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SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 1380–1383

Synthesis and antihyperglycemic evaluation of various protoberberine derivatives

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Received 17 June 2005; revised 29 October 2005; accepted 14 November 2005
Available online 15 December 2005

Abstract—Various berberine derivatives (**2**–**17**) were synthesized and their antihyperglycemic activities were evaluated in a model of β-cell-membrane chromatography and a model of alloxan-induced diabetes mice. The results indicated that compounds **5** and **14** exhibited antihyperglycemic activity. Their structure–activity relationships were discussed.

Type 2 diabetes (T2DM) affects a large proportion of the adult population and is closely associated with other conditions, including obesity, dyslipidemia, and cardiovascular disease.[1–4] It is well known that the loss of the insulin secretion function and insulin resistance contribute to this disease.[5,6] Sulfonylureas, which are widely used as hypoglycemic agents for T2DM, strongly inhibit ATP-sensitive K^+ channel activity by binding to sulfonylurea receptors in pancreatic β-cells.[7,8] Pancreatic β-cells and related sulfonylurea receptors are the targets on which sulfonylurea molecules and other hypoglycemic agents act. Pancreatic β-cells and sulfonylurea receptors were used to investigate the binding interactions between active ligands and their related targets.[9–11] The reported methods that have been used to analyze interactions between β-cell sulfonylurea receptors and drug molecules include: direct measurement of K^+ ion currents,[12] radioligand binding assays,[13] and column-based counting of flow-through radioactivity.[14] In this study, cell-membrane chromatography (CMC)[15] was used to investigate the binding interactions between β-cell-related receptors and drug molecules. CMC is a bio-affinity chromatographic method, in which bioactive cell membranes are immobilized on the silica carrier as a stationary phase. In this chromatographic system, the active molecules that have binding affinity to the cell membrane and its related targets will be retained. The interaction characteristics are reflected directly by the elution data.[15–18]

Berberine **1**, a major compound present in *Coptis chinensis France* (a Chinese traditional medicine), has been used for the treatment of diarrhea and other gastrointestinal diseases in China and other Asian countries.[19,20] It has been reported that berberine **1** has hypoglycemic effects.[21] However, it is not applicable to use berberine **1** for the treatment of diabetes as, when it is used as a hypoglycemic agent for an extended period, serious side effects occur due to its antibacterial activity. In this study, in order to find new structures that have high a hypoglycemic effect but little antibacterial activity, 16 protoberberine derivatives were synthesized with berberine as a lead compound, and their hypoglycemic effects were screened in a CMC model and then in an alloxan-induced diabetic model, and their structure–activity relationships were analyzed.

Berberine **1** has a dibenzo[*a*,*g*]quinolizidine ring system structure. The quaternary salts that have an aromatic C ring and a 2,3-methylenedioxy moiety are essential components for the antibacterial activities of protoberines.[22–24] Therefore, compounds **2**–**4** were derived from replacing the methylenedioxy function of berberine by –OH, $–OCH_3$, and $–OCOCH_3$, respectively.

Compounds **5**–**10**, which have different substitutes at positions C-9, C-10 and C-11, were prepared via the total synthetic route from the starting material of homopiperonylamine and different aromatic aldehydes. The aromatic quaternary nitrogen was reduced to tertiary nitrogen (**11**,**16**), then alkylated to produce *N*-alkyl

Keywords: Protoberberine derivatives; Hypoglycemic effect; Cell membrane chromatography.
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doi:10.1016/j.bmcl.2005.11.045

derivatives **12**–**14** and **17**, and oxidized to N-oxide compound **15**. Their structures are summarized in Table 1. The general synthetic route that is used to synthesize the designated compounds is outlined in Scheme 1.

Protoberberines **1**–**17** were tested in vitro against *Escherichia coli*. The minimum inhibitory concentration (MIC) of each compound was determined using a twofold serial-dilution technique.[25] The MIC value of compound **1** was 5 μg/mL. There was still no antibacterial activity when the concentration of compounds **2**–**17** was raised to 50 μg/mL. The protoberberines **2**–**17** do not possess as much antibacterial potential compared with berberine 1. This result corresponds with the findings reported in the literature.[22–24]

Compounds **1**–**17** were screened using a model of pancreatic-islet β-cell-membrane chromatography.[18,26] The binding affinity of the compounds on β-cell membrane and membrane receptors in the CMC system was reflected by the logarithm of capacity factor ($\log k'$). The results indicated that compounds **1**, **5**, **10**, **14**, **15**, and **17** exhibited retentive behavior similar to that of gliquidone in the model. The others had no retention under the same conditions (Table 2). The chromatographic conditions were given in reference.[26]

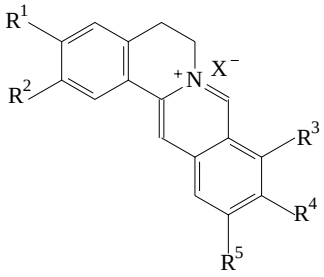
The hypoglycemic effect of compounds that have retentive behavior (**1**, **5**, **10**, **14**, and **15**) was tested in alloxan-induced diabetes mice, as shown in Table 3. Compound **17** was not tested because of its obvious toxicity at experimental dosages.

