberine would make a tremendous impact on their cytotoxicity [8]. Modifications on the A-ring of protoberberine alkaloids could significantly enhance the cleavable complex formation that occurred between DNA and topoisomerases [9]. Some berberine derivatives modified at these positions had been designed and synthesized, and all compounds were tested through the Epstein-Barr virus early antigen (EBV-EA) activation assay [10], an effective indicator for the evaluation of anti-tumor-promoting activity. The inhibitory activity of 13-alkyl and 8-alkyl-12-bromo derivatives decreased as the C-13 or C-8 alkyl chain was extended by one carbon. The investigation

1389-5575/11 $58.00+.00



1 **2**

Fig. (2). Structures of compounds **1** and **2**.

also indicated that the aromatic ring with methylenedioxyl group was required.

No matter in the presence or absence of metal cations, berberine and its derivatives would induce and stabilize the formation of anti-parallel G-quadruplex of telomeric DNA [11-13]. Among them, 9-*O*-substituted and 9-*N*-substituted berberine derivatives possessed higher affinity activity. Introducing a side chain with terminal amino group on 9-position of berberine might markedly increase interaction between the derivatives and G-quadruplex, such as compounds **3**, **4**, and **5**.

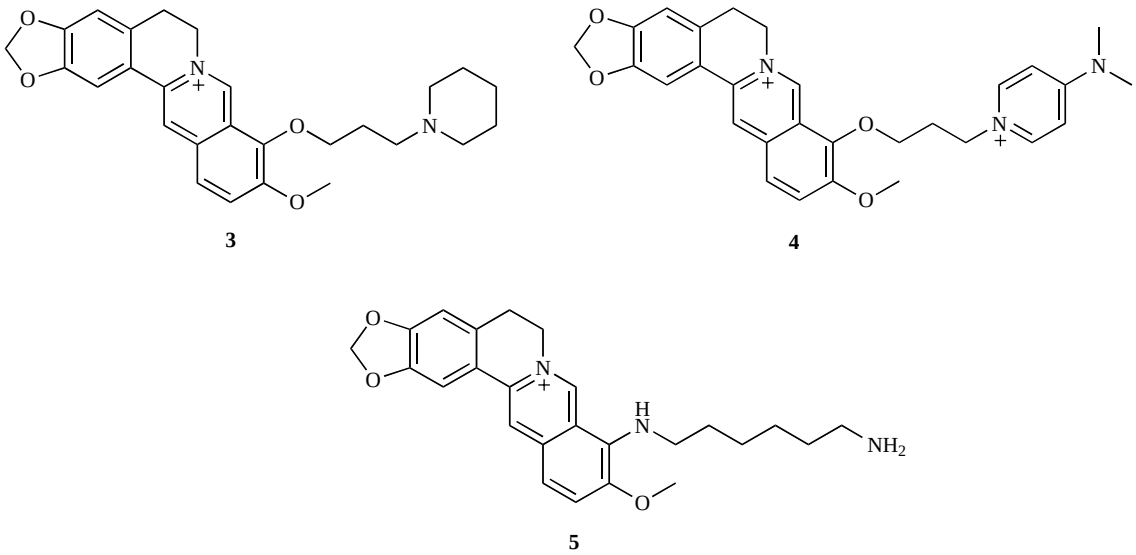
Berberine homodimers (**6**), berberine-jatrorrhizine heterodimers (**7**) and jatrorrhizine homodimers (**8**) showed much higher DNA-binding affinities than their monomeric components [14]. The systematic study of the optimal chain length suggested that ethylidene chain was the best linker. At the same time, berberine-containing dimers emerged higher binding affinities than jatrorrhizine homodimers with the same linker.

2. STRUCTURAL MODIFICATION FOR ANTI-MICROBIAL ACTIVITY

To investigate the mechanism of antimicrobial activity of berberine, LC/ESI-MS combined with principal component analysis was adopted to study metabolic profiles of *Staphylococcus aureus*, treated by berberine and nine antibacterial agents [15]. According to the result, the mode of antimicrobial activity of berberine should be similar with that of Rifampicin and Norfloxacin and its target was possibly on nucleic acid. Moreover, berberine was penetrating cations and substrate of an multidrug resistance pumps, which could be used for reversal of bacterial cells actively extruding these cations [16].

It was strongly proved that the quaternary nitrogen atom was required for enhancing activity [17]. The 13-ethyl-9-ethoxyl (**9**), 13-ethyl (**10**), and 13-methyl (**11**) homologs showed the increased antibacterial activity against *Staphylococcus aureus* by eight-, four- and two fold over the parent 13-alkyl-substituted and 13-unsubstituted protoberberinium salts, respectively. Replacement of methoxyl group by a methylenedioxy group at the C-2 and the C-3 of ring A could also increase their antibacterial activity. Further investigation [18, 19] showed that introduction of hydrocarbon groups at position C-8 such as 8-alkyl and 8-phenyl-substituted berberines and their 12-bromo derivatives could increase the antimicrobial activity.

A series of 9-*O*-acyl- and 9-*O*-alkyl- berberine derivatives were synthesized and evaluated for antimicrobial activity against Gram-positive, Gram-negative bacteria and fungi [20]. Octanoyl, decanoyl and lauroyl derivatives among the acyl analogs, and hexyl, heptyl, octyl, nonyl, decyl and undecyl derivatives among the alkyl analogs all exhibited strong antimicrobial activity against Gram-positive



3 **4** **5**

Fig. (3). Structures of compounds **3**-**5**.

