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BERBERINE ANALOGUES: PROGRESS TOWARDS VERSATILE APPLICATIONS

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Abstract – The promising activities shown by analogues of natural product berberine in numerous categories such as anti-hyperglycaemic, anti-cancer, anti-inflammatory, anti-alzheimer's disease and anti-microbials, have made it an indispensable structure for development of new therapeutic agents. The compounds bearing various substituents on the berberine skeleton have exhibited a wide spectrum of biological activities. Short reviews on biological importance of this core structure have been showcased in literature. However, systematic review on the research advances of structure-activity relationship of berberine and its analogues seems lacking. In the present review, various analogues of berberine with different pharmacological activities were summarized and their SAR were discussed as well. The current review would provide useful information for further studies on structural modifications on berberine for discovering new drug leads.

CONTENTS

1. INTRODUCTION

Berberine (**1**) is a quaternary ammonium salt from the protoberberine group of isoquinoline alkaloids. It is found in such plants as *Berberis aristata*, *Hydrastis canadensis*, *Phellodendron amurense*, *Coptis chinensis*, *Tinospora cordifolia*, and to a smaller extent in *Argemone mexicana* and *Eschscholzia californica*.[1] BBR is widely used as a traditional medicine to treat diarrhea and gastrointestinal disorders.[2,3] In recent years, it has been also reported that BBR showed various biological activities like antibacterial,[4,5] antifungal,[6] antimalarial,[7] antileishmanial,[8] anticancer,[9-12] anti-alzheimer's disease,[13,14] antiviral,[15,16] cholesterol lowering effect[17] and hypoglycemic effect.[18] The research in development of bioactive molecules from berberine was accelerated during the past decade. Optimization of substituents around berberine has resulted in many lead compounds in a wide range of therapeutic areas. Some reviews on involvement of berberine derivatives in antimicrobial activity is available in literature.[19, 20] Some reports on all activities associated with berberine derivatives are also reported,[21-24] but it seems no comprehensive report on varied activities of berberine derivatives is available in literature to date. Hence, this manuscript describes synthetic strategies, medicinal aspects and SAR of berberine analogues that is covered from the year 2005 to present.

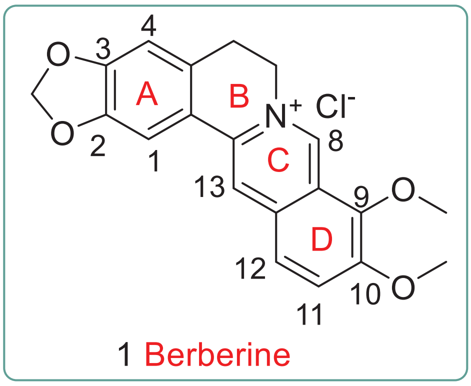
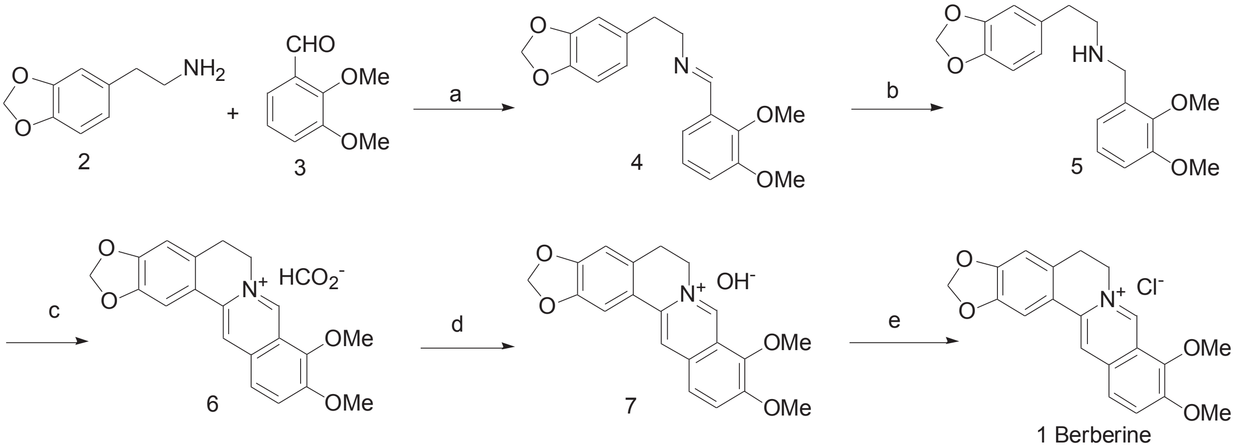


Figure 1. The structure of berberine

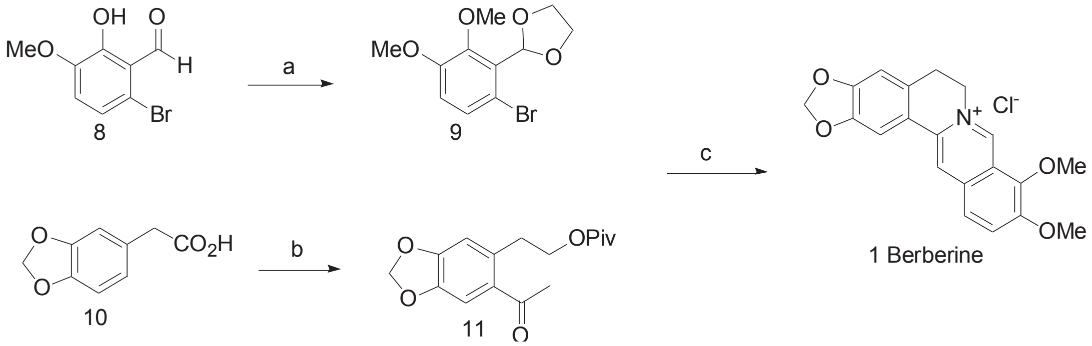
2. SYNTHESIS

The first total synthesis of berberine was reported by Kametani et al. in 1969, which provided berberine iodide in low yield from a commercially unavailable starting material.[25] Yang et al. synthesized a series of berberine analogues, in which berberine could be synthesized through a five-step process (Scheme 1).[17]



Scheme 1. Reagents and conditions: a) 100 ºC, 8 h; b) $NaBH_4$, MeOH, reflux, 5 h; c) glyoxal, HCO_2H, $CuSO_4$, HCl, 100 ºC, 5 h; d) MeOH, H_2O, CaO, rt, 2 h; e) EtOH, HCl, rt, 0.5 h.

Recently, Gatland et al. reported a concise synthesis of berberine by palladium catalyzed enolate arylation to form the isoquinoline core (Scheme 2).[26] This modular route also allows the rapid synthesis of other members of the protoberberine family (e.g., pseudocoptisine and palmatine) by substitution of the readily available aryl bromide and ketone coupling partners.



Scheme 2. Reagents and conditions: a) 1) K_2CO_3, MeI, 2) $HOCH_2CH_2OH$, PTSA; b) 1) $BH_3{\cdot}THF$, 2) Piv-Cl, pyridine, 3) Ac_2O, $ZnCl_2$; c) (5mol%) [$(Amphos)_2PdCl_2$], Cs_2CO_3, THF, 90 °C then add NH_4Cl, EtOH/H_2O, 90-110 °C. Piv=pivaloyl, PTSA=p-aratoluenesulfonic acid, THF=tetrahydrofuran.

3. APPLICATION IN MEDICINAL CHEMISTRY

There is a plethora of information highlighting various biological applications of berberine as a result of certain alterations carried out on structure of berberine (Figure 2). Some of the multitudes of activities encompassed in detail are: anti-hyperglycaemic activity, anti-Alzheimer's disease, anti-cancer activity, anti-microbial activity, binding with DNA, antiparasitic activity, anticoagulant activity, anti-inflammatory activity, antiprotozoal activity, LDLR up-regulators.

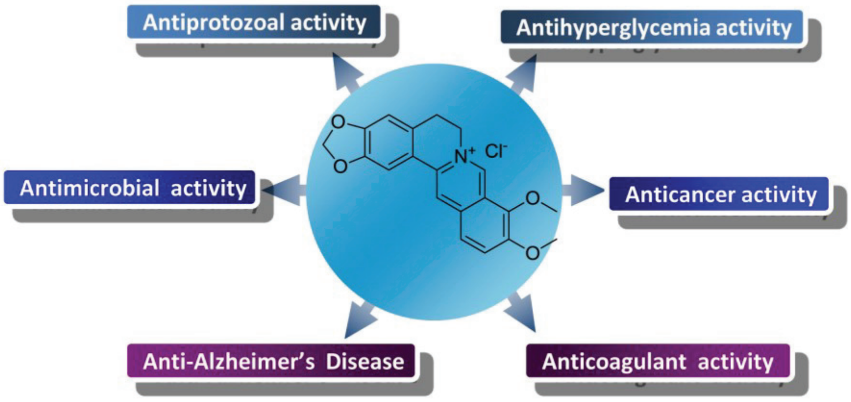


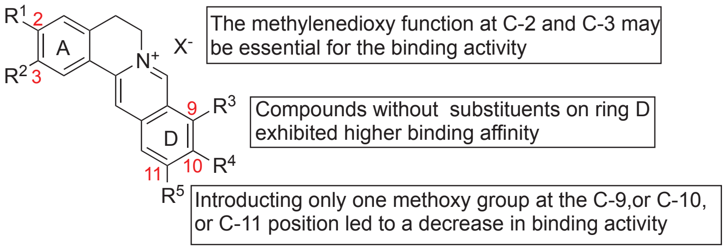
Figure 2. Berberine, a multifunction molecule

3-1. Anti-hyperglycemic Activity

Diabetes mellitus is a group of syndromes characterized by hyperglycaemia.[27] Non-insulin dependent diabetes mellitus (NIDDM, or type 2 diabetes (T2D)) is the most prevalent type. Early stage type 2 diabetes may be managed well with diet and exercise.[28] However, in addition to lifestyle and dietary changes, insulin or oral hypoglycaemic agents like insulin sensitizers either alone or in combination are still needed for controlling over blood glucose levels.[28] Berberine is the main active constituent of

Rhizoma coptidis, a traditional Chinese herb for the medication of diabetes with a history that can be traced to as early as 500 AD.[29,30] The beneficial metabolic effects of berberine have been reported by several groups in clinical studies with type 2 diabetes mellitus patients since 1988.[31-34]

In 2006, Bian et al. synthesized protoberberine derivatives and tested their hypoglycemic effects.[35] In this study, cell-membrane chromatography and alloxan-induced diabetes mice were employed to evaluate the hypoglycemic effects of protoberberine derivatives. The binding affinity of the compounds on β-cell membrane and membrane receptors in the cell-membrane chromatography system was reflected by the logarithm of capacity factor (log *k'*). The results indicated that compounds **1**, **12-15** exhibited retentive behavior similar to that of gliquidone in the model. Then hypoglycemic effect of these compounds (**12-15**) that have retentive behavior was tested in alloxan induced diabetes mice (Figure 3).

