Design, Synthesis, and Pharmacological Evaluation of Haloperidol Derivatives as Novel Potent Calcium Channel Blockers with Vasodilator Activity

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Abstract

Several haloperidol derivatives with a piperidine scaffold that was decorated at the nitrogen atom with different alkyl, benzyl, or substituted benzyl moieties were synthesized at our laboratory to establish a library of compounds with vasodilator activity. Compounds were screened for vasodilatory activity on isolated thoracic aorta rings from rats, and their quantitative structure–activity relationships (QSAR) were examined. Based on the result of QSAR, N-4-tert-butyl benzyl haloperidol chloride (16c) was synthesized and showed the most potent vasodilatory activity of all designed compounds. 16c dose-dependently inhibited the contraction caused by the influx of extracellular Ca²⁺ in isolated thoracic aorta rings from rats. It concentration-dependently attenuated the calcium channel current and extracellular Ca²⁺ influx, without affecting the intracellular Ca²⁺ mobilization, in vascular smooth muscle cells from rats. 16c, possessing the N-4-tert-butyl benzyl piperidine structure, as a novel calcium antagonist, may be as effective as a calcium channel blocker in cardiovascular disease.

Introduction

Calcium is essential for life and is the most common signal transduction element in cells [1,2]. The contraction of all types of muscle primarily depends on increased intracellular Ca²⁺ [3]. Calcium channel blockers constitute an important class of cardio-vascular drugs. Members of this class are clinically useful in the treatment of cardiovascular disorders in which calcium plays a regulatory role. However, most cannot be used to resolve clinical problems such as ischemia, reperfusion injury, and vascular structure remodeling [4]. Furthermore, some of them have serious side effects. Therefore, better calcium channel blockers are still needed.

Haloperidol is a widely used antipsychotic whose therapeutic properties have been associated with its D₂ antagonist activity on the basis of the so-called dopamine hypothesis [3]. N-Benzyl haloperidol chloride (4) (Figure 1A), a derivative of haloperidol, was synthesized at our laboratory and was found to have a vasodilator effect [6]. Our previous study revealed that 4's inhibitory effect on the influx of extracellular Ca²⁺ might be related to vasodilatory mechanisms [7]. Furthermore, the N-benzyl piperidine structure of 4 is completely different from that of the clinically used calcium channel blockers, which belong to one of the following three chemical classes: the dihydropyridines, the phenylalkylamines, and the benzothiazepines [8]. Therefore, a novel calcium channel antagonist may be obtained by molecular manipulation of the structure of 4, which itself is not a sufficiently potent vasodilator.

With the aim of exploring the structure–activity relationship of 4 and obtaining more efficient calcium channel blockers, we had cultured single crystals to determine the structure of 4. Furthermore we designed the series of compounds shown in Table S1. In addition, we revealed some haloperidol derivatives (compounds 6a–6c, 9a–9c in Table S1) had vasodilator activity in previous study [9]. The piperidine scaffold, which we consider a supporting structure for novel calcium channel antagonists, was decorated at the nitrogen atom of piperidine with different substituent groups. We screened these compounds for vasodilatory activity in isolated rat thoracic aorta rings held in a Ca²⁺-containing high-K⁺ solution. A detailed quantitative structure–activity relationship (QSAR) analysis of this series of haloperidol derivatives was subsequently performed to study the physicochemical properties that were responsible for their activity. Base on the result of QSAR, we synthesized N-4-tert-butyl benzyl haloperidol chloride (16c) which was found to posses a good vasodilative activity in KCl-constricted vessels in line with expectation.

Many studies have demonstrated that vasorelaxation in KCl-constricted vessels can be ascribed directly to the inactivation of voltage-dependent Ca²⁺ channels (VDCCs) [10]. Hence, 16c was
further investigated its inhibitory action on the contraction caused by an influx of exogenous Ca\(^{2+}\) in thoracic aorta rings to prove that its vasodilator activity was related to Ca\(^{2+}\). Then, the inhibitory effects of \(16c\) on the activity of VDCCs, the extracellular Ca\(^{2+}\) influx, and the intracellular Ca\(^{2+}\) release in vascular smooth muscle cells (VSMCs) were explored in detail to reveal the calcium antagonist mechanism.

