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Design and synthesis of tetrahydroisoquinoline derivatives as potential multidrug resistance reversal agents in cancer

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ABSTRACT

Exploration for new MDR-modulator utilizing tetrahydroisoquinoline as scaffold disclosed 6,7-dimethoxy-1-(3,4-dimethoxy)benzyl-2-(*N*-*n*-octyl-*N*′-cyano)guanyl-1,2,3,4-tetrahydroisoquinoline (**7**) as a readily accessible medicinal lead. Compound **7** possessed potent MDR reversal activity in the range of the reference compound verapamil, and had not cardiovascular activity compared to verapamil.

Multidrug resistance (MDR)[1] is a major problem in cancer treatment. The typical MDR in tumor cells is mainly associated with a reduced intracellular drug accumulation and an increased cellular drug efflux. This phenomenon can be related to the overexpression of the energy-dependent efflux pump, P-glycoprotein (P-gp),[2] a 170-kDa protein that belongs to the ATP-binding cassette superfamily of transporters. Intense efforts to overcome MDR by influencing transporter expressions via signal transduction pathways or by direct transcriptional control have not been successful in clinical trials.[3] A number of compounds, so-called chemosensitizers, are able to reverse the effect of Pgp on MDR.[4] Tsuruo and coworkers[5] were the first to demonstrate the ability of the calcium channel blocker, verapamil, to reverse MDR. The cardiovascular action of verapamil derivatives has always represented a problem in the development of MDR modulators possessing this structure and many efforts have been devoted to identifying more selective compounds.

Several bisbenzylisoquinoline alkaloids as tetrandrine and berbamine show anti-MDR properties and calcium antagonistic activity in various degrees.[6] More than 100 tetrahydroisoquinoline derivatives were designed and synthesized for the search of novel calcium channel blockers by simplifying and optimizing tetrandrine in our group.[7–12] A series of *N*-cyanoguanyl-substituted tetrahydroisoquinoline derivatives had strong calcium antagonistic activities but showed almost no cardiovascular activities. Their MDR reversal activities in vitro were evaluated, and the results showed that these compounds exerted different degrees of MDR reversal activities. Particularly, the activity of 6,7-dimethoxy-1-(3,4-dimethoxy)benzyl-2-(*N*-*n*-octyl-*N*′-cyano)guanyl-1,2,3,4-tetrahydro-isoquinoline (**7**) was comparable to that of the control verapamil.[13]

In this letter, the representative compound **7** was synthesized and evaluated in several assays of MDR reversal and cardiovascular activities in vitro and in vivo.

The synthetic route to the target compound **7** is outlined in Scheme 1. The synthesis of compound **7** utilized 3,4-dimethoxyphenylethyamine **1** and 3,4-dimethoxy-phenylacetic acid **2** as the starting material, which were converted to**3** at 190 °C under N_2 protection in 84% yield. Treatment of **3** with $POCl_3$ in toluene under reflux provided **4** in 97% yield, followed by reduction with the yield of 64%. Next, the reaction at the N-position of compound **5** was achieved with dimethyl cyanocarbonimidodithioate in 25% yield. Reaction of key intermediate **6** with *n*-octylamine in toluene under reflux gave **7** with the yield of 34%. Compound **7** was characterized by IR, ^{1}HNMR, mass spectra, and elemental analysis.[14]

The in vitro MDR reversal activities of compound **7** against MCF-7, MCF-7/ADR, and K562/A02 cell lines were evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay[15] with verapamil as reference drug (Tables 1 and 2). The results showed that compound **7** exhibited a well-defined trend in MDR reversal activities.