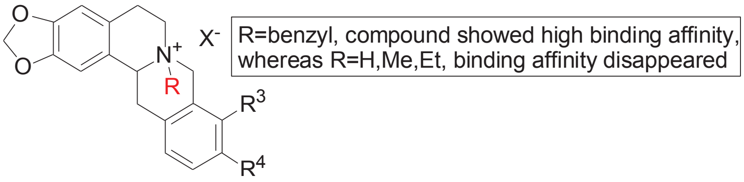


The methylenedioxy function at C-2 and C-3 may be essential for the binding activity

Compounds without substituents on ring D exhibited higher binding affinity

Introducting only one methoxy group at the C-9,or C-10, or C-11 position led to a decrease in binding activity

						Blood glucose (mg/dL)				
Compound	R^1 R^2	R^3	R^4	R^5	log *k'*	Dose (mg/kg)	n	Initial	Final	Reduction
normal							10	122 ± 23	110 ± 20	-14.4 ± 2.3
control							13	580 ± 16	558 ± 7	-22 ± 8
1	OCH_2O	OMe	OMe	H	1.32	200	14	572 ± 9	511 ± 11	-61 ± 3
12	OCH_2O	H	H	H	1.44	200	13	591 ± 14	536 ± 4	-67 ± 4
13	OCH_2O	OH	OMe	H	1.09	200	12	562 ± 11	574 ± 9	-12 ± 5
Gliquidone					1.59	200	13	563 ± 5	408 ± 3	-162 ± 5



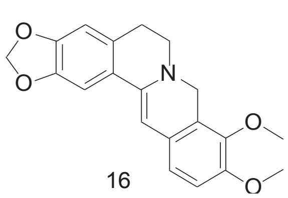
R=benzyl, compound showed high binding affinity, whereas R=H,Me,Et, binding affinity disappeared

Blood glucose (mg/dL)

Compound	R^3	R^4	R	log *k'*	Dose (mg/kg)	n	Initial	Final	Reduction
normal						10	122 ± 23	110 ± 20	-14.4 ± 2.3
control						13	580 ± 16	558 ± 7	-22 ± 8
14	OMe	OMe	$CH_2C_6H_5$	1.73	200	13	558 ± 3	412 ± 5	-146 ± 3
15	OMe	OMe	O	1.52	200	14	572 ± 6	546 ± 4	-23 ± 3
Gliquidone				1.59	200	13	563 ± 5	408 ± 3	-162 ± 5

Figure 3. SAR and antihyperglycemic activity for berberine derivatives

In 2008, Turner et al. reported that dihydroberberine (dhBBR, **16**) showed remarkable improvement in vivo efficacy in treatment of insulin-resistant rodents.[36] In diet-induced obese rats, 125 mg/kg twice a day was necessary for berberine to reduce random fed and fasting blood glucose, and 380 mg/kg/day was required to reduce plasma triglyceride and relieve insulin resistance. However, dhBBR (**16**) effectively counteracted the increased insulin resistance and triglyceride accumulation in adipose tissue at a much lower dosage (100 mg/kg/day).



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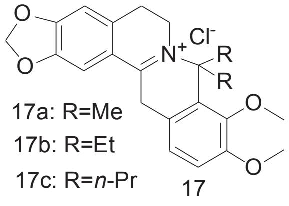

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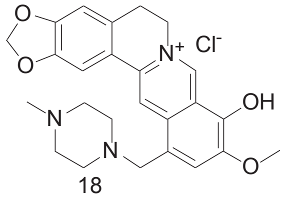
Later, Cheng et al. found that dhBBR is readily transformed into BBR after oral administration via acid-catalyzed aromatization under acidic condition in the stomach and a majority (90%) of absorbed drug in rat plasma was found to be berberine instead of dhBBR.[29, 36] In their continuous work, Cheng et al. proposed to prevent aromatization of dhBBR by 8,8-disubstitution with alkyl groups and further optimized dhBBR into the more aqueous soluble and acid tolerable 8,8-dialkyldihydroberberine hydrochlorides **17**.[29] 8,8-dimethyldihydroberberine (**17a**) induced a stronger dose-dependent Amp activated protein kinase (AMPK)/acetyl-CoA carboxylase (ACC) phosphorylation than 8,8-dipropyldihydroberberine (**17c**), while 8,8-diethyldihydroberberine (**17b**) displayed the strongest effect. On the other hand, 8,8-dimethyldihydroberberine (**17a**) and 8,8-dipropyldihydroberberine (**17c**) showed no or negligible cell toxicity at up to 40 μM after treatment for 24 h, whereas 8,8-diethyldihydroberberine (**17b**) exhibited toxicity at as low as 10 μM. Thus, 8,8-dimethyldihydroberberine (**17a**) was selected for further pharmacological analysis due to its acceptable reactivity and low toxicity. In addition, 8,8-dimethyldihydroberberine (**17a**) also showed higher aqueous solubility, stability, and bioavailability compared to the other dhBBRs.

Recently, Li et al. synthesized 12-(substituted aminomethyl)berberrubine derivatives and evaluated their anti-diabetic activity against type 2 diabetes mellitus.[37] Compound **18** bearing an *N*-methyl-piperazine-4-methyl group at C-12, exerted the most powerful anti-diabetic activity (Figure 4).

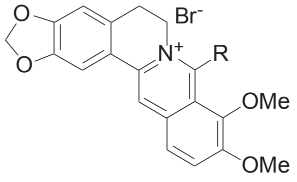


18

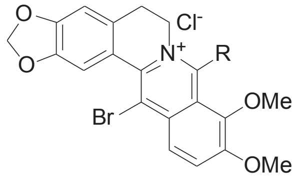
Compound	Concentration (μmol/mL)	Relative sensitization rate (%)	Sensitization rate (%)
18	10	126.29	48.55
	1.0	90.23	41.90
	0.1	-1.09	
1	10	74.23	43.28
	1.0	13.27	21.30
	0.1	-2.93	
Insulin	1.0		48.21

Figure 4. Relative sensitization rates of compound **18** to 3T3-L1 adipocytes and sensitization rates of compound **18** to L6 myotubes

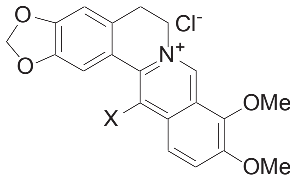
Ding et al. synthesized a series of berberine analogues and studied their effects on glucose consumption in vitro.[38] The glucose-lowering effect of chloroberberine (**21c**) and bromoberberine (**21b**) was better than non-modified berberine under the concentrations studied (0.5, 1, 2, 4 μg/mL). Compared to the control, glucose consumption of HepG2 cells treated with chloroberberine (**21c**) at 1 μg/mL was increased by 90%. 8-Alkylberberine (**19**) and 8-alkyl-13-bromoberberine (**20**) did not exhibit any glucose-lowering effect. Furthermore, MTT assay showed that berberine and its analogues exerted cytotoxicity against HepG2 cells in a dose-dependent manner and alkyl side substitution at C-8 position (**19**) could increase cytotoxicity of alkylberberine. The cytotoxicity of alkyl bromoberberine (**20**) to HepG2 cells was lower than that of alkylberberine (**19**) under the same concentration. Iodoberberine (**21a**) and chloroberberine (**21c**) exhibited nearly no cytotoxicity in low concentration (<4 μg/mL). Thus, it is postulated that halogenated berberine analogues could protect HepG2 cell line.



19a:R=$CH_2(CH_2)_2Me$
19b:R=$CH_2(CH_2)_6Me$
19c:R=$CH_2(CH_2)_{10}Me$



20a:R=$CH_2(CH_2)_2Me$
20b:R=$CH_2(CH_2)_6Me$
20c:R=$CH_2(CH_2)_{10}Me$

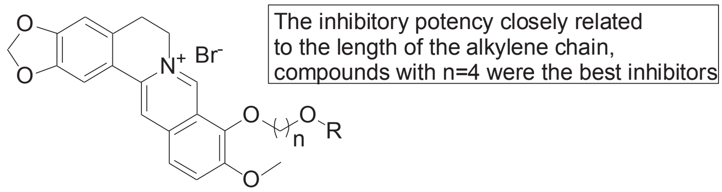


21a:X=I
21b:X=Br
21c:X=Cl

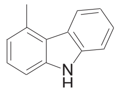
3-2. Anti-Alzheimer's Disease

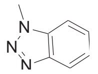
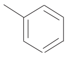
Alzheimer's disease (AD) is a progressive neurodegenerative disorder that, as of 2010, has affected approximately 36 million people.[39] The etiology of AD is still elusive, and multiple factors, such as β-amyloid (Aβ) deposits, τ-protein aggregation, oxidative stress, and low levels of acetylcholine (ACh), have been suggested contributing to the development of AD.[40]

In 2010, Huang et al. first reported berberine analogues as potent acetylcholinesterase inhibitors and these 9-O substituted berberine analogues exhibited mix-competitive binding mode by a kinetic study of acetylcholinesterase (AChE) and butyrycholinesterase (BuChE).[41] The inhibitory activity of these berberine analogues were evaluated toward AChE from electric eel and BuChE from equine serum, using galanthamine as the reference standard. Berberine-carbazole hybrids **22**, showed a remarkably low inhibitory activity toward AChE compared to the bicyclic heterodimeric hybrids in series **23** and monocyclic heterodimeric hybrids in series **24**. On the other hand, berberine-carbazole hybrids **22** exhibited a better activity toward BuChE than that of AChE. Some of the SAR features are summarized in Figure 5.



The inhibitory potency closely related
to the length of the alkylene chain,
compounds with n=4 were the best inhibitors

Compound	R	n	AChE[a] IC_{50}(μM) ± SEM	BuChE[b] IC_{50}(μM) ± SEM
22		4	0.856 ± 0.096	0.099 ± 0.006

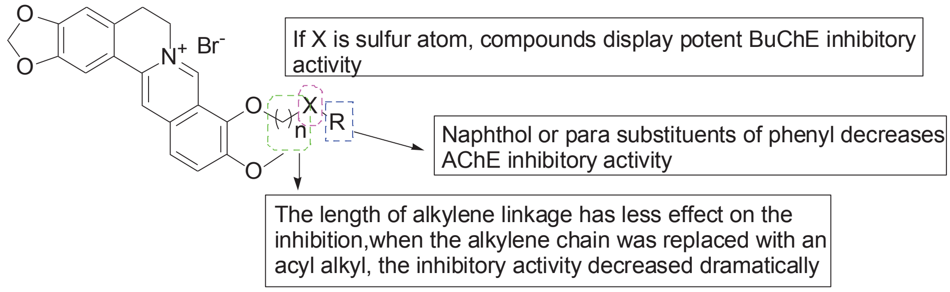
23		4	0.224 ± 0.019	4.17 ± 0.391
24		4	0.097 ± 0.005	4.89 ± 0.035
1			0.374 ± 0.024	18.2 ± 0.683
Galanthamine			0.623 ± 0.099	15.7 ± 0.787

[a] 50% inhibitory concentration (means ± ± SEM of three experiments) of AChE from electric eel.

[b] 50% inhibitory concentration (means ± SEM of three experiments) of BuChE from equine serum.