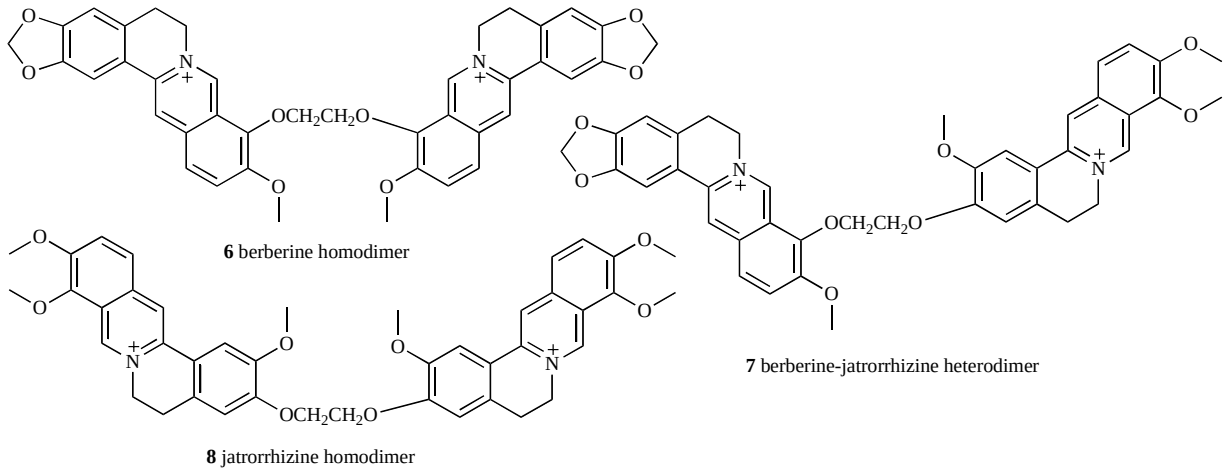


Fig. (4). Structures of compounds **6**-**8**.

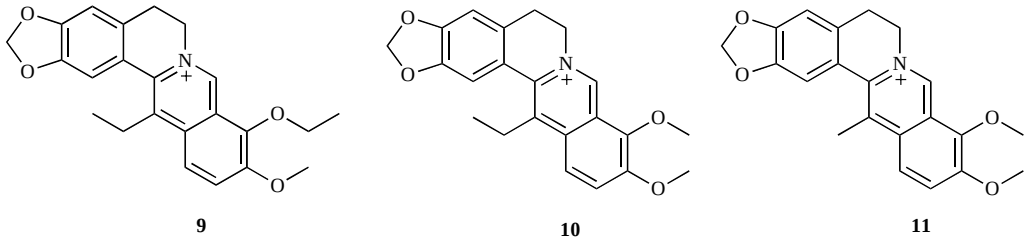


Fig. (5). Structures of compounds **9-11**.

bacteria and fungi, but poor activity against Gram-negative bacteria. Too short or too long substituent length would decrease their activity. As a whole, alkyl analogs were more active than acyl analogs for antimicrobial activity. These results suggested that the presence of lipophilic substituents with moderate sizes might be crucial for the optimal antimicrobial activity, such as compounds **12** and **13**.

The alkyl substituents at C-8 significantly increased the antimicrobial activity of berberine as the length of aliphatic chain was elongated, but gradually decreased its activity when the alkyl chain exceeded eight carbon atoms, especially for Gram-positive bacteria [21]. At the same time, the toxicity decreased gradually with the elongation of the aliphatic chain. In all compounds, 8-octylberberine (**14**) displayed the highest antimicrobial activity.

When the 9-*O*-methyl of 13-(4-isopropylbenzyl) berberine was replaced by various acyl, alkyl, and benzyl groups, a novel series of 9-*O*-alkyl-13-(4-isopropylbenzyl)