The in vivo efficacy of compound **7** was evaluated by using the resistant K562/A02 cell xenografts SCID nude mice.[16] The results displayed that compound **7** had no direct effect on K562/A02 cell growth. The antitumor activity of adriamycin (ADM) was signifi-

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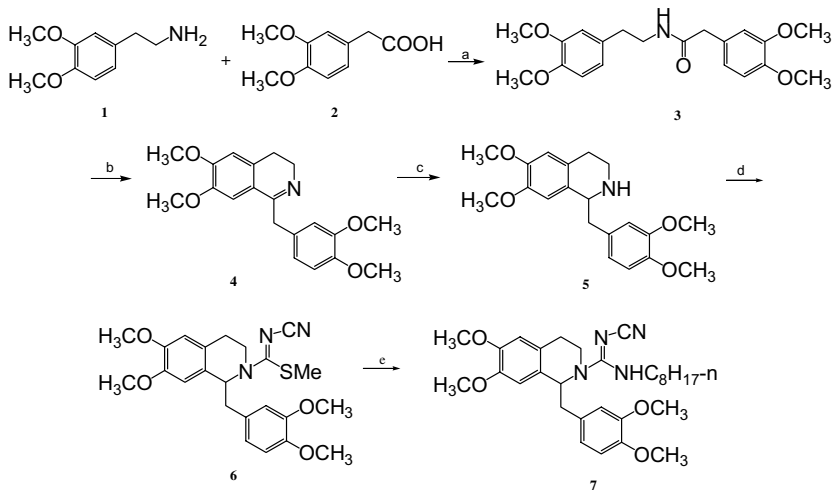

Scheme 1. Reagents and conditions: (a) N_2, 190 °C, 3 h; (b) $POCl_3$, toluene, N_2, 110 °C, reflux, 2.5 h; (c) KBH_4, diethylamine, methanol, rt, 22 h; (d) dimethyl cyanocarbonimidodithioate, toluene, reflux, 30–50 h; (e) n-$C_8H_{17}NH_2$, toluene, reflux, 14–72 h.

Table 1
Inhibitory effects of compound **7** on the proliferation of MCF-7 cell line and MCF-7/ADR cell line by MTT assay($\bar{X} \pm s, n = 5$)

Compound[#]	IC_{50}[*] MCF-7	MCF-7[##]	IC_{50}[*] MCF-/ADR	MCF-7/ADR[##]
Control[**]	96.2 ± 2.6		6864.2 ± 4.9	
Verapamil	92.8 ± 1.8	1.0	689.2 ± 8.6	10.0
7	79.7 ± 0.9	1.2	226.8 ± 3.5	30.3

[*] IC_{50} of adriamycin (nmol/L).
[**] 0.01‰ DMSO.
[#] Compounds were tested at 10 μmol/L.
[##] Reversal fold.

Table 2
Inhibitory effects of compound **7** on the proliferation of K562/A02 cell line by MTT assay($\bar{X} \pm s, n = 5$)

Compound[#]	Reversal adriamycin		Reversal vincristine	
	IC_{50}[*]	[##]	IC_{50}[*]	[##]
Control[**]	18.13 ± 1.2		12.23 ± 0.4	
Verapamil	0.40 ± 0.2	45.30	1.55 ± 0.1	7.90
7	0.38 ± 0.3	47.71	1.33 ± 0.1	9.20

[*] IC_{50} of adriamycin (μmol/L).
[**] 0.01‰ DMSO.
[#] Compounds were tested at 10 μmol/L.
[##] Reversal fold.

Table 3
The effects of compound **7** on K562/A02 cell growing of bearing cancer mice ($\bar{X} \pm s, n = 8$)

Group	Dose (mg/kg)	Tumor weight (g)	Inhibitory ratio (%)
0.9% NaCl	—	3.54±1.1[*]	−63.1
0.9% NaCl + ADM	—	2.15±0.4[#]	0.9
Verapamil	8	3.61±1.2[*]	−66.4
Verapamil + **7**	8	0.3±0.2[**,##]	86.2
ADM	2	2.17 ± 0.4[#]	
7	8	3.46±1.2[*]	−59.5
	2	3.52±1.1[*]	−62.2
7 + ADM	8	0.32±0.2[**,##]	85.3
	4	0.4±0.3[**,##]	81.6
	2	0.64±0.3[**,##]	70.5

[*] $P < 0.05$.
[**] $P < 0.01$ vs ADM.
[#] $P < 0.05$.
[##] $P < 0.01$ vs 0.9%NaCl.