Figure 5. SAR of berberine analogues as anti-Alzheimer's disease agents

Later, in continuation of their work, Huang et al. synthesized new berberine analogues as dual inhibitors of acetylcholinesterase and butyrylcholinesterase.[42] Among these analogues, compound **25**, berberine linked with 3-methylpyridinium by a 2-carbon spacer, was found to be a potent inhibitor of AChE, and compound **26**, berberine linked with 2-thionaphthol by a 4-carbon spacer, acted as the most potent inhibitor for BuChE (Figure 6). Kinetic studies and molecular modeling simulations of the AChE-inhibitor complex indicated that a mixed-competitive binding mode existed in these berberine analogues.



If X is sulfur atom, compounds display potent BuChE inhibitory activity

Naphthol or para substituents of phenyl decreases AChE inhibitory activity

The length of alkylene linkage has less effect on the inhibition,when the alkylene chain was replaced with an acyl alkyl, the inhibitory activity decreased dramatically

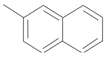
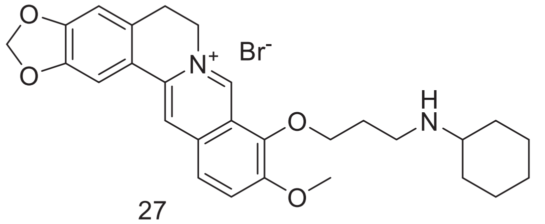
Compound	R	n	X	AChE IC_{50}(μM) ± SEM	BuChE IC_{50}(μM) ± SEM
25		2	-	0.048 ± 0.003	2.46 ± 0.141
26		4	S	1.35 ± 0.092	0.078 ± 0.011
24				0.097 ± 0.005	4.89 ± 0.035
1				0.374 ± 0.024	18.2 ± 0.683
Galanthamine				0.623 ± 0.099	15.7 ± 0.787

Figure 6. SAR and in vitro inhibition of compounds **1**, **24**, **25**, **26** and galanthamine on AChE and BChE activities

Further, the same research group synthesized and evaluated berberine analogues with substituted amino groups linked at the 9-position using different carbon spacers as inhibitors of acetylcholinesterase/butyrylcholinesterase.[43] Compound **27** with a cyclohexylamino group linked to berberine by a three-carbon spacer, gave the most potent inhibitor activity against AChE (Figure 7).

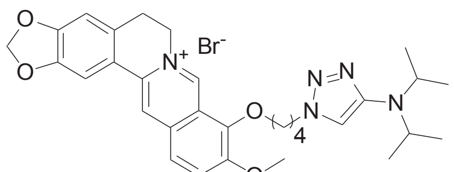


27

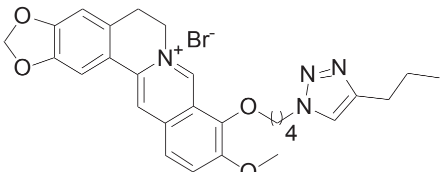
Compound	AChE	BuChE
	IC$_{50}$(μM) ± SEM	IC$_{50}$(μM) ± SEM
27	0.020 ± 0.002	4.17 ± 0.391
1	0.374 ± 0.024	18.2 ± 0.683
Tacrine	0.311 ± 0.009	0.041 ± 0.003

Figure 7. In vitro inhibition of compounds **1**, **27** and tacrine on AChE and BChE activities

In 2011, Shi et al. designed and established triazole-containing berberine analogues as acetylcholinesterase and amyloid-β aggregation inhibitors and the triazole moiety of berberine derivatives displayed a face-to-face π-π stacking interaction in a 'sandwich' form in catalytic sites of AChE, which was confirmed by molecular modeling studies.[44] Among these analogues, compound **28** was found to be a potent inhibitor of AChE and compound **29** showed the highest potency of amyloid-β aggregation inhibition (Figure 8).



28



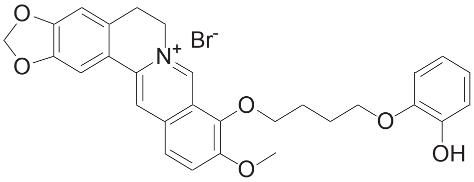
29

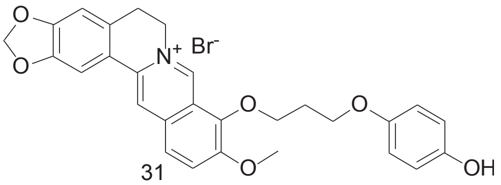
Compound	AChE	BuChE	Aβ aggregation inhibition ratio (%)
	IC$_{50}$(μM) ± SEM	IC$_{50}$(μM) ± SEM	20 μM
28	0.044 ± 0.001	6.21 ± 0.127	52.8
29	0.201 ± 0.017	2.02 ± 0.0500	77.9

1	0.374 ± 0.024	18.2 ± 0.683	36.3
Galanthamine	0.623 ± 0.099	15.7 ± 0.787	n.t.

Figure 8. In vitro inhibition of compounds **1**, **28**, **29** and galanthamine for AChE and BChE and Aβ aggregation

Jiang et al. synthesized and reported novel berberine-benzenediol derivatives, berberine-melatonin hybrids, berberine-ferulic acid hybrids as multiple cholinesterases (ChEs) inhibitors.[45] These compounds were also evaluated for the capacity to prevent amyloid-β (Aβ) aggregation and antioxidant activity. All of these compounds exhibited antioxidant activity and inhibited Aβ aggregation better than unconjugated berberine, although some of them had a reduced ability to inhibit AChE. The hydroquinone-berberine hybrid, compound **31**, had the greatest ability to suppress Aβ aggregation. It was also an excellent antioxidant and a reasonable AChE and BuChE inhibitor (Figure 9). In addition, compound **30** was a potent AChE inhibitor and a strong antioxidant and it also inhibited Aβ aggregation.


30

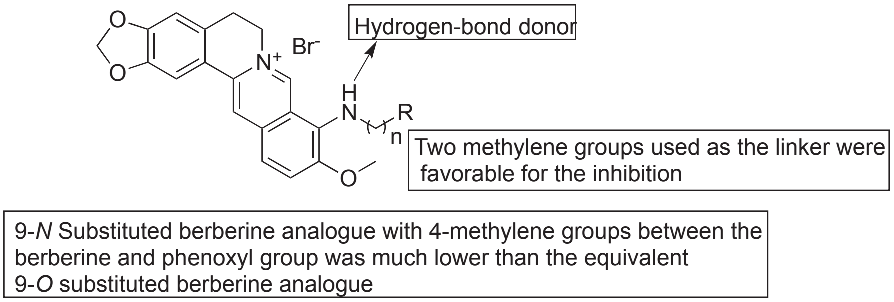

31

Compound	AChE	BuChE	Trolox equiv[a]	Aβ aggregation inhibition ratio (%)
	IC$_{50}$(μM) ± SEM	IC$_{50}$(μM) ± SEM		20 μM
30	0.123 ± 0.003	2.09 ± 0.14	3.54 ± 0.27	84.5
31	0.460 ± 0.013	2.06 ± 0.035	1.66 ± 0.106	92
24	0.097 ± 0.005	4.89 ± 0.035	1.0 ± 0.02	57.4
1	0.374 ± 0.024	18.2 ± 0.683	0.4 ± 0.01	36.3
Galanthamine	0.623 ± 0.099	15.7 ± 0.787	n.t.	n.t.

[a] Data are expressed as mmol of trolox equivalent/mmol of tested compound (means ± SEM of three experiments).

Figure 9. In vitro inhibition of compounds **1**, **24**, **30**, **31** and galanthamine for AChE and BChE and Aβ aggregation and antioxidant activities

Shan and his research group synthesized 9-N-substituted berberine analogues as antioxidant and inhibitors of acetylcholinesterase, butyrylcholinesterase and amyloid-β aggregation.[46] By a simple structure-activity relationship analysis, although some of the 9-N-substituted berberine analogues exhibited slightly poorer AChE inhibitory activities,[46] most of them exhibited much better antioxidant and anti-Aβ aggregation activities than BBR and 9-*O*-substituted berberine analogues (Figure 10).



Hydrogen-bond donor

Two methylene groups used as the linker were favorable for the inhibition

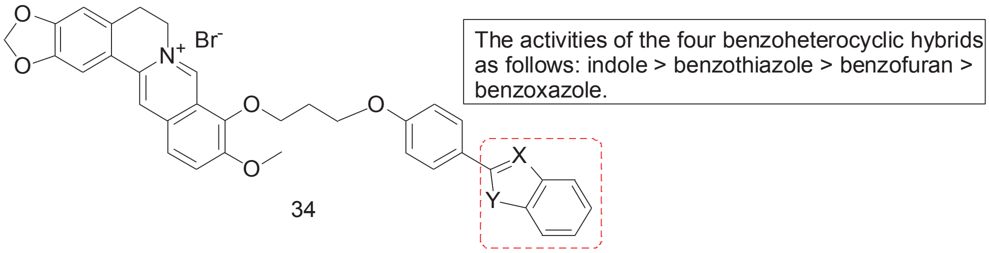
9-*N* Substituted berberine analogue with 4-methylene groups between the berberine and phenoxyl group was much lower than the equivalent 9-*O* substituted berberine analogue

Compound	n	R	AChE IC_{50}(μM) ± SEM	BuChE IC_{50}(μM) ± SEM	Trolox equiv	Aβ aggregation Inhibition ratio (%) 20 μM	IC_{50} (μM)
1			0.374 ± 0.024	18.2 ± 0.683	0.4 ± 0.01	36.3	n.t.
24			0.097 ± 0.005	4.89 ± 0.035	1.0 ± 0.02	57.4	-
32	4	$-OC_6H_5$	0.349 ± 0.020	1.201 ± 0.028	3.68 ± 0.23	-	-
33	2	$2\text{-}MeC_6H_4$	0.027 ± 0.002	0.713 ± 0.016	4.05 ± 0.09	95.0	2.73

n.t.: not tested

Figure 10. SAR and 9-N-substituted berberine analogues as anti-Alzheimer's disease agents

In 2012, Huang and co-workers disclosed synthesis and anti-Alzheimer's disease evaluation of new berberine-phenyl-benzoheterocyclic hybrids and they tested the series of synthesized 9-*O*-substituted berberine derivatives for cholinesterase activity and amyloid aggregation inhibitory.[39] The result is shown in Figure 11.



The activities of the four benzoheterocyclic hybrids as follows: indole > benzothiazole > benzofuran > benzoxazole.