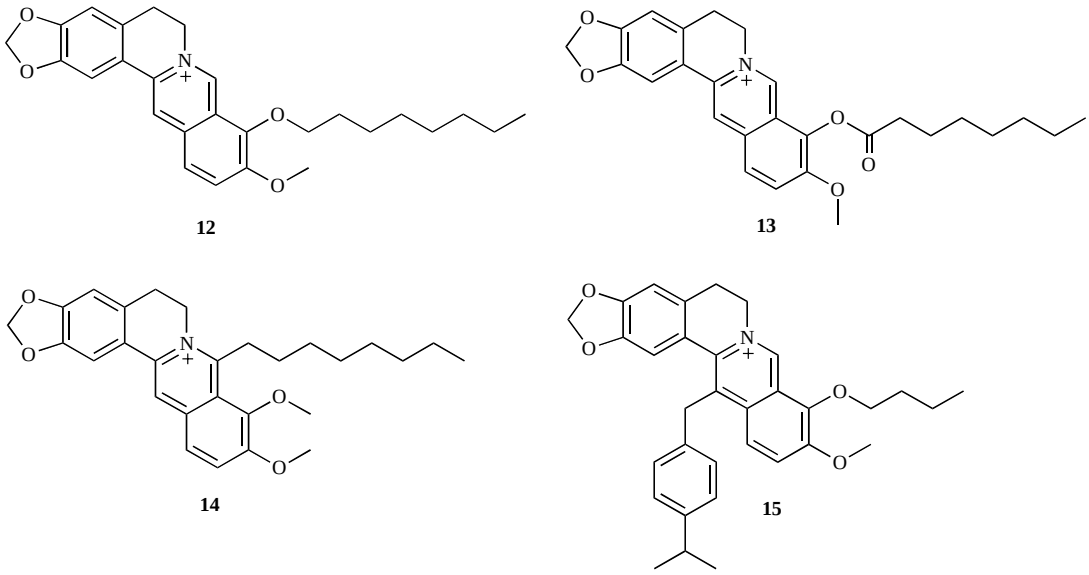
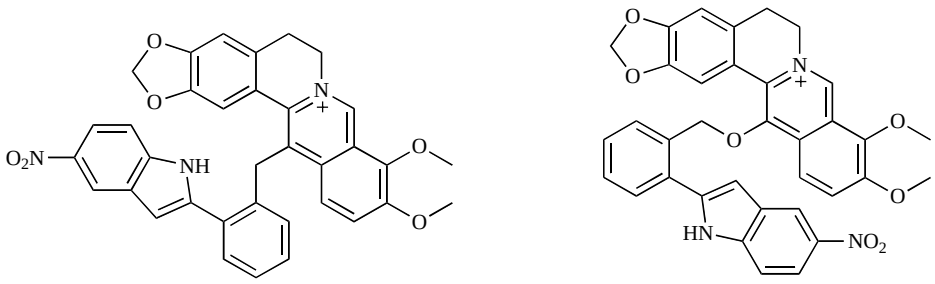


Fig. (6). Structures of compounds **12-15**.



16 **17**

Fig. (7). Structures of compounds **16** and **17**.

berberine derivates were obtained, and showed the enhanced antifungal activities against various human pathogenic fungi. Among them, 9-*O*-butyl-13-(4-isopropylbenzyl)berberine (**15**) exhibited the most potent antifungal activities, with MIC against *Cryptococcus neoformans*, *Candida* species and *Aspergillus* species of 0.25–1 μg/ml, 2–4 μg/ml, respectively [22].

A covalently linked combination of berberine at C-13 position and 2-phenyl-5-nitro-1*H*-indole at the ortho position of the indolic 2-phenyl ring *via* a methylene linking group gave a high antibacterial hybrid (**16**) [23]. An increase of linker chain length by introduction of an oxygen atom produced a new hybrid (**17**) which had stronger antibacterial activity and MDR pump inhibitory potency than hybrid **16**.

3. STRUCTURAL MODIFICATION FOR ANTI-ALZHEIMER'S DISEASE

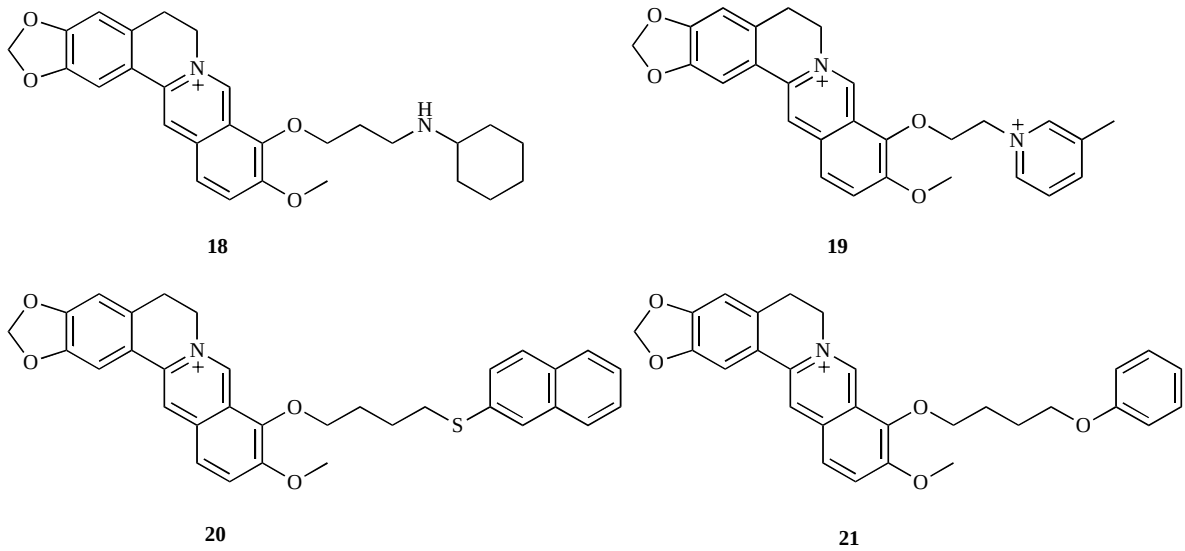
Alzheimer's disease (AD), the most frequent and predominant cause of dementia in the elder population, is a progressive neurodegenerative disorder. Growing evidences suggested that β-amyloid (Aβ) deposits, oxidative stress, τ-protein aggregation, and low levels of acetylcholine (ACh) played significant roles in the pathophysiology of the disease [24]. Based on these mechanisms, different treatment approaches and major therapeutic strategies could be adopted.