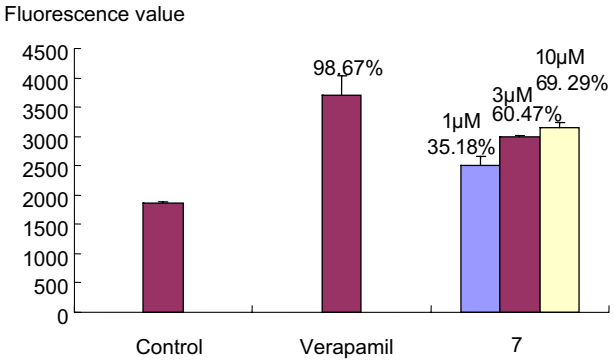

Figure 1. The effects of compound **7** on cellular Rh123 accumulation in MCF-7/ADM ($n = 3$).

cantly potentiated by the coadminstration of compound **7** (8, 4 and 2 mg/kg) in SCID nude mice (Table 3).

The rhodamine 123 (Rh123) accumulation assay was used to measure the P-gp inhibitory activity of compound **7**. The uptake of Rh123 in cells was followed by monitoring the fluorescence signal with the method described.[17] The results showed that fluorescence value of compound **7** was increased obviously compared to

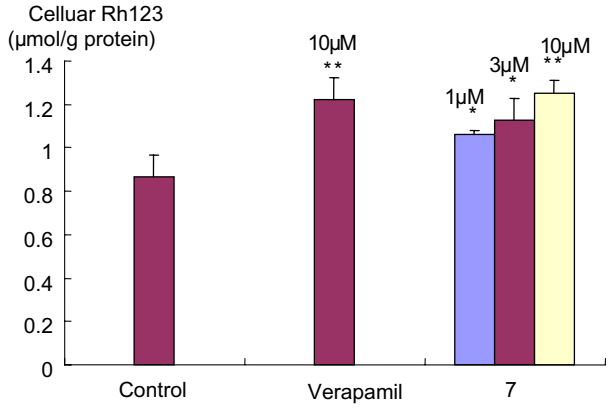

Figure 2. The effects of compound **7** on Rh123 accumulation in RBMEC ([*]$P < 0.05$, [**]$P < 0.01$ vs control) ($n = 3$).

control. P-gp function in MCF-7/ADM cells was inhibited after the treatment with compound **7**. When compound **7** was added at a concentration of 1, 3 and 10 μmol/L, the inhibitory ratio was 35.18%, 60.47%, and 69.29%, respectively (Fig. 1).

P-gp, which actively transport agents from the cerebral endothelial cells back into the bloodstream, have a major influence on blood–brain barrier (BBB) permeability to hydrophobic and amphiphilic drugs.[18] In order to further investigate the effect of compound **7** on P-gp, the rat brain microvascular endothelial cells (RBMEC)[19–21] for BBB model have been used. After incubated with RBMEC, compound **7** of 1, 3, and 10 μmol/L could increase the intracellular accumulation of Rh123 significantly[22] (Fig. 2).

The increase in the efflux of drugs is the major feature of P-gp mediated MDR. Considering that Rh123 efflux is more sensitive than that of anticancer drugs, the efflux inhibitory activity of the compound with Rh123 as a substrate was chosen to quantify the MDR reversal activity.[23] This test was carried out on RBMEC cell line. A time-dependent Rh123 content curve was performed with verapamil as reference drug (Fig. 3). The results showed that Rh123 efflux was decreased in the dose-dependent manner.