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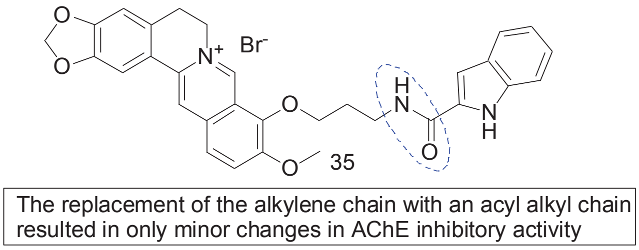
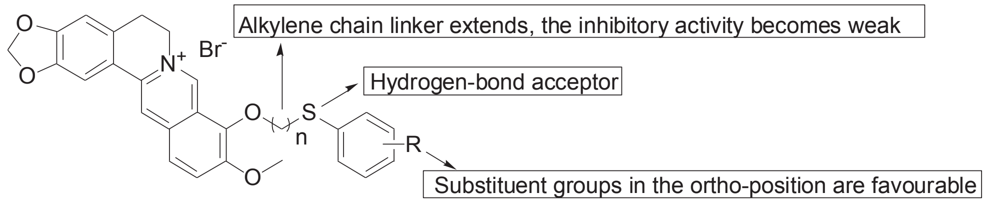


The replacement of the alkylene chain with an acyl alkyl chain resulted in only minor changes in AChE inhibitory activity

Compound	X	Y	AChE	BuChE	Aβ aggregation inhibition ratio (%)	
			IC_{50}(µM) ± SEM	IC_{50}(µM) ± SEM	20 µM(%)	IC_{50}(µM)
1			0.374 ± 0.024	18.2 ± 0.683	36.3	-
24			0.097 ± 0.005	4.89 ± 0.035	57.4	-
34	C	NH	0.774 ± 0.013	0.711 ± 0.048	-	4.69 ± 0.50
35			0.976 ± 0.060	2.19 ± 0.127	73.5	-

Figure 11. SAR for berberine-phenyl-benzoheterocyclic hybrids derivatives as anti-Alzheimer's disease agents

In 2013, Su et al. evaluated and assessed the berberine-thiophenyl hybrids as multi-functional agents: Inhibition of acetylcholinesterase, butyrylcholinesterase, and Aβ aggregation and antioxidant activity.[47] Hydrogen peroxide scavenging activity assay indicated these compounds could efficiently eliminate H_2O_2. SAR studies are portrayed in Figure 12.



Alkylene chain linker extends, the inhibitory activity becomes weak

Hydrogen-bond acceptor

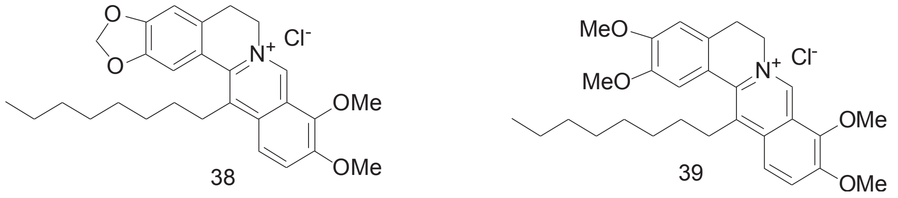
Substituent groups in the ortho-position are favourable

Compound	n	R	AChE	BuChE	Trolox equiv	Aβ aggregation inhibition ratio (%)
			IC_{50}(µM) ± SEM	IC_{50}(µM) ± SEM		20 µM
1			0.374 ± 0.024	18.2 ± 0.683	0.4 ± 0.01	36.3
24			0.097 ± 0.005	4.89 ± 0.035	1.0 ± 0.02	57.4
36	2	*o*-methyl	0.077 ± 0.010	1.360 ± 0.018	1.11 ± 0.08	73.4
37	2	*o*-chloro	0.042 ± 0.007	0.662 ± 0.046	0.75 ± 0.08	65.1

Figure 12. SAR and berberine-thiophenyl hybrids as anti-Alzheimer's disease agents

3-3. Anti-cancer Activity

In 2012, Zhang et al. investigated 13-*n*-hexyl/13-*n*-octylberberine and palmatine analogues and examined their cytotoxic activities in seven human cancer cell lines (7701QGY, SMMC7721, HepG2, CEM, CEM/VCR, KIII, Lewis) by MTT assays.[48] 13-*n*-Octyl-berberine (**38**) and 13-*n*-octyl-palmatine (**39**) exerted the most potent antitumor activities, with IC_{50} values of 0.02 ± 0.01-13.58 ± 2.84 μM against various cancer cell lines, and 6-fold stronger antitumor activities than berberine and palmatine. Anticancer activity of compounds **38** and **39** was investigated *in vivo* in the murine sarcoma S180 xenografted model on male Kunming mice using intraperitoneal (ip) injection using berberine, palmatine and cyclophosphamide as the positive control (Figure 13). Although compound **38** possessed the topmost antitumor activity, it had perspicuously higher toxicity than berberine, palmatine and cyclophosphamide.



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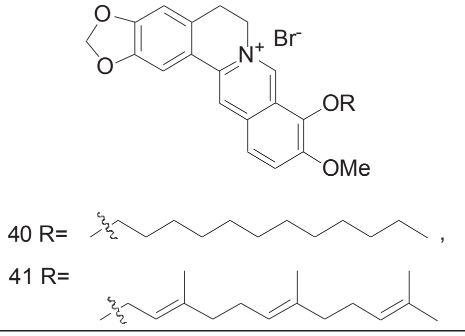
Compound	Dose (mg/kg)	Injection	Number of mice		Tumor inhibitory rate (%)
			Start	End	
control	-	iv	10	10	-
Cyclophosphamide	30	iv	10	10	80.61
Berberine	30	ip	10	9	42.99
Palmatine	30	ip	10	10	34.09
38	1	ip	10	8	53.52
	2.5	ip	10	5	59.86
39	5	ip	10	9	42.05
	10	ip	10	5	50.96

Figure 13. Structure and tumor inhibitory rate of berberine and palmatine and their analogues **38**，**39** in the murine sarcoma S180 xenografted model

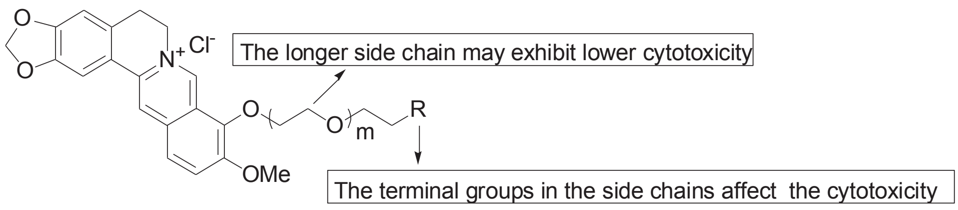
In 2013, Lo et al. synthesized several alkylated and terpenylated berberine analogues to evaluate their efficacy as preferential cytotoxic agents against human cancer HepG2 and HT-29 cells.[49] They concluded that the lipophilic substituent of 9-*O*-alkyl- and 9-*O*-terpenylberberine analogues played a crucial role in inhibiting the human cancer cell growth and its activity could be maximized with the optimized substituent type and chain length. The compounds as presented in Figure 14 showed maximum inhibitory action as compared to the reference Cisplatin.



	IC₅₀ (μM)	
Compound	HepG2	HT-29
1	8.32 ± 2.11	8.45 ± 0.35
40	0.10 ± 0.03	0.28 ± 0.04
41	0.08 ± 0.02	0.27 ± 0.07
Cisplatin	36.00 ± 3.10	24.10 ± 0.10

Figure 14. In-vitro cytotoxic activity of 9-*O*-alkyl- and 9-*O*-terpenylberberine analogues

Liu et al. designed 9-substituted berberine analogues with polyethylene glycol side chain and tested them in vitro cytotoxic activity against four human tumor cell lines: granulocyte leukemia (HL-60), gastrocarcinoma (BGC-823), carcinoma (Bel-7402), and nasopharyngeal carcinoma (KB) with MTT assay.[50] 9-Substituted berberine analogues with different lengths of polyethylene glycol side chains and terminal groups displayed selective cytotoxicity against tested cells lines, both the length of the polyethylene glycol side chain and the nature of the terminal group exhibited important effects on the cytotoxicity (Figure 15).



The longer side chain may exhibit lower cytotoxicity

The terminal groups in the side chains affect the cytotoxicity

			IC₅₀ (μM)			
Compound	m	R	HL-60	BGC-823	Bel-7402	KB
1			5.83 ± 0.26	7.04 ± 0.29	16.28 ± 0.68	6.03 ± 0.24
Berberrubine			11.36 ± 0.42	7.66 ± 0.34	34.31 ± 0.53	6.92 ± 0.36

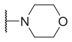
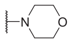
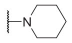
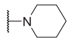
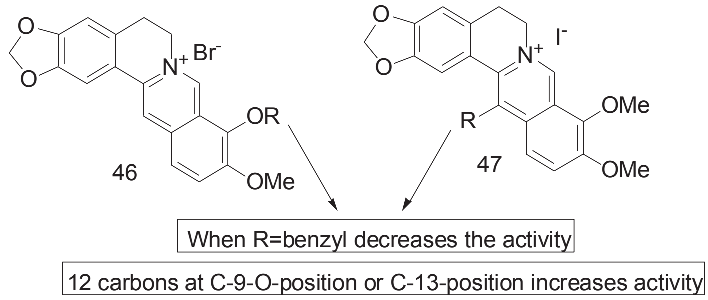
42	0		2.71 ± 0.23	2.82 ± 0.31	11.58 ± 0.65	5.46 ± 0.48
43	1		6.51 ± 0.47	10.17 ± 0.62	27.53 ± 0.86	17.12 ± 0.63
44	0		16.39 ± 0.68	11.89 ± 0.56	9.44 ± 0.38	9.93 ± 0.43
45	1		46.80 ± 1.02	22.18 ± 1.17	17.86 ± 0.76	19.68 ± 0.85

Figure 15. SAR and cytotoxicity for compounds against tumor cell lines

Recently, Fu et al. synthesized lipophilic berberine analogues and evaluate their antiglioma effect on rat C6 and human U87 glioma cells.[51] Several compounds presented dose dependent repression against proliferation (IC_{50}, 1.12-6.12 μM) (Figure 16). Furthermore, preliminary research about the underlying mechanism for the enhanced antiglioma ability indicated that these analogues preferentially localized into mitochondria, inducing the up-regulation of reactive oxygen species production.

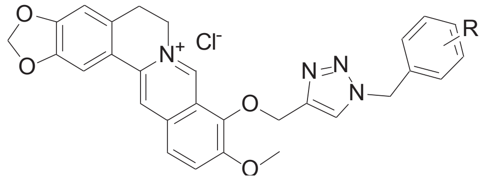


		IC_{50} (μM)	
Compound	R	C6	U87
BBR		>20	>20
46a	$(CH_2)_{11}Me$	1.24 ± 0.13	4.55 ± 0.28
46b	$CH_2C_6H_5$	11.48 ± 2.05	>20
47a	$(CH_2)_{11}Me$	1.65 ± 0.38	3.09 ± 0.54
47b	$CH_2C_6H_5$	14.4 ± 1.48	>20

Figure 16. SAR and survival rate (IC_{50}, μM) of berberine and compounds **46**, **47**

Jin et al. designed and synthesized a series of novel derivatives of phenyl-substituted berberine triazolyls as anticancer agents and all of the compounds were evaluated against MCF-7 (breast), SW-1990 (pancreatic), and SMMC-7721 (liver) and the noncancerous human umbilical vein endothelial cell (HUVEC) cell lines.[52] Compound **48** showed the most potent inhibitory activity against the SW-1990 and

SMMC-7721 cell lines and compound **49** exhibited the most potent inhibitory activity against the MCF-7 cell line (Figure 17). Meanwhile, compound **48** and compound **49** showed low cytotoxicity in the HUVEC cell line.