Berberine could reduce Aβ levels at the range of berberine concentration without cellular toxicity by modulating APP processing in human neuroglioma H4 cells which stably express Swedish-type of APP [25]. The IC_{50} for extracellular Aβ production was 5 μM.

Increasing the level of ACh through inhibition of the acetylcholinesterase (AChE) was another way for the therapeutics of AD. A new series of berberine derivatives were designed and synthesized as dual inhibitors of AChE and BuChE [26-28]. Through systematic studies of the optimal chain length at C-9 and the functions at the end of its chain, four potent inhibitors **18**, **19**, **20**, **21** were obtained with the IC_{50} of 0.02, 0.048, 0.078, 0.097 μM, correspondingly. A kinetic study indicated that the berberine derivatives caused a mixed type of inhibition and could interact with both peripheral anionic site and catalytic active site.

4. STRUCTURAL MODIFICATION FOR HYPOGLYCEMIC ACTIVITY

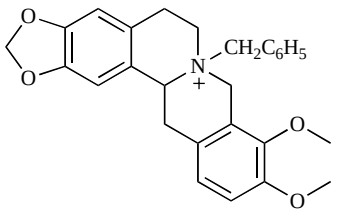
Clinical use of berberine for type 2 diabetes mellitus has been reported since 1988, although its mechanism of



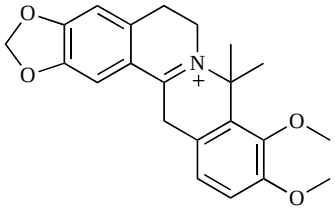
18 **19**

20 **21**

Fig. (8). Structures of compounds **18**-**21**.



22



23

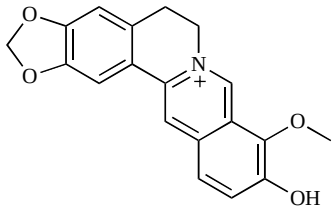
Fig. (9). Structures of compounds **22** and **23**.

antihyperglycemic effects is still uncertain. Basically, berberine inhibits α-glucosidase to reduce intestinal absorption of monosaccharides. In addition, berberine regulates peroxisome proliferator-activated receptors and the expression of positive transcription elongation factor b in diabetic adipocytes [29].

Due to its antibacterial activity, berberine cannot be used for the treatment of diabetes for an extended period. Fourteen berberine derivatives were synthesized and their antihyperglycemic effect was undertaken in alloxan-induced mice [30]. The results suggested that the methyllenedioxy function at C-2 and C-3 might be indispensable for binding activity of protoberberine to β-cell membrane. Quaternary salts also played an important role in binding interaction. *N*-hydrophobic group was found to possess higher affinity. Compound **22** is the most effective derivative in lowering blood glucose.

The 8,8-dimetheyldihydroberberine (**23**) was synthesized with better stability and bioavailability over berberine and dihydroberberine [31]. In diet-induced obese, **23** decreased tissue triglyceride accumulation and insulin resistance, as well as improved glucose tolerance.

Berberine was investigated to possess up-regulating activity not only on insulin receptor (InsR) but also low-density-lipoprotein receptor (LDLR) [32]. According to structure-activity relationships analysis, some appropriate modifications on phenyl ring A or D of berberine might keep the up-regulatory activities. 10-hydroxylberberine (**24**) presented activities on the gene expression of either LDR or InsR.



24

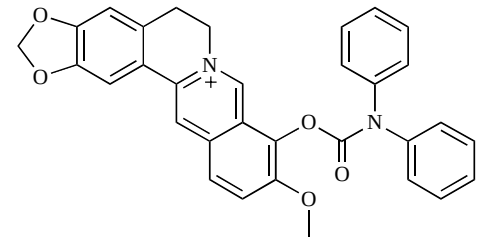
Fig. (10). Structure of compound **24**.

5. STRUCTURAL MODIFICATION FOR ANTI-INFLAMMATORY ACTIVITY

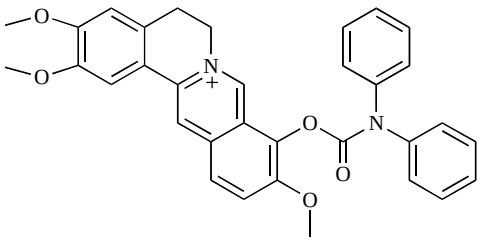
The definite mechanism that berberine repressed inflammatory responses remained unclear. But it had been reported that berberine could inhibit the expression of iNOS, COX-2, IL-1, IL-6 and NF-κB activation. At the same time, it reduced the LPS-induced intestinal damage by elevating the activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), and by suppressing the activation of toll-like receptor 4 (TLR4) [33-36] .

The investigation about the effects of 13-alkyl-substituted berberine on the expression of iNOS showed that the concentration of 50% inhibition of NO production (IC_{50}) by 13-methylberberine and 13-ethylberberine was 11.6 μM and 9.32 μM**,** respectively [37].