In parallel with the mechanisms of MDR modulation it could be speculated that the ability of the drugs to interact with the membrane phospholipids could also play a role.[24] Drug/membrane interactions could lead directly (changes in membrane permeability and fluidity) and/or indirectly (inhibition of P-gp phosphorylation via inhibition of PKC, changes of the conformation and functioning of the membrane-integrated proteins via changes in the structure organization of the surrounding membrane bilayer) to the reversal of MDR.[25] Diphenylhexatriene(DPH) was chosen to quantify the membrane lipid fluidity.[26] The results showed that the membrane lipid fluidity in K562 cell line was not affected by compound **7** while that in K562/A02 cell line was decreased after incubated with 10 μmol/L compound **7** ($P < 0.05$) (Fig. 4). The results indicated that the mechanism of compound **7** may inhibit P-gp transport function through decreasing membrane lipid fluidity indirectly.

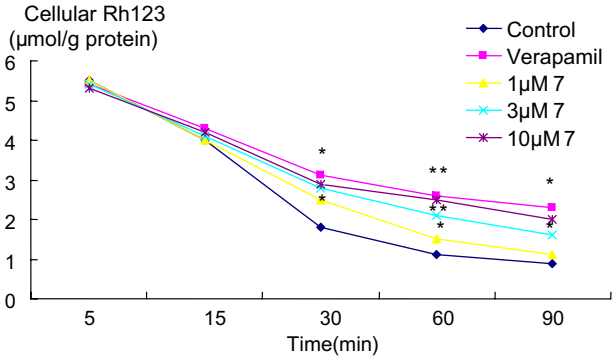

Figure 3. The effects of compound **7** on Rh123 efflux in RBMEC ($^{*}P < 0.05$, $^{**}P < 0.01$ vs control) ($n = 3$).

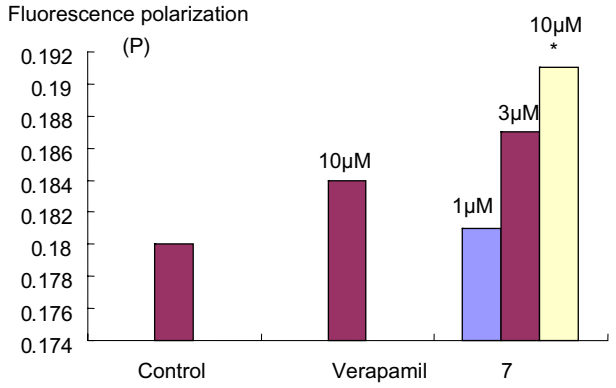

Figure 4. The effects of compound **7** on membrane lipid fluidity in K562/A02 ($^{*}P < 0.05$ vs control).

Table 4
The inhibitory ratio of compound **7** on aorta contractile activity ($\bar{X} \pm s, n = 3$)

Group	Concentration	20 mmol KCl	80 mmol KCl
Verapamil	10^{-6} mol/L	36.3±0.2%	46.3 ± 0.1%
7	10^{-6} mol/L	0 ± 0.0%	0 ± 0.0%

Table 5
Antiarrhythmic action of compound **7** by aconitine nitrate ($\bar{X} \pm s, n = 3$)

Group	Dose (mg/kg)	The dose of aconitine nitrate when antiarrhythmic (μg/kg)		
		VP	VT	VF
Control		20.2±0.1	28.9±0.3	37.0±0.2
7	2	22.4±0.1	26.7±0.1	31.5±0.1
Verapamil	2	30.3±0.2	44.0±0.2	55.0±0.1

The in vitro vascular contractile activity of compound **7** were performed with aorta isolated from SD rats (250–350 g).[27] Verapamil was used as reference drug. The results showed that compound **7** had no vascular contractile activity (Table 4).

The in vivo arrhythmogenesis was evaluated following the method described.[28] The dose of aconitine nitrate was determined after drug injection by echocardiography when ventricular premature beat (VP), sustained tachycardia (VT), and fibrillation (VF) happened. The results showed that compound **7** had lower arrhythmogenic effect than verapamil (Table 5).