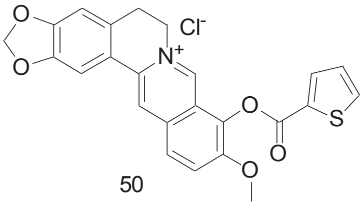
Compound	R	IC$_{50}$ (μM)			
		MCF-7	SW-1990	SMMC-7721	HUVEC
48	4-C(Me)$_3$	15.80 ± 2.14	8.54 ± 1.97	11.87 ± 1.83	25.49 ± 3.24
49	2,4,6-3Cl	12.57 ± 1.96	13.32 ± 2.36	18.68 ± 2.69	30.47 ± 3.47
BBR		121.91 ± 11.26	27.64 ± 3.04	68.06 ± 7.76	18.33 ± 2.31

Figure 17. Structure and in vitro cytotoxicity (IC$_{50}$, μM) of the compounds **48**, **49** against three human cancer cell lines and the human umbilical vein endothelial cell line

3-4. Anti-microbial Activity

3-4-1. Antiviral Activity

In 2011, Bodiwala et al. reported the anti-human immunodeficiency virus (HIV) activities of berberine and berberrubine along with berberine-9-*O*-esters analogues.[15] Berberine was found to be the most active agent against HIV-1 NL4.3 in CEM-GFP cell line. Berberrubine and two other compounds were found to be less active than berberine, and they were also less toxic than berberine (Figure 18). Enzyme based assay suggested that the anti-HIV activity of berberine and its analogs might be due to reverse transcriptase inhibitory activity and some additional mechanisms.



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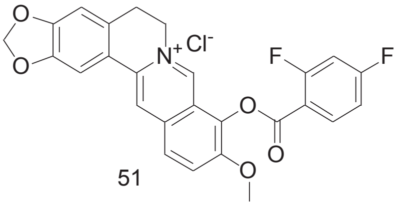

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Compound	Anti-HIV activity in CEM-GFP cell line				HIV-1 reverse transcriptase activity		
	Conc. (μM)[a]	EC$_{50}$ (μM)[b]	CC$_{50}$ (μM)[c]	TI[d]	μg/reaction	Inhibition	IC$_{50}$ (μg/reaction)
BBR	0.29	0.13	2.09	16.07	20	92%	2.1 ± 0.5
Berberrubine	15.52	2.8	169.2	60.42	20	99%	6.1 ± 0.1

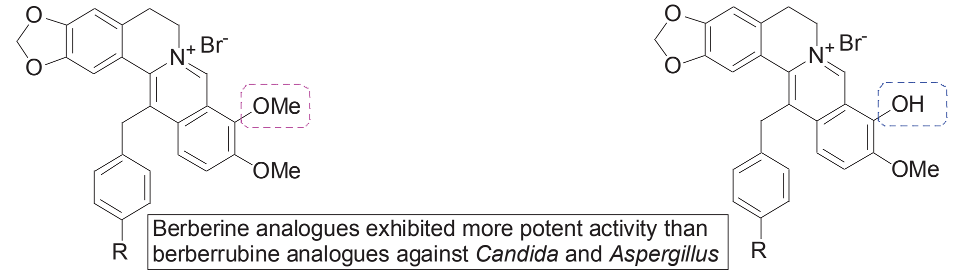
50	4.62	1.82	91.87	50.47	20	99%	3.8 ± 0.2
51	4.32	0.95	57.14	60.16	20	99%	3.5 ± 0.1
Azidothymidine	5	1.05 ± 0.07	24.06 ± 0.63	22.91			
Nevirapine					0.1	95.5%	0.041

[a] Highest noncytotoxic concentration.
[b] EC_{50}= concentration of compound to achieve 50% inhibition of infected cells.
[c] CC_{50}=concentration of compound indicating 50% cytotoxicity in uninfected cells.
[d] TI =CC_{50}/EC_{50}.

Figure 18. Structure and anti-HIV activity of berberine and its analogues

3-4-2. Antifungal Activity

In 2006, Kim et al. reported the antifungal activities of 13-(substituted benzyl)berberine and berberrubine analogues and the synthesized compounds exhibited more potent antifungal activities than berberine and berberrubine.[6] Among them, the analogues bearing electron-withdrawing groups, at the *para*-position were less active than the derivatives bearing bulky hydrocarbon groups (Figure 19).



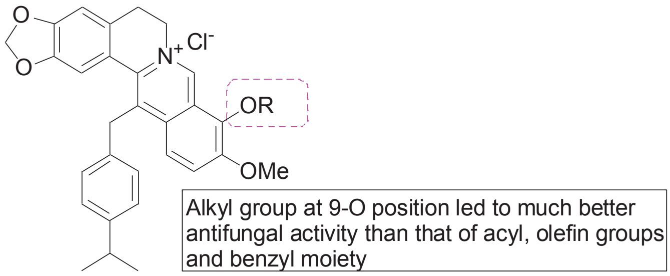
Berberine analogues exhibited more potent activity than berberrubine analogues against *Candida* and *Aspergillus*

51 52

Compound	R	MIC[a] (μg/mL)						
		C A	C T	C L	C K	A F	A T	C N
1		128	16	>128	32	>128	>128	64
Berberrubine		>128	128	>128	>128	>128	>128	>128
52a	C(Me)$_3$	4	2	8	8	64	64	4
52b	CH(Me)$_2$	4	1	8	4	64	32	4
53a	C(Me)$_3$	16	16	8	32	>128	>128	8
53b	CH(Me)$_2$	16	16	8	16	>128	>128	4
Amphotericin B		0.5	0.5	1	0.5	1	16	0.25

[a]MIC: Minimum inhibitory concentration
C A-*Candida albicans* ATCC 10231, C T-*Candida tropicalis* ATCC 13803, C L-*Candida lusitaniae* ATCC 42720, C K-*Candida krusei* ATCC 6258, A F-*Aspergillus fumigatus* ATCC 16424, A T-*Aspergillus terreus* ATCC 46941, C N-*Cryptococcus neoformans* ATCC 36556.

Figure 19. Structures and in vitro antifungal activity for 13-(substituted benzyl)berberine analogues (**52**) and berberrubine derivatives (**53**)



Alkyl group at 9-O position led to much better antifungal activity than that of acyl, olefin groups and benzyl moiety

Compound	R	MIC (μg/mL)						
		CA	CT	CL	CK	AF	AT	CN
54a	Me	4	1	8	4	64	32	4
54b	$CH_2(CH_2)_2Me$	0.5	0.25	1	1	2	4	1
54c	$CH_2CH=CHMe$	0.5	0.5	1	1	2	4	1
Amphotericin B		0.5	0.5	1	0.5	1	16	0.25

C A-*Candida albicans* ATCC 10231, C T-*Candida tropicalis* ATCC 13803, C L-*Candida lusitaniae* ATCC 42720, C K-*Candida krusei* ATCC 6258, A F-*Aspergillus fumigatus* ATCC 16424, A T-*Aspergillus terreus* ATCC 46941, C N-*Cryptococcus neoformans* ATCC 36556.

Figure 20. SAR and in vitro antifungal activities for 9-substituted-13-(4-isopropylbenzyl)berberine analogues

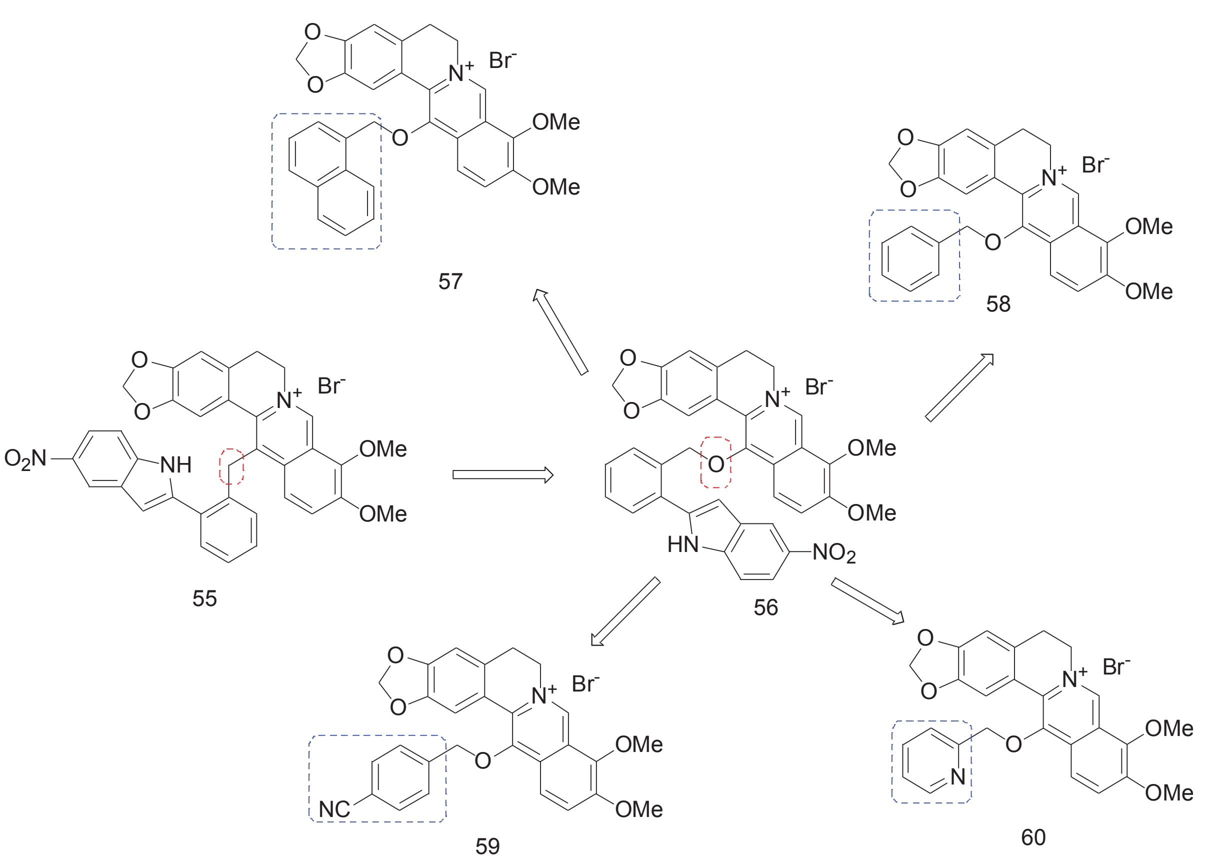
Structurally, berberine analogues are different from berberrubine analogues only by the presence of a methyl group on 9-hydroxy, thus, the capped hydroxy group should be perceived to be important for the enhanced antifungal activity. To further investigate the antifungal activity of the capping group, in 2010, Kim et al. synthesized 13-(4-isopropylbenzyl)berberine analogs in which the methyl group at 9-O position was replaced with various moieties such as alkyl chains, olefins and aromatic groups.[53] From the SAR study, alkyl chains, especially butyl group, led to the most enhanced activity (Figure 20). The acute toxicity test of compound **54b** showed that it was considerably safe in vivo up to 900 mg/kg in oral administration.