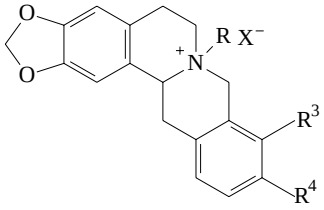
The results derived from CMC screening indicated that the methylenedioxy function at C-2 and C-3 may be essential for the binding activity of protoberberines to β-cell membranes. The binding activity disappeared when the methylenedioxy ring of berberine was opened and the methylenedioxy function was replaced by groups of hydroxyl (**2**), methyloxyl (**3**), and acetoxyl (**4**), respectively.

The quaternary salts have an aromatic C ring, which is a necessary structure of berberine derivatives for antibacterial activity.[22–24] When the quaternary salts were reduced to tertiary amines, their binding affinity to β-cell membranes disappeared. This suggests that the electrostatic effect of positively charged nitrogen atoms in the aromatized C ring may play an important role in binding interactions. However, when tetrahydroberberine was alkylated to quaternary ammonium salts, the binding activity changed with different alkyl derivatives. *N*-Methyl **12** and *N*-ethyl **13** displayed no binding activity. This could be explained by the fact that the electrostatic effects of positively charged nitrogen atoms were shielded by the methyl or ethyl groups. However, *N*-benzyl **14** and **17** possessed high binding affinity. In this case, the interaction forces may be different due to the hydrophobic properties of the benzyl group. These results suggested that the electrostatic effects of positively charged nitrogen atoms may be non-specific binding forces for berberine and that the quaternary ammonium salt structure (that has an aromatic C ring) is not necessary for the hypoglycemic effects of berberine derivatives to occur.

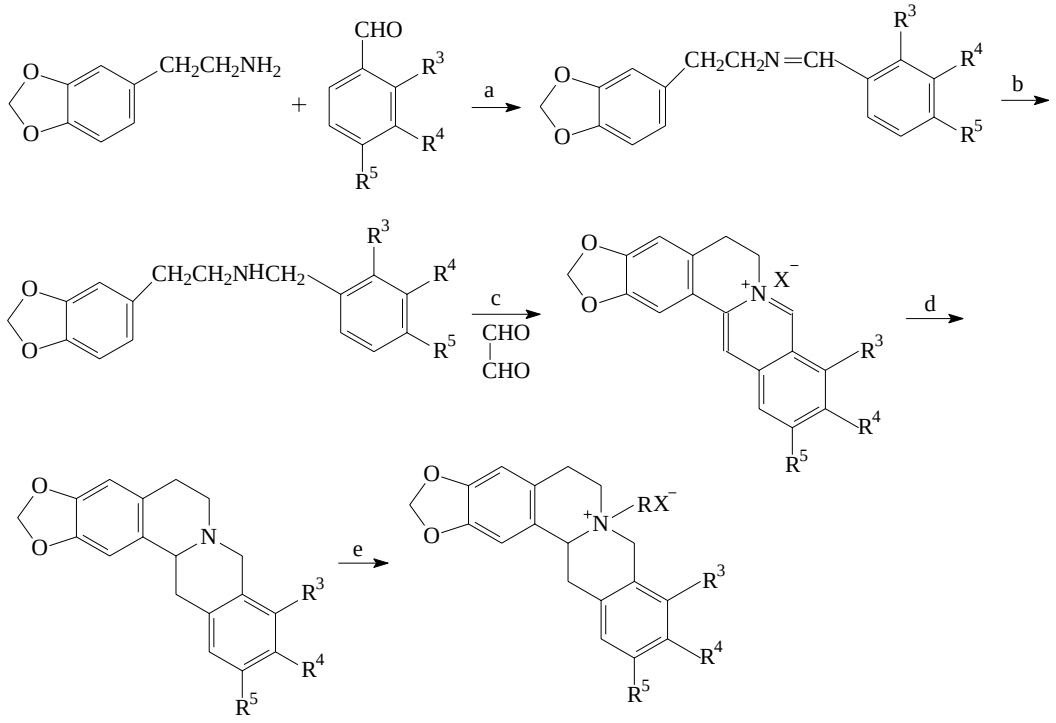
Berberine **1** showed two methoxyl groups at positions C-9 and C-10 (ring D); it also possessed high binding affinity and hypoglycemic activity. Compound **5**, without a substituent on ring D, also possessed high binding affinity and hypoglycemic activity. However, introduction of a methoxyl group at the C-9 position of compound **5** led to a decrease in binding activity. Similar results were observed when a methoxyl group was introduced at the C-10 position, the C-11 position and a benzyloxy group at the C-11 position of compound **5** (compounds **8**, **9**, and **10**, respectively). This indicated that binding affinity