**Results**

**Synthesis**

The reaction pathways used to synthesize the designed compounds (compounds 1–16) were described in Schemes S1, S2, and the chemical and physical characteristics of the compounds were reported in Table S1. The key intermediate haloperidol (4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidyl]-1-(4-fluorophenyl)-butan-1-one) could be obtained via piperidyl alkylation reaction according to the literature [11]. Commercially available alkyl and benzyl halides were used. Some of benzyl halides (compounds 1–V) were synthesized from the corresponding benzaldehyde compounds (Scheme S1). The quaternary ammonium salt derivatives of haloperidol (compounds 1–16) were synthesized from haloperidol by alkylation or benzylation at the nitrogen atom using a suitable alkyl or benzyl halide (Scheme S2).

In order to know about the structure of 4 as the leading compound clearly, we had cultured single crystals and determined the crystal structure by X-ray single crystal diffraction (Figure 1B, Table S2). The chemical structures of these compounds were characterized by \(^1\)H NMR spectra (Table S3). \(16c\) was illustrated as a representation in Figure 2.

**Determination of vasorelaxation**

All synthesized compounds caused a concentration-dependent relaxation of aortic rings that had been precontracted to varying extents with a high level of K\(^+\) (80 mM). The vehicle (0.1% DMSO) used for all compounds had no effect on vascular tone. Concentration–response curves were determined for each compound, and the potency was expressed as the IC\(_{50}\) value in the presence and absence of endothelium (Table S4). From Table S4, we can see no significant IC\(_{50}\) difference between aortic rings with intact endothelium or denuded endothelium. Concentration–response curves showed no significant shift in the absence of endothelium, than in the presence of endothelium, which suggested no nitric oxide involvement in the vasodilatation (data not shown).

**QSAR study of some derivatives.** A detailed QSAR analysis of a series of haloperidol derivatives (compounds 4–15), splitting into a testing set of 3 compounds which are randomly selected and training set of 26 compounds had been performed to determine the physicochemical properties responsible for their activity. The physicochemical parameters used were listed in Table 1, namely the hydrophobic constant \(\pi\), Hammett \(\sigma\), molar refractivity (MR), and were compiled from the literature [12–14]. The IC\(_{50}\) values were transformed to pIC\(_{50}\) (negative logarithm of IC\(_{50}\)). The QSAR results indicated that activity was best modeled by molecular steric parameters represented by MR, hydrophobic parameters represented by \(\pi\). The best QSAR obtained was:

\[
pIC_{50}=0.238MR_{p}+0.181\pi-0.195MR_{o}+5.061
\]

The subscripts in the parameter relate to the position of the substituent considered. A determinants of coefficient \(R^2\) is 0.787 for 26 compounds in training set, and the residuals of compounds 9b,11b,14c as testing set are 0.03176; 0.1224, 0.00163. It confirmed that the equation was of some value in predicting activity (Table 1, Figure 3).

**The inhibitory action of \(16c\) on the contraction caused by KCl and the influx of extracellular Ca\(^{2+}\)**

The results indicated \(16c\) showed the most significant vasorelaxation effect on KCl-dependent contraction compared with compounds 1–15, and the IC50 value of \(16c\) was 0.95 \(\mu\)M (Table 1). And \(16c\) was chosen for further research.