3-4-3. Antibacterial Activity

The gradually generated resistance by human pathogenic bacteria to antibacterial agents is a serious and growing health issue of the current biomedical community.[54] A number of resistance mechanisms have been employed by bacteria to counter antibacterial challenge, and one of them is the overexpression of transmembrane protein-based efflux pumps which can pump out various antibacterials from inside of cells, thus lowering the concentration of antibacterials to sub-lethal levels.[55] Therefore, it is of great necessity to keep looking for new antibacterial agents. Berberine analogues are attracting significant interests because BBR has been served as a natural antibacterial agent for decades. Recently, compound **55**, a covalently linked combination of berberine and 2-phenyl-5-nitro-1*H*-indole, was reported as the first hybrid working as anti-infective/multidrug resistance (MDR) pump inhibitor, while 2-phenyl-5-nitro-1*H*-indole was a synthetic NorA inhibitor which potentiates the antibacterial activity of BBR by reducing its efflux from bacterial cells.[56] Compound **55** showed a superior antibacterial activity with over 200-fold greater than that of BBR against *S. aureus* overexpressing NorA strain while kept insensitive to multi-drug resistance (MDR) efflux.

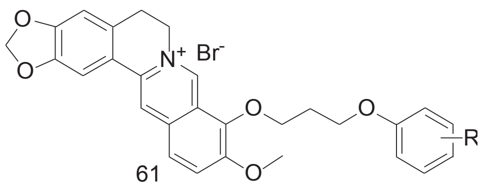
In order to further understand the dispositional and structural requirements for the efflux pump blocking moiety in berberine-based hybrids, compound **56**, an analogue of **55** in which a methylene ether linking group was introduced, was synthesized and evaluated by Samosorn et al.[5] Four other related compounds were also synthesized by simple modification of the indolic moiety with aryl- or heteroarylmethyl and naphthylmethyl groups to assess aromatic ring effect in this region (Figure 21). It was found that compound **56** was more potent as an antibacterial than **55** with MIC values up to 1.8-fold greater, and over 382-fold greater in activity than BBR against NorA overexpressing *S. aureus* strain K2378. Removal of the indole moiety of **56** would lower the antibacterial activity but still retain the NorA pump inhibitory activity, suggesting that the indole moiety is not necessary for NorA pump inhibition but is required for high antibacterial activity of the 13-substituted berberine hybrids.

Sun et al. designed a series of 9-phenoxyalkyl berberine analogues as potent FtsZ (Filamenting temperature-sensitive mutant Z, an essential and highly conserved bacterial cytokinesis protein) inhibitors.[57] The 9-phenoxyalkyl substituted derivatives exhibited potent antimicrobial activity against Gram-positive bacterial strains such as ampicillin- and methicillin-resistant *S. aureus*, and broader spectrum of activity than the parent compound berberine. Biochemical evaluations demonstrated that the new berberine derivatives target the bacterial filamenting temperature-sensitive mutant Z protein. Compound **62** without the 9-phenoxy group was less potent than compounds **61**, suggesting that the aromatic ring plays an important role on the antibacterial activity. Compounds **61a** and **61b** with chloro- and nitro-substituents showed slightly stronger antibacterial activity than the other berberine derivatives (Figure 22).



	MIC (µg/mL)					
Compound	S. *aureus* K1758 DnorA	S. *aureus* 8325-4wild-type	S. *aureus* K2378 NorA++	E. *coli* BW25113 DTolC	E. *coli* K12 wild-type	E. *faecalis* OG1RF
55	3.1	3.1	3.1	>150	>150	6.3
56	1.7	1.7	1.7	n.t.	n.t.	3.4
57	5.5	10.9	21.9	10.9	>174.8	n.t.
58	12.0	24.0	95.8	12.0	>191.6	n.t.
59	91.4	182.8	>182.8	91.4	182.8	n.t.
60	191.2	>191.2	>191.2	191.2	>191.2	n.t.
1	40	325	>650	325	>2600	650

Figure 21. Antimicrobial activity of 13-substituted berberines analogues



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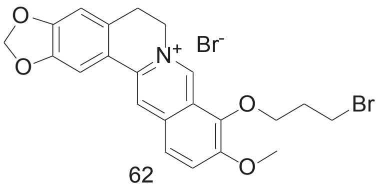

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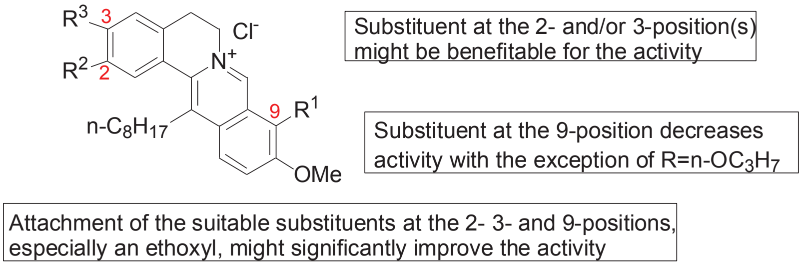
		MIC (μg/mL)					
Compound	R	*B.subtilis* 168	*S. aureus* ATCC 29213	*E.faecium* ATCC 49624	*S.epidermidis* ATCC 12228	*E.coli* ATCC 25922	IC_{50}(μM)
61a	4-Cl	4	2	4	2	32	37.8
61b	4-NO_2	8	2	8	2	32	43.4
62		112	112	>196	112	384	240.43
1		128	128	>196	128	>500	272

Figure 22. MIC of 9-phenoxyalkyl berberine analogues against some bacterial strains and their IC_{50} against *S. aureus* FtsZ GTPase

3-4-4. Antitubercular Activity

In 2012, Liu et al. synthesized twenty-eight new 13-*n*-octylberberine analogues and evaluated their activities against drug-susceptible *Mycobacterium tuberculosis* (*M. tuberculosis*) strain H37Rv.[58] Among these compounds, compound **67** was the most effective anti-tubercular agent with a MIC value of 0.125 mg/mL (Figure 23). Importantly, compound **67** exhibited more potent effect against rifampicin (RIF)-and isoniazid (INH)-resistant *M. tuberculosis* strains than both rifampicin and isoniazid, suggesting a new mechanism of action.



Substituent at the 2- and/or 3-position(s) might be benefitable for the activity

Substituent at the 9-position decreases activity with the exception of R=n-OC_3H_7

Attachment of the suitable substituents at the 2- 3- and 9-positions, especially an ethoxyl, might significantly improve the activity

Compound	R^1	R^2	R^3	MIC (μg/mL)
1	OMe	OCH_2O		2.0
63	OEt	OCH_2O		4.0
64	OMe	OH	OH	16.0
65	OMe	OEt	OEt	0.5
66	OBu-*n*	OH	OBu-*n*	8.0

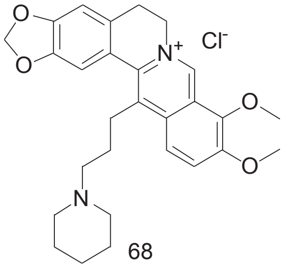
67	OEt	OEt	OEt	0.125
Isoniazid				0.0625
Rifampicin				0.0625

Figure 23. SAR and anti-mycobacterial activities of compounds against *M. tuberculosis* strain H37Rv

3-5. Binding with DNA

3-5-1. G-quadruplex Binding Ligands

In 2006, Neidle's group reported a 13-substituted berberine analogue **68** as telomerase inhibitor by binding to G-quadruplex.[59] As compound **68** had a common interaction with G-quadruplex, in 2007, Zhang et al. demonstrated 9-O-substituted berberine analogues as potent G-quadruplex stabilizing ligands in telomeric DNA.[60] The interaction of berberine and its 9-substituted analogues with human telomeric DNA d[$G_3(T_2AG_3)_3$](telo 21) has been investigated via CD spectroscopy, fluorescence spectroscopy, PCR-stop assay, competitive dialysis, and telomerase repeat amplification protocol (TRAP) assay. Some of the important SAR features are represented in Figure 24.



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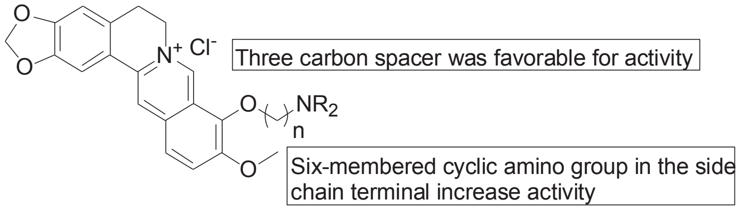
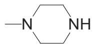
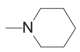


Compound	n	-NR_2	$K \times 10^{-6}(M^{-1})^a$	$IC_{50}(\mu mol/L)^b$	$^{Tel}IC_{50}(\mu mol/L)^c$
69	3		3.0	0.7	7.5
70	3		3.5	0.7	12.5
1			2.0	2.1	75

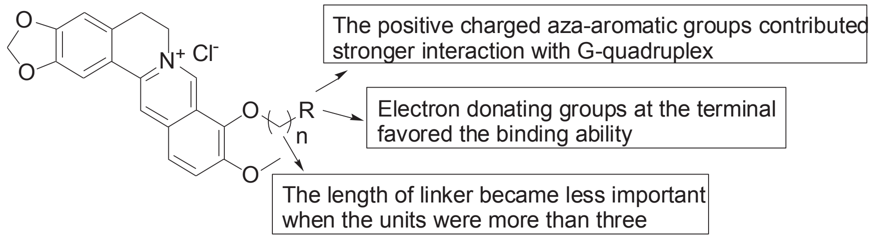
[a] Binding constants (K, M^{-1}) by fluorescence titration.

[b] IC_{50}(μM): The IC_{50} values of compounds in PCR-stop assay

[c] $^{Tel}IC_{50}$(μM): The IC_{50} values of compounds in TRAP assay

Figure 24. SAR and binding constants (K_b, M^{-1}), PCR-stop assay data (IC_{50}) and TRAP assay data ($^{Tel}IC_{50}$) of berberine and its analogues

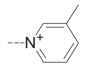
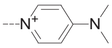
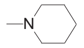
In 2009, Ma et al. synthesized and evaluated 9-O-substituted berberine analogues containing aza-aromatic terminal group as highly selective telomeric G-quadruplex stabilizing ligands.[61] These derivatives showed excellent selectivity for telomeric G-quadruplex DNA over duplex. The SAR and results of activities are laid out in Figure 25.