Table 1. Protoberberinum salts





Compound	R^1	R^2	R^3	R^4	R^5	Compound	R^3	R^4	R
1	OCH_2O		OMe	OMe	H	**11**	OMe	OMe	H
2	OH	OH	OMe	OMe	H	**12**	OMe	OMe	Me
3	OMe	OMe	OMe	OMe	H	**13**	OMe	OMe	Et
4	OCOMe	OCOMe	OMe	OMe	H	**14**	OMe	OMe	$CH_2C_6H_5$
5	OCH_2O		H	H	H	**15**	OMe	OMe	O
6	OCH_2O		OMe	H	H	**16**	H	H	H
7	OCH_2O		H	OMe	H	**17**	H	H	$CH_2C_6H_5$
8	OCH_2O		H	H	OMe				
9	OCH_2O		H	H	$OCH_2C_6H_5$				
10	OCH_2O		OH	OMe	H				



Scheme 1. Synthesis of compounds **2**–**17**. Reagents and conditions: (a) 110 °C, 1 h, 85%; (b) $NaBH_4$, CH_3OH, reflux, 2 h, 85%; (c) HOAc, $CuSO_4$, NaCl, 50 °C 0.5 h, then 85 °C, 6 h, 60%; (d) Zn, HCl, reflux, 3 h, 65%; (e) 42 °C, 2 h, 86%.

Table 2. Retention features of compounds **1**–**17** in the model of β-cell membrane chromatography

Compound:	**1**	**2–4**	**5**	**6–9**	**10**	**11–13**	**14**	**15**	**16**	**17**	GQ
$\log k'$	1.32	—	1.44	—	1.09	—	1.73	1.32	—	1.56	1.59

Table 3. Effect of compounds on hyperglycemia in mice with alloxan-induced diabetes ($\bar{x} \pm s$)

Group	Dose (mg/kg)	*n*	Blood glucose (mg/dL)		
			Initial	Final	Reduction
Normal		10	122 ± 23	110 ± 20	−14.4 ± 2.3
Control		13	580 ± 16	558 ± 7	−22 ± 8*
1	200	14	572 ± 9	511 ± 11	−61 ± 3
5	200	13	591 ± 14	536 ± 4	−67 ± 4*
10	200	12	562 ± 11	574 ± 9	−12 ± 5
14	200	13	558 ± 3	412 ± 5	−146 ± 3**
15	200	14	572 ± 6	546 ± 4	−23 ± 3
GQ	200	13	563 ± 5	408 ± 3	−162 ± 5

* $p < 0.01$ versus normal.
** $p < 0.05$ versus control.

may relate to substituents on ring D. This feature could not be concluded here, as further study results are required.

Of the compounds that had retentive behavior, compounds **1**, **5**, and **14** showed significant hypoglycemic activity. Hypoglycemic activity of compound **17** was not tested due to its obvious toxicity at experimental dosages. Compound **14** presented the highest hypoglycemic activity. The relative coefficient between the $\log k'$ values and the reduction values of compounds **1**, **5**, **10**, **14**, and **15** was 0.8714. This indicated that there was a good correlation between the β-CMC model and the mouse model with alloxan-induced diabetes.

As a bio-affinity screen model, the CMC model could be used to investigate ligands that have potential binding affinity with targets on the cell membrane.[15,16,18] The retentive behavior of the active compounds in the pancreatic-islet CMC model (shown in Table 1) indicated that these compounds have a binding affinity for targets in pancreatic-islet β-cell membranes that are similar to those of gliquidone. They may produce hypoglycemic effects through similar mechanisms as sulfonylurea. The hypoglycemic effects can be confirmed in diabetes animal experiments. Although it cannot be confirmed that the binding site is a sulfonylurea receptor, compounds that have no interaction with β-cell membranes were excluded rapidly from the CMC model. Therefore, the number of compounds that are available for animal experiments is greatly reduced. In the CMC model, the separation and purification of receptor proteins are not necessary, and the fast and efficient characteristics of chromatography make it a time- and resource-efficient method for screening new drugs.

From the binding activity–structure point of view, some features can be pointed out. On the basis of the results

derived from CMC screening with protoberberinium, it seems that the substations that exist on ring A are important for binding activity. The binding affinity disappeared when the methylenedioxy function at C-2 and C-3 was replaced by other groups. The binding interactions were affected by the station and environment of N-7. The aromatic quaternary nitrogen may not be the structure of berberine derivatives that is required for hypoglycemic effects to occur. For quaternary salts, which were produced by alkylation of tetrahydrogen berberine, the hydrophobic force of alkyl groups may play an important role in their binding interaction. Of all the synthesized protoberberiniums, compound **14** was the most effective in lowering blood glucose.

Acknowledgment

This work was supported by the Natural Science Foundation of Shaanxi Province (No. 2003C205).

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26. Note: the chromatographic conditions in the CMC model: rabbit pancreatic-islet β-cell membrane column (50 mm × 2.0 mm i.d.) was used. The mobile phase was 25 mM ammonium sulfate buffer (pH 7.4), with a flow rate of 0.5 mL/min. The detection wavelength was 262 nm, at a column temperature of 37 ± 0.5 °C.