KCl-dependent contraction is due to the influx of extracellular Ca\(^{2+}\) through VDCCs [10]. We first investigated the ability of \(16c\) to inhibit the contraction induced by the exogenous application of Ca\(^{2+}\) in a Ca\(^{2+}\)-free high-K\(^+\) solution. As Figure 4 showed, \(16c\) at different concentrations (0.1–10 \(\mu\)M) significantly impaired the response to Ca\(^{2+}\) (3 \(mM\)) to 87.49%–11.02% of the initial response. The IC\(_{50}\) value was 0.76 \(\mu\)M, similar to the result obtained from the determination of vasorelaxation (Table S1, Table 1). The results indicated that \(16c\) inhibited the responses to Ca\(^{2+}\) in a concentration-dependent manner. So we deduced that \(16c\) significantly inhibited the response to Ca\(^{2+}\) influx from the extracellular space.

**Effects of \(16c\) on [Ca\(^{2+}\)]\(_i\), in arterial VSMCs.**

The Ca\(^{2+}\) responses of arterial VSMCs to KCl (80 mM) were studied after incubation with \(16c\). \(16c\) did not affect basal [Ca\(^{2+}\)]\(_i\) (29.8±1.2 vs 30.7±1.4 \(nM\) for \(16c\) and control, respectively, \(n=25\), \(p>0.05\)). The response of the control cells to KCl was characterized by a sharp increase in [Ca\(^{2+}\)]\(_i\) (from 30.18±2.82 to 103.34±7.26 \(nM\), mean ±S.E. \(n=24\)) followed by a sustained plateau (106.64±6.24 \(nM\) at 200 s). This response was totally inhibited by \(16c\) at 0.1, 1, 10 \(\mu\)M (both KCl \(\text{prok}\) and KCl \(200 \mu M\) vs 89.76±4.96, 57.24±3.64, 42.32±2.65 \(nM\), respectively \(n=24\), \(p<0.05\) vs control values; Figure 5).

**Effect of \(16c\) on L-type Ca\(^{2+}\) channel activity.** We then applied the patch-clamp technique to measure the effect of \(16c\) on the activity of VDCCs on the primary cells. In our study, \(I_B\) was elicited by depolarization from the depolarizing potential of −30 mV to +50 mV. The peak \(I_B\) was elicited at the potential of 0 mV. Barium currents were reversibly inhibited by 1 \(\mu\)M nimodipine, and augmented by 50 nM Bay K 8644 (data not shown). \(16c\) (10, 1, 0.1 \(\mu\)M) reduced the peak current to 21.3±5.1% (\(p<0.01\), \(n=5\)), 39.4±6.2% (\(p<0.01\), \(n=5\)), 89.8±15.1% (\(p<0.01\), \(n=5\)) of control values, respectively (Figure 6A). The VSMCs showed current/voltage relationships typical of high voltage-activated L-type Ca\(^{2+}\) channels, with apparent threshold and reversal potentials of approximately −30 and +50 mV, respectively (Figure 6B). No change in leak current.
was observed during the application of the compounds, which suggests that at these concentrations, the compounds had no “detergent-like” effect on cell plasma membrane. Half-maximal inhibition of the Ca\(^{2+}\) channel current was observed at about 1 \(\mu\)M.

**Discussion**

Calcium channel blockers play an important role in cardiovascular diseases, but better drugs are still needed for some clinical problem. In our study, we found some Haloperidol derivatives showed vasorelaxation activity to varying degree. We synthesized a series of haloperidol derivatives and we used the test of vasodilator effect on the rat isolated thoracic aorta rings with a high level of K\(^+\) (80 mM) to screen molecules for further studies, and examined the structure-activity relationship of compounds. Studies have demonstrated that the contraction of vascular smooth muscle is initiated by an increased intracellular calcium level [15–17], which may be achieved in two ways: extracellular Ca\(^{2+}\) influx from VDCCs evoked by depolarization with high potassium concentration, and intracellular Ca\(^{2+}\) release from the intracellular stores [18,19]. From the result, we can deduce these compounds’ inhibition on the contraction of the vascular smooth muscle might relate to the extracellular Ca\(^{2+}\) influx from VDCCs evoked by depolarization with high K\(^+\).