The positive charged aza-aromatic groups contributed stronger interaction with G-quadruplex

Electron donating groups at the terminal favored the binding ability

The length of linker became less important when the units were more than three

Compound	n	R	K×10^{-6}(M^{-1})[a]	IC_{50}(μM)[b]	$^{Tel}IC_{50}$(μM)[c]
71	3		7.3	6	14
72	3		12	4	14
70	3		3.0	7	16

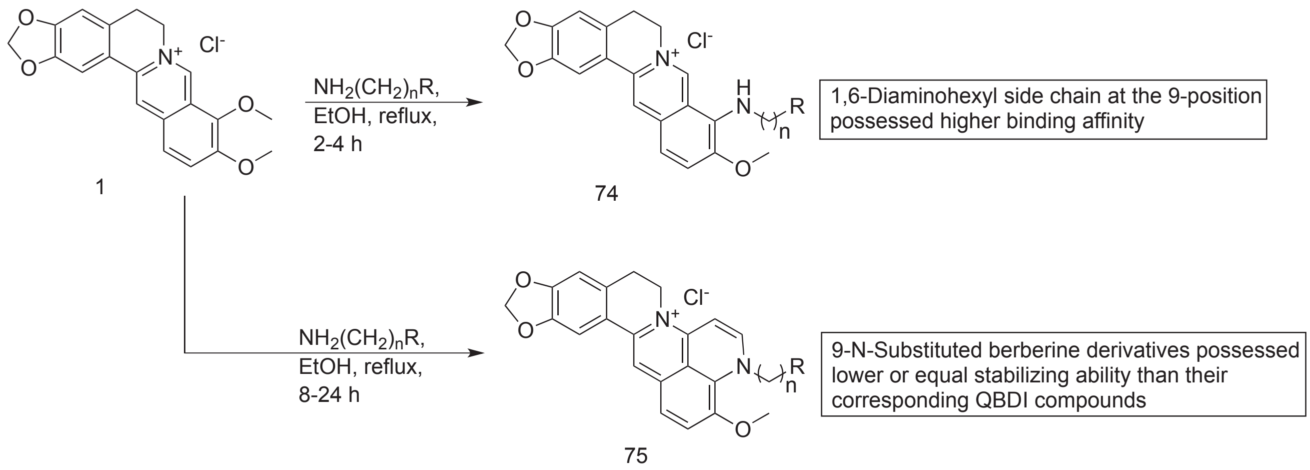
[a] Binding constants (K, M^{-1}) by fluorescence titration.

[b] IC_{50}(μM): The IC_{50} values of compounds in PCR-stop assay.

[c] $^{Tel}IC_{50}$(μM): The IC_{50} values of compounds in TRAP assay.

Figure 25. SAR of 9-O-substituted berberine analogues as selective telomeric G-quadruplex stabilizing ligands

Further, the same research group designed and synthesized 9-N-substituted berberine analogues and their quinolino-benzo-[5,6]-dihydroisoquindolium compounds as G-quadruplex binding ligands (Figure 26).[62-63] 9-N-Substituted berberine analogues possessed lower or equal stabilizing ability than their corresponding quinolino-benzo-[5,6]-dihydroisoquindolium compounds, which might be due to their maximizing π-π stacking interaction with the G-quartet.



1,6-Diaminohexyl side chain at the 9-position possessed higher binding affinity

9-N-Substituted berberine derivatives possessed lower or equal stabilizing ability than their corresponding QBDI compounds

	$^aIC_{50}$(μM)	$^bIC_{50}$(μM)

Compound	n	R		HL60	ECV-304	NCI-82	GLC-H460
74a	3	NH_2	2.0	5.5	>100	4	9
75a	3	NH_2	1.9	2	>100	10	13

HL60, NCI-82,GLC-H460 - tumor cells

ECV-304 - normal epithelial cell

[a]IC_{50}(μM): The IC_{50} values of compounds in PCR-stop assay

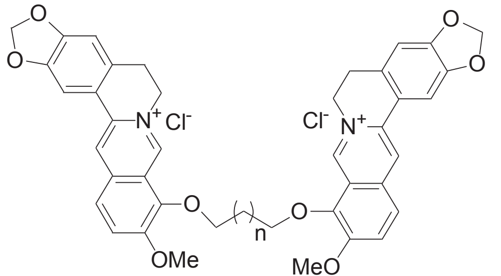
[b]IC_{50}(μM): The IC_{50} values of compounds against tumor cells

Figure 26. 9-O-Substituted berberine derivatives and their effect on tumor cells

3-5-2. Binding Activities toward DNA

The development of efficient DNA-binding agents with improved binding affinities and site specificities toward target DNA attracts much interests of many researchers. Such agents have potential applications, including elucidating the action mechanism of antitumor and antivirus drugs and developing new chemotherapeutic agents.[64-68] Berberine also works as a DNA binder and its binding affinity has been extensively explored by several analytical techniques. Thus, berberine becomes attractive as a versatile platform for the development of more efficient DNA-binding agents.

In 2005, Chen et al. described the facile synthesis of five novel berberine dimers and their strong affinities toward doublestranded DNA.[69] These dimmers exhibited higher binding affinities with two double helical oligodeoxynucleotides, d(AAGAATTCTT)$_2$ and d(TAAGAATTCTTA)$_2$ than their monomeric parent berberine (Figure 27).



76

Relative binding affinities: n=1> n=0 >n=2 >n=3 >n=4

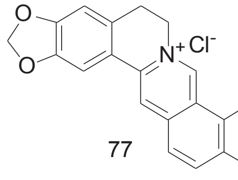
Compound	N	[a]D(AAGAATTCTT)$_2$		[a]D(TAAGAATTCTT)$_2$	
		K_a	RA[b]	K_a	RA[b]
1		$(1.24 \pm 0.12) \times 10^4$	1	$(2.93 \pm 0.31) \times 10^4$	1
76a	0	$(1.18 \pm 0.10) \times 10^5$	9.5	$(1.62 \pm 0.35) \times 10^6$	54.9
76b	1	$(2.46 \pm 0.07) \times 10^5$	19.8	$(2.76 \pm 0.37) \times 10^6$	94.2

[a]In 50 Mm Tris-HCl (pH 6.35) at room temperature

[b]RA denotes relative affinity

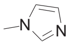
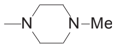
Figure 27. SAR and association constants (K_a, M^{-1}) of **76a-b** with d(AAGAATTCTT)$_2$ and d(TAAGAATTCTTA)$_2$

Pang et al. synthesized four 9-substituted berberine derivatives and their strong affinities toward calf-thymus DNA (CT DNA).[70] Spectrometric titration and ethidium bromide displacement experiments indicate that these berberine derivatives could enhance the CT DNA-binding ability. The most efficient binding activity was observed from the derivative **77b** for its less hindered protonated amino group, can interact more strongly with CT DNA through hydrogen bonding and enhanced electrostatic interactions in the measuring conditions. The association constants of compounds **77a-77d** were showed in Figure 28.



77

Compound with R=NH$_2$ could strongly bind with CT DNA

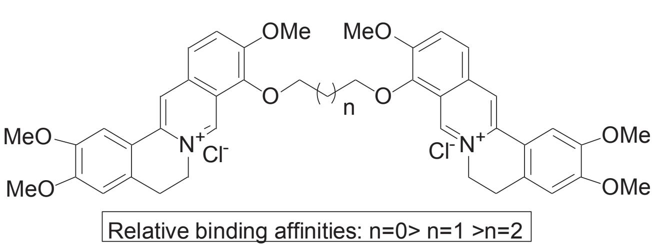
Compound	R	$K_a \times 10^{-4}$
1		1.12 ± 0.04^b
77a	OH	1.18 ± 0.07^b
		4.64 ± 0.25^a
77b	NH_2	33.65 ± 1.73^a
77c		3.56 ± 0.35^b
		5.10 ± 0.69^a
77d		6.08 ± 0.55^b
		8.24 ± 1.45^a

[a]Obtained from ethidium bromide displacement experiment.

[b]Obtained from spectrofluorimetric titration experiment.

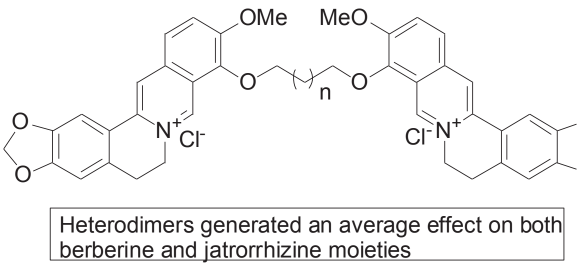
Figure 28. Association constants (K_a, M^{-1}) of **1** and **77a–d** with CT DNA

In 2006, Long et al. synthesized jatrorrhizine homodimers and berberine jatrorrhizine heterodimers and investigated their binding activities toward calf thymus (CT) DNA and three double-stranded oligodeoxynucleotides, d(AAGAATTCTT)$_2$, d(TAAGAATTCTTA)$_2$, and d(TTAAGAATTCTTAA)$_2$.[71] The relative affinity of some compounds are listed in Figure 29.



Relative binding affinities: n=0> n=1 >n=2

78



Heterodimers generated an average effect on both berberine and jatrorrhizine moieties

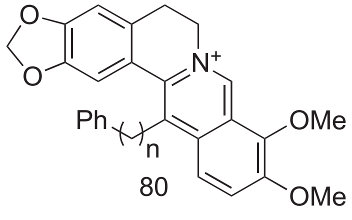
79

Compound	n	RA[a] d(AAGAATTCTT)$_2$	RA d(TAAGAATTCTTA)$_2$	RA d(TTAAGAATTCTTAA)$_2$
78a	0	15.6	5.5	10.7
79a	1	10.6	15.4	22.9
76b		19.8	94.2	114.1
1		1.0	1.0	1.0
Palmatine		1.0	1.0	1.0

[a] RA denotes relative affinity

Figure 29. SAR and relative affinity of compounds **1**, **78a**, **79a**, **76b** and Palmatine with double-stranded oligodeoxynucleotides

Bhowmik et al. evaluated and assessed the DNA binding affinity of berberine analogues with alkyl chains of varying length and a terminal phenyl group at the C-13 position.[72] The phenylalkyl substitution at the C-13 position significantly enhanced the DNA binding affinity and the analogue with three-carbon spacer showed the highest binding affinity (Figure 30).



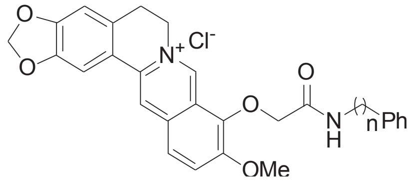
80

		$K_i \times 10^{-5}$ (M^{-1})	
Compound	n	spectrophotometry	spectrofluorimetry
80	3	11.01 ± 0.09	10.03 ± 0.08

Figure 30. Binding parameters for the association of compound **80** with the CT DNA

Basu et al. synthesized three 9-substituted berberine analogues (**81-83**, Figure 31), which displayed the

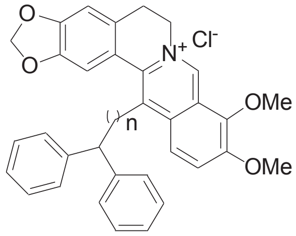
DNA-binding affinity by more than six times compared with berberine.[73]



Compound	n	$K_a \times 10^{-5}$
1		1.28 ± 0.05
81	0	7.96 ± 0.04
82	1	6.33 ± 0.07
83	2	5.18 ± 0.02

Figure 31. Binding parameters for the association of compounds with the CT DNA

Bhowmik et al. reported 13-diphenylalkyl berberine analogues and their binding affinity with DNA.[74] The binding affinity was enhanced with the increase in chain length up to a critical length of $(CH_2)_3$ in all the cases, after which the binding affinity decreased (Figure 32).