N,*N*-Diphenylcarbamoyl derivative (**25**) of 9-demethyl-berberine and *N*, *N*-Diphenylcarbamoyl derivative (**26**) of 9-demethylpalmatine showed ten times stronger inhibitory activities against the induction of edema on mouse ear by application of 12-*O*-tetradecanoylphorbol-31-acetate (TPA) than corresponding mother compounds had [38].



25

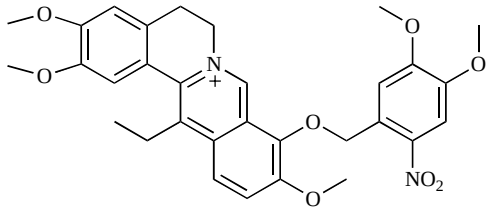


26

Fig. (11). Structures of compounds **25** and **26**.

The $P2X_7$ receptor ($P2X_7R$), a plasma membrane receptor for extracellular adenosine-5-triphosphate (ATP) dominantly expressed in inflammation-related cells, had been known as essential regulator of both IL-1 maturation and externalization [39, 40]. Therefore, blocking of IL-1 pathway through the inhibition of $P2X_7$ had been investigated. Berberine bearing alkyl groups at the C-13 position, especially 2-nitro-4, 5-dimethoxy-benzyl group substituted at C-9 position, such as compound **27**, exhibited potent inhibitory efficacy as $P2X_7$ antagonists [41]. The IC_{50} of **27**

on ethidium accumulation in hP2X$_7$-expressing HEK293 cells was 0.17 μM, and its inhibition (%) against BzATP-stimulated IL-1β released by LPS/IFNγ-differentiated human THP-1 cells was 83%.

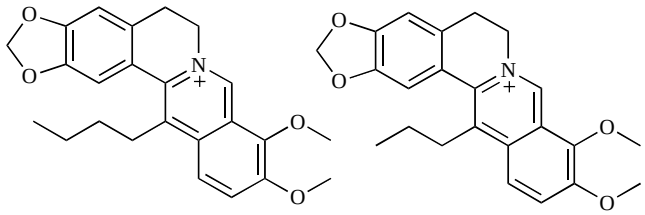


27

Fig. (12). Structure of compound **27**.

6. STRUCTURAL MODIFICATION FOR ANTIMALARIAL ACTIVITY

In 1986, Elford [42] reported that berberine chloride at 50 μM was found to completely block protein synthesis in *Plasmodium falciparum*. Although antimalarial effects had been ascribed to berberine, little quantitative experimental data supported this point of view. In 1988, ten protoberberine derivatives [43] were tested *in vitro* against two clones of human malaria, *P. falciparum* D-6 and W-2. In the vitro screen, a proportion of these derivatives exhibited potency comparable to that of quinine. However, none of them was active *in vivo* against *P. berghei*. This investigation suggested that a dihydro or quaternary protoberberine structure is necessary for the potent compounds.

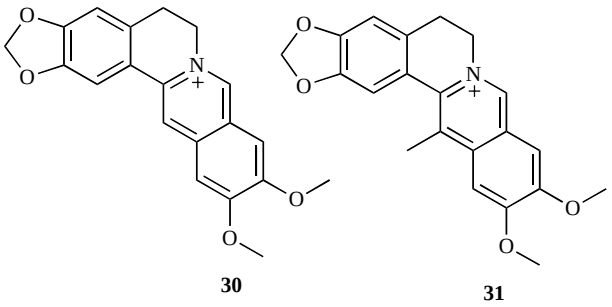


28 **29**

Fig. (13). Structures of compounds **28** and **29**.

Thirty-nine protoberberine derivatives were evaluated for antimalarial activity *in vitro* against *Plasmodium falciparum* in 1988 [44]. The results demonstrated that the activity of the quaternary protoberberinium salts like berberine was higher than that quaternary salts such as the *N*-metho salts or *N*-oxides. Meanwhile, the type of *O*-alkyl substituents on ring A and D as well as the nature and the bulk of the substituents at the C-13 position would influence the activity. Among the 13-alkylberberines and 13-alkylpalmatines, 13-butylberberine (**28**) and 13-propylpalmatine (**29**) were the most active compounds.

Further exploration was done to find out the influence of the type of oxygen substituents on ring A, C and D and the position of the oxygen functions on ring D [45]. Shifting the oxygen functions at C-9 and C-10 to C-10 and C-11 would strongly increase the activity. Derivatives bearing a methylenedioxy function at ring A showed higher activity than those methoxy groups at the same positions. The most potent derivatives **30**, **31** were shown in Fig. (**14**).



30 **31**

Fig. (14). Structures of compounds **30** and **31**.

7. OTHERS

The anti-human cytomegalovirus activity of berberine and its structurally related compounds had been tested and reported [46], which indicated the anti-HCMV activity (IC_{50} 0.68 μM) of berberine was quivalent to ganciclovir (IC_{50} 0.91 μM). The mechanism might be that berberine interfered with intracellular events after virus penetration into the host cells and before viral DNA synthesis.

A series of new protoberberine quaternary ammonium compounds were synthesized for the effect on ouabain-induced arrhythmia [47]. Proportion of these compounds possessed the prophylaxis effect. Moreover, *N*-(4-chlorobenzyl)tetrahydroberberrubine had the most significant action, with $ED_{50(VF)}$ of 1.03×10^{-6} mol/kg.