Furthermore, most published QSARs show the importance of particular physicochemical parameters in describing activity [20]. We observed a correlation between the pharmacological activities and structures in this study. The best QSAR obtained was: 

\[
pIC_{50} = 0.238 \text{ MR}_x + 0.181 \pi - 0.195 \text{ MR}_3 + 5.061.
\]

This result showed that the main factors governing activity were the MR term of the specific position of the substituent which will determine the fit at the receptor site and the hydrophobic parameters which determine the ability of the compounds to transport the cellular...
may be due to a conformational preference of 4-phenyl piperidine steric parameter. It is used to model the intermolecular effects a steric parameter was consistently expressed in a regression receptor binding. This was inferred from the fact that MR term, as parameters and their position appears to be in their ability to the cellular membrane. In addition, compound 4 displayed higher membrane and to bind in the hydrophobic space of the proteins in the cellular membrane. In addition, compound 4 displayed higher vasodilation activity than compounds 1–3 (from Table S1). These results also indicated that for this series of derivatives, the MR term of a particular molecule was the significant element of the substituent. The importance of both the substituent steric parameters and their position appears to be in their ability to maintain the molecule in an orientation that is conducive to receptor binding. This was inferred from the fact that MR term, as a steric parameter was consistently expressed in a regression analysis. The equation also highlighted the importance of the substituent for vasodilator activity in the ortho- and para-positions. The MR parameter needs some discussion at this point. MR has been viewed as a measure of bulk and as a “rough and ready” steric parameter. It is used to model the intermolecular effects between a ligand and receptor. From the x-ray structure of 4 (Figure 1B), it is easy to understand that the negative ortho effect may be due to a conformational preference of 4-phenyl piperidine ring for the substituted benzyl group. It is, therefore, most probably due to direct steric hindrance interfering with drug receptor binding [21]. Although a more complex problem involving, interpretation of a positive coefficient with MR revealed that the steric effect at the para-position of the aryl ring was advantageous to the activity. In summary, the result showed activity may be further improved if substituents with greater hydrophobic parameters in the para-position are considered (such as p-(CH2)4N(CH3)3, p-(CH2)4C6H5). The compound with p-(CH2)3N(CH3)3 had a lower molecular weight than the others (such as p-(CH2)3N(CH3)3, p-(CH2)3C6H5). So this compound (16c) was designed and synthesized, and 16c showed the most potent vasorelaxation effect agreeing with the predicted effect (experimental IC50: 0.95 μM, predicted IC50: 1.36 μM, Table 1).

As KCl-dependent contraction is due to the influx of extracellular Ca2+ through VDCCs [10], it is likely that the haloperidol derivatives, such as 16c, may have effects on the inhibition of the influx extracellular Ca2+, and the blockade of calcium channels. The ability