In summary, tetrahydroisoquinoline derivative **7** was synthesized and evaluated for its MDR reversal and cardiovascular activities in vitro and in vivo. Compound **7** might exhibit MDR reversal activity by directly modulating the function of P-gp or indirectly inhibition of P-gp transport function through decreasing membrane lipid fluidity. Compound **7** may be a promising MDR reversal drug candidate. Further biological evaluation, pharmacokinetics study, long-term toxicity, and mechanistic studies on this new compound are currently in progress and will be reported in due course.

Acknowledgment

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14. *Selected spectroscopic data: Compound* **7**: IR (KBr, ν): 3380, 3000, 2920, 2860, 2162, 1610, 1570, 1520 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 6.40–6.84 (m,

5H, Ar–H), 4.95–5.03 (m, 1H, C_1–H), 3.78 (s, 3H, OCH_3), 3.86 (s, 9H, 3× OCH_3), 2.77–3.66 (m, 8H, C_3–H, C_4–H, $ArCH_2$, $NHCH_2$), 0.88–1.62 (m, 15H, $(CH_2)_6CH_3$); MS(ESI, *m*/*z*): 523 ($[M+H]^+$, base peak); Anal. Calcd for $C_{30}H_{42}N_4O_4$: C 68.94, H 8.10, N 10.72; found: C 68.58, H 8.13, N 10.65.