Tetrahydroprotoberberines are novel antagonists on dopamine receptors in the brain. To quantize the interaction between tetrahydroprotoberberine and dopamine D_2 receptor, Free-Wilson method was used [48]. The result demonstrated that OH group at C-2 and Cl at C-12 were beneficial, the steric hinder at C-11 was unfavourable, and substituents at C-10 had little effect on the affinities.

CONCLUSION

As illustrated in the above descriptions, the multiple activities of berberine are closely related to its structure. The quaternary salts and the nature of substituents at different position greatly influenced the activities. Although it possesses adverse pharmacological properties, modern medicinal chemistry-based molecular modification will play an important role in overcoming these disadvantages for new drug discovery.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (81072554).

REFERENCES

[1] Li, B.; Zhu, W.L.; Chen, K.X.; Advances in the study of berberine and its derivatives. *Acta Pharm. Sin*., **2008**, *43*, 773-787.

[2] Tang, J.; Feng, Y.B.; Tsao, S.W.; Wang, N.; Curtain, R.; Wang, Y.W. Berberine and Coptidis Rhizoma as novel antineoplastic agents: A review of traditional use and biomedical investigations. *J. Ethnopharmacol*., **2009**, *126*, 5-17.

[3] Kettmann, V.; Kostalova, D.; Hoeltje, H.D. Human topoisomerase I poisoning: docking protoberberines into a structure-based binding site model. *J. Comput. Aid Mol. Des*., **2004**, *18*, 785-796.

[4] Inoue, K.; Kulsum, U.; Chowdhury, S.A.; Fujisawa, S.I.; Ishihara, M.; Yokoe, I.;Sakagami, H. Tumor-specific cytotoxicity and

apoptosis-inducing activity ofberberines. *Anticancer Res*., **2005**, 4053-4059.

[5] Teresa, S.; Paulo, O.; Vilma, S.; Ed, P.; Donna, P.; Jon, H. Different concentrations of berberine result in distinct cellular localization patterns and cell cycle effects in a melanoma cell line. *Cancer Chemother. Pharmacol*., **2008**, *61*, 1007-1008.

[6] Goncalo, C.; Pereira, F.; Branco, A.C.; Matos, P.; Donna, P.; Edward, P.; Teresa, S.; Vilma, S.; Maria, S.; Antonio, M.; Jon, H.; Paulo, O. Mitochondrially targeted effects of berberine [natural yellow 18, 5,6-dihydro-9,10-dimethoxybenzo(g)-1,3-benzodioxolo(5,6-a) quinolizinium] on K1735-M2 mouse melanoma cells: comparison with direct effects on isolated mitochondrial fractions. *J. Pharmacol. Exp. Ther*., **2007**, *323*, 636-649.

[7] Iwasa, K.; Moriyasu,M.; Yamori, T.; Turuo, T.; Lee, D.U.; Wiegrebe,W. *In vitro* cytotoxicity of the protoberberine-type alkaloids. *J. Nat. Prod*., **2001**, *64*, 896-898

[8] Orfila, L.; Rodriguez, M.; Colman, T.; Hasegawa, M. ; Merentes, E.; Arvelo, F. Structural modification of berberine alkaloids in relation tocytotoxic activity *in vitro*. *J. Ethnopharmacol*., **2000**, *71*, 449-456.

[9] Makhey, D.; Li, D.J.; Zhao, B.P.; Sim, S.P; Li, T.K,; Liu, A.; Liu ,L. F.; LaVoiea, E. J. Substituted benzo[i]phenanthridines as mammalian topoisomerase-targeting agents. *Bioorg. Med. Chem*., **2003**, *11*, 1809-1820.

[10] Cui, W.H.; Iwasa ,K.; Tokuda ,H.; Kashihara ,A.; Mitani ,Y.; Hasegawa , T.; Nishiyama , Y.; Moriyasu, M.; Nishino, H.; Hanaoka ,M.; Mukai ,C.; Takeda, K. Potential cancer chemopreventive activity of simple isoquinolines, 1-benzylisoquinolines, and protoberberines. *Phytochemistry*, **2006**, *67*, 70-79.

[11] Zhang, W.J.; Ou, T.M.; Lu,Y.J.; Huang, Y.Y.; Wu, W.B.; Huang, Z.S.; Zhou, J.L.; Wong, K.Y.; Gu, L.Q. 9-Substituted berberine derivatives as G-quadruplex stabilizing ligands in telomeric DNA. *Bioorg. Med. Chem*., **2007**, *15*, 5493-5501.

[12] Ma,Y.; Ou, T.M.; Hou, J.Q.; Lu,Y.J.; Tan, J.H.; Gu, L.Q.; Huang, Z.S. 9-*N*-Substituted berberine derivatives: Stabilization of G-quadruplex DNAand down-regulation of oncogene c-myc. *Bioorg. Med. Chem*., **2008**, *16*, 7582-7591.

[13] Ma, Y.; Ou, T.M.; Tan, J.H.; Hou, J.Q.; Huang, S.L.; Gu, L.Q.; Huang, Z.S. Synthesis and evaluation of 9-*O*-substituted berberine derivatives containing aza-aromatic terminal group as highly selective telomeric G-quadruplex stabilizing ligands. *Bioorg. Med. Chem. Lett*., **2009**, *19*, 3414-3417.