| Compound | π | σ | MRo | MRm | MRp | lgIC50 (M)* | lgIC50 (M)* Predicted |
|----------|---|---|-----|-----|-----|-------------|---------------------|
| 4        | 0 | 0 | 0.1 | 0.1 | 0.1 | −4.9965     | −5.06832            |
| 5a       | −0.67 | −0.2 | 0.28 | 0.1 | 0.1 | −4.8877     | −4.91286            |
| 5b       | −0.67 | 0.12 | 0.1 | 0.28 | 0.1 | −4.9597     | −4.94857            |
| 5c       | −0.67 | −0.37 | 0.1 | 0.1 | 0.28 | −4.9974     | −4.99122            |
| 6a       | 0.71 | 0.40 | 0.6 | 0.1 | 0.1 | −5.07       | −5.09602            |
| 6b       | 0.71 | 0.37 | 0.1 | 0.2 | 0.1 | −5.2097     | −5.19522            |
| 6c       | 0.71 | 0.23 | 0.1 | 0.1 | 0.6 | −5.3001     | −5.33352            |
| 7b       | 0.64 | 0.39 | 0.1 | 1.25 | 0.1 | −5.0074     | −5.18271            |
| 7c       | 0.64 | 0.31 | 0.1 | 0.1 | 1.25 | −5.2111     | −5.45515            |
| 8a       | 0.88 | 0.97 | 0.5 | 0.1 | 0.1 | −5.0301     | −5.14624            |
| 8b       | 0.88 | 0.64 | 0.1 | 0.5 | 0.1 | −5.3595     | −5.22561            |
| 8c       | 0.88 | 0.56 | 0.1 | 0.1 | 0.5 | −5.44       | −5.32037            |
| 9a       | 0.56 | 0.93 | 0.56 | 0.1 | 0.1 | −5.07       | −5.07715            |
| 9b*      | 0.56 | 0.8 | 0.56 | 0.1 | 0.1 | −5.1999     | −5.16814            |
| 9c       | 0.56 | 0.73 | 0.1 | 0.1 | 0.56 | −5.27       | −5.27739            |
| 10a      | 0.14 | 0.98 | 0.09 | 0.1 | 0.1 | −5.02       | −5.09333            |
| 10b      | 0.14 | 0.92 | 0.1 | 0.09 | 0.1 | −5.0798     | −5.09335            |
| 10c      | 0.14 | 0.93 | 0.1 | 0.1 | 0.09 | −5.07       | −5.09098            |
| 11a      | −0.28 | 1.03 | 0.74 | 0.1 | 0.1 | −4.9698     | −4.8913             |
| 11b*     | −0.28 | 0.86 | 0.74 | 0.1 | 0.1 | −5.1402     | −5.01778            |
| 11c      | −0.28 | 0.73 | 0.1 | 0.1 | 0.74 | −5.27       | −5.1699             |
| 12c      | 1.53 | −0.15 | 0.1 | 0.1 | 1.50 | −5.6458     | −5.67345            |
| 13b      | −0.01 | 0.36 | 0.1 | 1.29 | 0.1 | −5.266      | −5.06654            |
| 13c      | −0.01 | 0.45 | 0.1 | 0.1 | 1.29 | −5.4485     | −5.34845            |
| 14a      | −0.57 | 1.28 | 0.63 | 0.1 | 0.1 | −4.7201     | −4.66129            |
| 14b      | −0.57 | 1.07 | 0.63 | 0.1 | 0.1 | −4.9299     | −4.96587            |
| 14c*     | −0.57 | 0.91 | 0.1 | 0.1 | 0.63 | −5.0899     | −5.08827            |
| 15a      | 1.02 | −0.17 | 1.03 | 0.1 | 0.1 | −5.1598     | −5.06611            |
| 15c      | 1.02 | −0.15 | 0.1 | 1.03 | 0.1 | −5.58       | −5.70995            |
| 16c      | 1.98 | −0.02 | 0.1 | 0.1 | 1.96 | −6.0222     | −5.86636            |

* lgIC50: The half maximal inhibitory concentration.
* MRi: molar refractivity in ortho-position, MRm: molar refractivity in meta-position, MRp: molar refractivity in para-position.
* Compounds as testing set and their predicted IC50 were calculated by QSARs model.
of 16c to inhibit the contraction induced by the exogenous application of Ca\(^{2+}\) then was confirmed in a Ca\(^{2+}\)-free high-K\(^{+}\) solution. This observation suggests that C3 might further inhibit the influx of Ca\(^{2+}\) through VDCCs.

An elevated [Ca\(^{2+}\)]\(_i\) level is the main trigger for VSMC contraction [22], and we showed that 16c had an inhibitory effect on the contraction induced by exogenous Ca\(^{2+}\). To reveal the molecular mechanism by which 16c inhibited vasoconstriction and to obtain more information about the effect of 16c on calcium ions, we studied the effect of 16c on Ca\(^{2+}\) signaling in VSMC by LSCM. The increase in [Ca\(^{2+}\)]\(_i\) is believed to result from Ca\(^{2+}\) influx, corresponding to the opening of the voltage-sensitive plasma membrane Ca\(^{2+}\) channels [23]. This response was inhibited by 16c, which is consistent with the results in intact

![Graph](image1)

**Figure 3.** Correlation between predicted activities (IC\(_{50}\)) by QSARs models and the experimental activities (IC\(_{50}\)) (for compounds of training set, \(R^2=0.787\); for randomly selected compounds 9b,11b,14c as testing set, Residue are 0.03176; 0.1224, 0.00163. Correlation is significant at the 0.01 level.).