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16. K562/A02 cells were grown in RPMI 1640-containing 10% fetal calf serum and adriamycin (ADM) with 1 μg/ml as a final concentration at 37 °C in a 5% CO_2 humidified atmosphere. For the log phase cells were inoculated subcutaneously in oxter in the density of 1×10^7/ml. When the tumors reached a mean diameter of 0.4–0.6 cm, the animals were randomized into groups of ten and treated with drugs in the intravenous injection three times a week. Control animals were treated with vehicle alone. Tumor weights were measured. The inhibition ratio (*I*%) of tumor growth was calculated using ((mean relative tumor weights of the treated group)/(the mean relative tumor weights of ADM group) − 1) × 100 (%).
17. MCF-7/ADM cells were grown in RPMI 1640-containing 10% fetal calf serum, ADM with 1 μmol/L as a final concentration at 37 °C in a 5% CO_2 humidified atmosphere. For the log phase cells were implanted in 96-well plates in the density of 4×10^4/ml. the original medium was removed and the cells were incubated in the 160 μL RPMI 1640 containing no serum for 30 min. Verapamil with 10 μmol/L as a final concentration and 10, 3 and 1 μmol/L compound **7** and the same volume of PBS were added to incubate for 30 min. Then rhodamine 123 with 10 μmol/L as a final concentration was added and incubation continued for 60 min at 37 °C. After being washed twice with phosphate-buffered saline (PBS), 1% of Trition X-100 cells were added to each well. The samples were resuspended in PBS for analysis. The intracellular fluorescence due to Rh123 ($\lambda_{excitation}$ = 488 nm, $\lambda_{emission}$ = 535 nm) was measured in a microplate spectrofluorometer. Assays were performed in triplication. The inhibitory ratio (*I*%) was calculated using ((mean absolute fluorescence value of the treated group − the mean absolute fluorescence value of control)/the mean absolute fluorescence value of control) × 100 (%), where absolute fluorescence value for (fluorescence value of each well) − (the bases of fluorescence value of 96-well plate).
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22. RBMEC cells were grown in DF containing 20% fetal calf serum at 37 °C in a 5% CO_2 humidified atmosphere. Primary culture RBMEC cells were cultured in 24-well plates. When cells were forming a 90% confluent monolayer, the original medium was removed and the cells were incubated in the 0.4 ml DF containing no serum for 30 min. Verapamil with 10 μmol/L as a final concentration and 10, 3, and 1 μmol/L compound **7** were added, the same volume of PBS and Rh123 with 5 μmol/L as a final concentration in each well was added at last to incubate for 90 min. The cells were centrifuged after adding ice-cold PBS in different time and washed three times with ice-cold PBS. Finally, 1% of Trition X-100 cells were added to each well. The intracellular fluorescence due to Rh123 ($\lambda_{excitation}$ = 488 nm, $\lambda_{emission}$ = 535 nm) was measured in a microplate spectrofluorometer. The content of protein was measured using the method of Coomassie brilliant blue for fluorescence intensity value. Assays were performed in duplicate, with at least three separate experiments.
23. Compound **7** was diluted in DMSO (10 mmol/L) and RBMEC cells were grown in DF containing 20% fetal calf serum at 37 °C in a 5% CO_2 humidified atmosphere. Primary culture RBMEC cells were cultured in 24-well plates. When cells were forming a 90% confluent monolayer, the original medium was removed and the cells were incubated in the 0.45-ml assay buffer (PH 7.4) and 0.05-ml Rh123 with 100 μmol/L for 30 min. After being washed three times with ice-cold PBS, the samples were resuspended in assay buffer (PH 7.4) with Rh123-free. Verapamil with 10 μmol/L as a final concentration and 10, 3, and 1 μmol/L compound **7** were added to incubate. The cells were centrifuged after adding ice-cold PBS in different time and washed three times with ice-cold PBS. Finally, 1% of Trition X-100 cells were added to each well. The intracellular fluorescence due to Rh123 ($\lambda_{excitation}$ = 488 nm, $\lambda_{emission}$ = 535 nm) was measured in a microplate spectrofluorometer. The content of protein was measured using the method of Coomassie brilliant blue for fluorescence intensity value. Assays were performed in duplicate, with at least three separate experiments.
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26. K562 and K562/A02 cells were grown in RPMI 1640-containing 10% fetal calf serum at 37 °C in a 5% CO_2 humidified atmosphere. For the log phase cells were implanted in 96-well plates in the density of 1×10^5/ml. A final concentration of 10, 3, and 1 μmol/L compound **7** was added, respectively. Ten micromoles per liter verapamil and PBS was added as reference. After 24 h of incubation. The cells were centrifuged and washed twice with ice-cold PBS, the samples were resuspended in 1 ml PBS. DPH buffer (1ml) with 2 μmol/L were added for 30 min. After being washed twice with ice-cold PBS, the samples were resuspended in 2 ml PBS and incubated for 10 min at 25 °C. Four direction of fluorescence polarization were measured by adding two polarizing filters vertically ($\lambda_{excitation}$ = 357.6 nm, bandpass = 10 nm, $\lambda_{emission}$ = 428.7 nm) in a microplate spectro-fluorometer immediately. To calculate the Fluorescence polarization (*P*) for expressing the membrane lipid fluidity (*P* relating with membrane fluidity in inverse ratio) by the equation: $P = (I_{VV} - G \times I_{VH})/(I_{VV} + G \times I_{VH})$; where I_{VH} and I_{VV} are fluorescence intensity value when excitation in vertical direction, *G* is correction factor ($G = I_{HV}/I_{HH}$), where I_{HV} and I_{HH} are fluorescence intensity value when excitation in horizontal direction. Assays were performed in duplicate, with at least three separate experiments.
27. A section of the aorta was cleared of adhering fat and connective tissue and was cut into transverse rings (4- to 5-mm-long). The endothelium was removed by rubbing the intimal surface with forceps. The segments were suspended under 1.0 g of tension by means of steel hooks in an organ bath containing 10 mL of a Krebs-bicarbonate-buffered solution of the following composition (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 5. The physiological solutions were maintained at 37 °C and bubbled continuously with a mixture of O_2 (95%) and CO_2 (5%). The isometric contractions of the aortic rings were measured with a force–displacement transducer. After 60 min of equilibration, the rings were exposed to 20 mM KCl or 80 mM KCl. When the tension had stabilized, the drugs were added to the bath at increasing concentrations until maximal relaxation. The relaxation response was expressed as the percentage of the contractile response to KCl.
28. SD rats (2.5–3.5 kg) were anesthetized with urethane (1.2 g/kg ip). The test compounds were dissolved in normal saline and administrated by femoral vein. Intravenous administration of aconitine nitrate (1 μg/0.2 ml/min) was kept constant velocity from femoral vein by peristaltic pump after 2 min.