[14] Long, Y.H.; Bai, L.P.; Qin, Y.; Pang, J.Y.; Chen, W.H.; Cai, Z.W.; Xu Z.L.; Jiang, Z.H. Spacer length and attaching position-dependent binding of synthesized protoberberine dimers to double-stranded DNA. *Bioorg. Med. Chem*., **2006**, *14*, 4670-4676.

[15] Yi, Z.B.; Yu, Y.; Liang, Y.Z.; Zeng, B. Evaluation of the antimicrobial mode of berberine by LC/ESI-MS combined with principal component analysis. *J. Pharm. Biomed. Anal*., **2007**, *44*, 301-304.

[16] Inna, S.; Maria, M.; Kim, L.; Vladimir, S. Transfer of cationic antibacterial agents berberine, palmatine, and benzalkonium through bimolecular planar phospholipid film and *Staphylococcus aureus* membrane. *IUBMB Life*, **2001**, *52*, 321-324.

[17] Iwasal, K.; Kamigauchil, M.; Uek, M.; Antibacterial activity and structure-activity relationships of berberine analogs. *Eur. J. Med. Chem*., **1996**, *31*, 469-478.

[18] Iwasa, K.; Hye-Sook, K.; Wataya, Y.; Lee, D.U.; Antimalarial activity and structure–activity relationships of protoberberine alkaloids. *Eur. J. Med. Chem*., **1998**, *33*, 65-69.

[19] Iwasa, K.; Lee, D.U.; Kang, S.I.; Wiegrebe, W. Antimicrobial activity of 8-alkyl- and 8-phenyl-substituted berberines and their 12- bromo derivatives. *J. Nat. Prod*., **1998**, *61*, 1150-1153.

[20] Kim, S.H.; Lee, S.J.; Lee, J.H.; Sun, W.S.; Kim. Antimicrobial activity of 9-*O*-acyl- and 9-*O*-alkylberberrubine derivatives. *Planta Med*., **2002**, *68*, 277-81.

[21] Yang, Y.; Ye, X.L.; Li, X.G.; Zhen, J.; Zhang, B.S.; Yuan, L.J. Synthesis and antimicrobial activity of 8-alkylberberine derivatives with a long aliphatic chain. *Planta Med*., **2007**, *73*, 602-604.

[22] Park, K. D.; Cho, S. J.; Moon, J. S.; Kim, S. U. Synthesis and antifungal activity of a novel series of 13-(4-isopropylbenzyl)berberine derivatives. *Bioorg. Med. Chem. Lett*., **2010**, *20*, 6551-6554.

[23] Siritron, S.; Bongkot, T.; Nussara, M.; Gabriele, C.; Danuta, T.; Kim, L.; Apichart, S.; Therdsak, P.; Karina, C.; Gornalle; Jennifer, L.; Becke; John B. Bremnere. Antibacterial activity of berberine-NorA pump inhibitor hybrids with a methylene ether linking group. *Bioorg. Med. Chem*., **2009**, *17*, 3866-3872.

[24] Scarpini, E.; Scheltens, P.; Feldman, H. Treatment of Alzheimer's disease: current status and new perspectives. *Lancet Neurol*., **2003**, *2*, 539-547.

[25] Asai, M.; Iwata, N.; Yoshikawa, A.; Aizaki, Y.; Ishiura, S.; Saido, T. C.; Maruyama, K. Berberine alters the processing of Alzheimer's amyloid precursor protein to decrease Aβ secretion. *Biochem. Biophys. Res. Commun*., **2007**, *352*, 498-502.

[26] Huang, L,; Luo, Z.H.; He, F.; Shi, A.; Qin, F.F.; Li, X.S. Berberine derivatives, with substituted amino groups linked at the 9-position, as inhibitors of acetylcholinesterase/butyrylcholinesterase. *Bioorg. Med. Chem. Lett*., **2010**, *20*, 6649-6652.

[27] Huang, L.; Shi, A.; He, F.; Li, X.S. Synthesis, biological evaluation, and molecular modeling of berberine derivatives as potent acetylcholinesterase inhibitors. *Bioorg. Med. Chem*., **2010**, *18*, 1244-1251.

[28] Huang, L.; Luo, Z.H.; He, F.; Lu, J.; Li, X.S. Synthesis and biological evaluation of a new series of berberine derivatives as dual inhibitors of acetylcholinesterase and butyrylcholinesterase. *Bioorg. Med. Chem*., **2010**, *18*, 4475-4484.

[29] Zhou, J.Y.; Zhou, S.W. Berberine regulates peroxisome proliferator-activated receptors and positive transcription elongation factor b expression in diabetic adipocytes. *Eur. J. Pharmacol*., **2010**, *649*, 390-397.

[30] Bian, X.L.; He, L.C.; Yang, G.D. Synthesis and antihyperglycemic evaluation of various protoberberine derivatives. *Bioorg. Med. Chem. Lett*., **2006**, *16*, 1380-1383.

[31] Cheng, Z.; Chen, A.F.; Wu, F.; Sheng, L.; Zhang, H.K.; Gu, M.; Li, Y.Y.; Zhang, L.N.; Hu, L.H.; Li, J.Y.; Li, J. 8,8-Dimethyldihydroberberine with improved bioavailability and oral efficacy on obese and diabetic mouse models. *Bioorg. Med. Chem*., **2010**, *18*, 5915-5924.