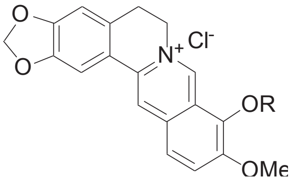
Compound	n	$K_a \times 10^{-5}$
84	0	0.48 ± 0.04
85	1	0.51 ± 0.06
86	2	6.89 ± 0.15
87	3	11.2 ± 0.55
88	4	8.58 ± 0.45
89	5	7.36 ± 0.41

Figure 32. Binding parameters for the association of compounds **84-89** with the CT DNA

3-6. Antiparasitic Activity

In 2013, Peter et al. generated a library of berberine derivatives and evaluated the in vitro inhibition on *T. gondii* and host cell cytotoxicity.[75] Different substitutions and their inhibitory doses (ID_{50}), toxic dose

(TD_{50}), and therapeutic indices (TI) are listed in Figure 33.

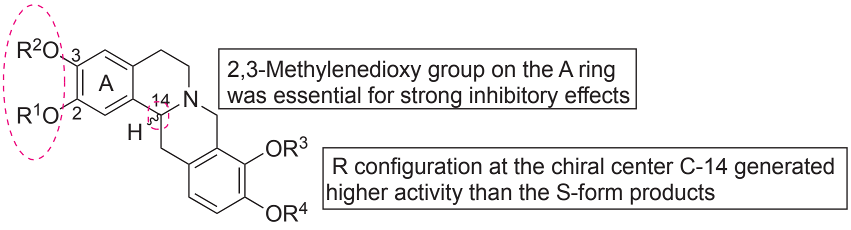


Compound	R	ID_{50} (μM)	TD_{50} (μM)	TI
1		0.58	0.85	1.5
Berberrubine		0.060	81	1300
90	$Me(CH_2)_2CO$	0.068	135	2000
91	$Me(CH_2)_4CO$	0.036	145	4000
Trimethoprim		15	>500	3

Figure 33. In vitro inhibition of *T. gondii* and host cell cytotoxicity for compounds

3-7. Anticoagulant Activity

Yu et al. reported some tetrahydroprotoberberines as new inhibitors on tissue factor procoagulant activity and these compounds were screened on the model of human THP-1 cells which were stimulated by lipopolysaccharide.[76] Among these tetrahydroprotoberberines, several interesting compounds were synthesized through microbial transformation. Effect of substitution on inhibitory activities has been summarized in Figure 34.



2,3-Methylenedioxy group on the A ring was essential for strong inhibitory effects

R configuration at the chiral center C-14 generated higher activity than the S-form products

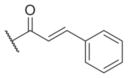
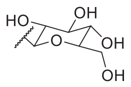
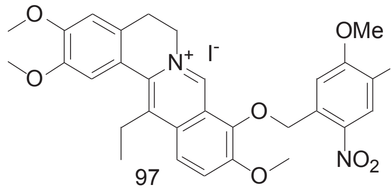
Compound	C-14	R^1	R^2	R^3	R^4	IC_{50} (nM)
92	±	$-CH_2-$		Me	Me	8.35
93	+	$-CH_2-$		H	Me	6.75
94	±	$-CH_2-$			Me	3.75
95	-	$-CH_2-$			Me	8.79
Curcumin						199.63

Figure 34. SAR of tetrahydroprotoberberines and their IC_{50} values on TF procoagulant activity

3-8. Anti-inflammatory Activity

The P2X7 receptor (P2X7R), 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.[77,78] In Lee's work, a novel series of protoberberine-based P2X7 antagonists with modifications at the 9- and 13- positions of berberine and palmatine was synthesized and evaluated in ethidium accumulation and IL-1β release assays.[79] Compounds with alkyl groups at the 13-position of berberine (**96a** and **96b**) showed high potency in both assay systems as P2X7 antagonists (Figure 35). In particular, compound **97**, which contains an ethyl group at the 13-position and a 2-NO_2-4,5-dimethoxy-benzyl group at the 9-position, was discovered as the most potent antagonist of all QPAs tested, and the antagonistic potency was comparable to the positive control, KN-62 (Figure 35).



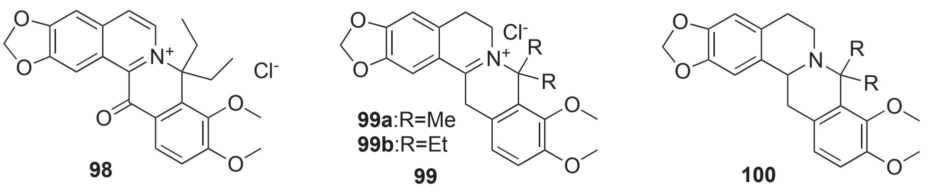
97

Compound	IC_{50} (μM)	%inhibition
96a	0.35 ± 0.04	73 ± 14
96b	0.44 ± 0.15	71 ± 0.30
97	0.17 ± 0.07	83 ± 3
KN-62	0.34 ± 0.03	79 ± 4

Figure 35. Ethidium accumulation and IL-1β release assays of compounds **96a**, **96b**, **97** and KN-62

3-9. Antiprotozoal Activity

Bahar et al. reported the antiprotozoal activity of 8,8-dialkyldihydroberberines (8,8-DDBs, **98**).[80] The compound retained the potent antiparasitic activity and displayed efficacy in a murine visceral leishmaniasis model (Figure 36). However, **98** could be given to mice only at 1 mg/kg/day when administering ip. In 2013, Endeshaw et al. disclosed the semisynthesis of 8,8-dialkyldihydroberberines (8,8-DDBs), and investigated their antiprotozoal activities (Figure 36).[81] Selected 8,8-DDBs showed efficacy in mouse models of visceral leishmaniasis and African trypanosomiasis, with 8,8-dimethyldihydroberberine chloride (**99a**, R=Me) reducing liver parasitemia by 46% in *L. donovani*-infected BALB/c mice when given at an intraperitoneal dose of 10 mg/kg/day for five days.



Compounds **99** exhibit more potent antiprotozoal activity than **100**

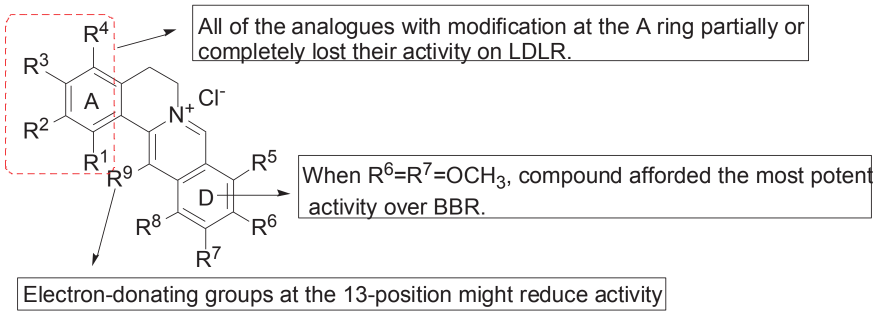
Compound	R	IC_{50} vs *Pf*	IC_{50} vs *Ld*	IC_{50} vs *Tbb*	IC_{50} vs Vero cells
1		800	17 000	1100	>200 000
98		20	290	2.0	39000
99b	Et	77	100	5.3	18000
Chloroquine		8.3	ND	ND	ND
Amphotericin B		ND	46	ND	ND
Suramin		ND	ND	130	ND
Podophyllotoxin		ND	ND	ND	17

Pf-P.falciparum. *Ld-L.donovani*. *Tbb-T.brucei brucei*.
ND-Not determined.

Figure 36. Structures and in vitro activity (nM) of berberine and 8,8-DDBs

3-10. LDLR Up-regulators

Li and co-workers synthesized berberine analogues and evaluated their up-regulatory activity on the low-density-lipoprotein receptor (LDLR) expression.[82-84] Of the BBR analogues, compound **101** exhibited higher activity on LDLR expression than **1** (Figure 37). Later, Wang et al. also reported that compound **101** exhibited the potential effect on Amp activated protein kinase activation as compared with BBR which suggested that compound **101** might be a multiple-target agent for treatment of metabolic syndrome.[85]



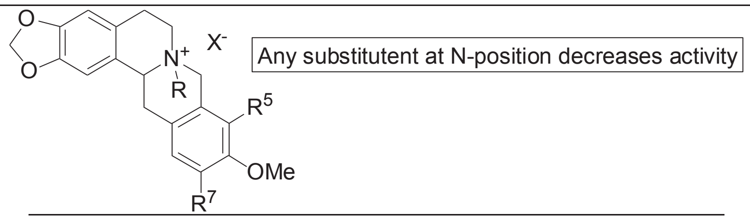
All of the analogues with modification at the A ring partially or completely lost their activity on LDLR.

When R^6=R^7=OCH_3, compound afforded the most potent activity over BBR.

Electron-donating groups at the 13-position might reduce activity

Compound	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	R^9	LDLRmRNA
BBR	H	OCH_2O		H	OMe	OMe	H	H	H	2.2 ± 0.3

101	H	OCH_2O		H	H	OMe	OMe	H	H	3.5 ± 0.9
102	OMe	OMe	OMe	H	OMe	OMe	H	H	H	0.88 ± 0.1
103	H	OCH_2O		H	OMe	OMe	H	H	Me	1.1 ± 0.1



Compound	R^5	R^7	R	X	LDLRmRNA
BBR	OMe	H	H	Cl	2.2 ± 0.3
104	H	OMe	H	Cl	3.5 ± 0.9
105	OMe	H	H		1.0 ± 0.1
106	H	OMe	Me	I	0.9 ± 0.1

Figure 37. SAR and LDLR up-regulating activities of BBR analogues

4. CONCLUSION

In this article, we have aimed to highlight the versatile role of BBR derivatives in the treatment of various disorders. An insight into the SAR of the compounds in each aspect of activity was also outlined. Despite the meticulous and target based research on the development of BBR with varied activities, their therapeutic focuses were still limited in laboratory with only few research has reached clinical trials. The present review would serve as a comprehensive compilation of various research reports of berberine and its analogues which may provide drug designers and medicinal chemist comprehensive information for development of clinically useful molecules.

ACKNOWLEDGEMENTS

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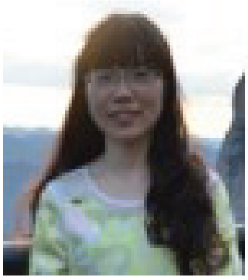

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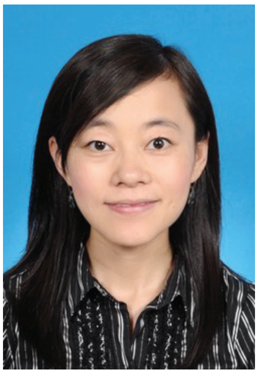

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