[32] Wang, Y.X.; Wang, Y.P.; Zhang, H.; Kong, W.J.; Li, Y.H.; Liu, F.; Gao, R.M.; Liu, T.; Jiang, J.D.; Song, D.Q. Synthesis and biological evaluation of berberine analogues as novel up-regulators for both low-density-lipoprotein receptor and insulin receptor. *Bioorg. Med. Chem. Lett*., **2009**, *19*, 6004-6008.

[33] Lee, I.A.; Hyun, Y.J.; Kim, D.H. Berberine ameliorates TNBS-induced colitis by inhibiting lipid peroxidation, enterobacterial growth and NF-κB activation. *Eur. J. Pharmacol*., **2010**, *648*, 162-170.

[34] Kuo, C.L.; Chi, C.W.; Liu, T.Y. The anti-inflammatory potential of berberine *in vitro* and *in vivo*. *Cancer Lett*., **2004**, *203*, 127-137.

[35] Zhang, Q.; Piao, X.L.; Piao, X.S.; Lu, T.; Wang, D.; Kim, S.W. Preventive effect of Coptis chinensis and berberine on intestinal injury in rats challenged with lipopolysaccharides. *Food Chem. Toxicol*., **2011**, *49*, 61-69.

[36] Lu, D.Y.; Tang, C.H.; Chen, Y.H.; Wei, I.H. Berberine suppresses neuroinflammatory responses through AMP-activated protein kinase activation in BV-2 microglia. *J. Cell. Biochem*., **2010**, *110*, 697-705.

[37] Lee, D.U.; Kang, Y.J.; Park, M.K.; Lee, Y.S.; Seo, H.G.; Kim, T.S.; Kim, C.H.; Chang. K.C. Effects of 13-alkyl-substituted berberine alkaloids on the expression of COX-II, TNF-a, iNOS, and IL-12 production in LPS-stimulated macrophages. *Life Sci*., **2003**, *73*, 1401-1412.

[38] Yasukawa, K.; Takido, M.; Ikekawa, T.; Shimada, F.; Takeuchi, M.; Nakagawa, S. Relative inhibitory activity of berberine-type alkaloids against 12-O-tetradecanoylphorbol-13-acetate-induced inflammation in mice. Chem. *Pharm. Bull*., **1991**, *39*, 1462-1465.

[39] Tsukimoto, M.; Harada, H.; Degawa, M. Role of purinoceptors in immune-mediated disease (therapies targeting the $P2X_7$ receptor). *Drug Discov. Today Ther. Strateg.*, **2007**, *4*, 33-37.

[40] Ferrari, D.; Pizzirani, C.; Adinolfi, E.; Lemoli, R. M.; Curti, A.; Idzko, M.; Panther, E.; Di Virgilio, F. The $P2X_7$ receptor: a key player in IL-1 processing and release. *J. Immunol*., **2006,** *176*, 3877-3883.

[41] Lee, G.E.; Lee, H.S.; Lee, S.D.; Kim, J.H.; Kim, W.K.; Kim, Y.C. Synthesis and structure–activity relationships of novel, substituted 5,6-dihydrodibenzo[a,g]quinolizinium $P2X_7$ antagonists. *Bioorg. Med. Chem. Lett*., **2009**, *19*, 954-958.

[42] Elford B.C. L-Glutamine influx in malaria-infected erythrocytes: a target for anti malarials . *Parasitol. Today*, **1986**, *2*, 310-312.

[43] Jonathan L. V.; Daniel, K. Protoberberine alkaloids as antimalarials. *J. Med. Chem.*, **1988**, *31*, 1085-1087.

[44] Iwasa, K.; Kim, H.S.; Wataya, Y.; Lee, D.U. Antimalarial activity and structure-activity relationships of protoberberine alkaloids. *Eur. J. Med. Chem.*, **1988**, *33*, 65-69.

[45] Iwasa, K.; Nishiyama, Y.; Ichimaru, M.; Moriyasu, M.; Kim, H.S.; Wataya, Y.; Yamori, T.; Takashi, T.; Lee, D.U. Structure-activity relationships of quaternary protoberberine alkaloids having an antimalarial activity. *Eur. J. Med. Chem.*, **1999**, *34*, 1077-1083.

[46] Hayashi, K.; Minoda, K.; Nagaoka, Y.; Hayashi, T.; Uesatob, S. Antiviral activity of berberine and related compounds against human cytomegalovirus. *Bioorg. Med. Chem. Lett.*, **2007**, *17*, 1562-1564.

[47] Zhang, C.; Huang, W.L. Synthesis and antiarrhythmic activity of protoberberine quaternary ammonium compounds. *J. Chin. Pharm. Univ.*, **2003**, *34*, 7-12.

[48] Fu, X.C.; Hu, Y.Z.; Zhou, Q.T.; Bai, D.L. Quantitative structure-activity relationships for tetrahydroprotoberberines to interact with the dopamine D2 receptor. *Chin. J. Med. Chem.*, **1999**, *32*, 111-113.

Received: March 20, 2011 Revised: June 01, 2011 Accepted: July 03, 2011