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![Graph](image2)

**Figure 4.** Cumulative concentration-response curves for Ca\(^{2+}\) in the presence of the vehicle and increasing concentrations of 16c (0.1–10 \(\mu\)M) in isolated endothelium-intact aortic rings \((n = 5)\). Results are the mean ±S.E. from 5 independent experiments.

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arteries (Figure 4). The results of the effect on KCl-dependent Ca$^{2+}$ signaling indicated that 16c decreased the influx of extracellular Ca$^{2+}$ caused by depolarization in VSMCs.

Based on these findings, we therefore postulate that the compounds targeted the plasma membrane, especially the VDCCs present in VSMCs. The patch-clamp technique was applied to measure the effect of 16c on the activity of VDCCs on the primary cells. 16c obviously suppressed the calcium channel current and the I-V relation of $I_{Na}$ indicated that the blocking effect of 16c on calcium channels was voltage-independent. Studies indicate two types (L and T) of calcium channel exist in VSMCs [24]. Under the condition of individual cell depolarization from a holding potential of $-40$ mV, the L-type calcium channel was activated, and the T-type Ca$^{2+}$ and Na$^+$ channels were inactivated. Moreover, TEA, a non-specific K channel blocker, was administered in the extracellular solution. The pipette solution was also filled with 4-AP (a K$^+$ channel blocker). So the outward K$^+$ currents were completely blocked [25]. In addition, Barium currents were reversibly inhibited by 1 μM nimodipine, and augmented by 50 nM Bay K 8644, an activator of L-type Ca$^{2+}$ channels [26] in our study. Therefore, the inward current we recorded under these conditions was an L-type Ca$^{2+}$ current. Obviously, 16c suppressed the L-type calcium channel current. In addition, we can infer the mechanism of 16c inhibition of L type Ca$^{2+}$ channels, from the structure of 4-phenyl piperidine which exists in the haloperidol derivatives. In our study, the structure-activity relationship analysis indicated the 16c and other haloperidol derivatives, which contain 4-phenyl piperidine ring possess calcium channel blocking (CCB) activity and the conformation of the 4-phenyl piperidine ring correlates with the CCB activity. The piperidine ring is the hydride 1, 4-DHP ring. Studies on the structure of nifedipine demonstrated the 1, 4-dihydropyridine (1, 4-DHP) ring system which was perpendicular to the C-4 substituted-phenyl ring, was the determinant of calcium channel antagonist activity [27]. Therefore we suppose the mechanism of 16c in the block of L-type Ca$^{2+}$ channel is probably

![Figure 5](image5.png)

**Figure 5.** Effect of 16c (0.1–10 μM) and the vehicle (0.1% DMSO) on KCl (80 mM)-induced changes of Ca$^{2+}$ concentration in vascular smooth muscle cells. A, representative Ca$^{2+}$ concentration response to KCl; B, dose-dependent inhibitory effect of 16c on the Ca$^{2+}$ concentration response to KCl. Results are the means ± S.E. (n = 24). *, p<0.01 compared with normal. **, p<0.01 compared with model (KCl).
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![Figure 6](image6.png)

**Figure 6.** Effect of 16c on L-type Ca$^{2+}$ channel currents in vascular smooth muscle cells (A) and on I–V relation of Ba$^{2+}$ currents in vascular smooth muscle cells (B). The step protocol of recording was described in methods section.
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similar to the nifedipine. The heterologous expressing such as Xenopus oocytes, will be performed to explore the exact mechanism in our future studies.

Ca^{2+} is considered to be an intracellular signal implicated in both vasoconstriction and proliferation [28]. However, it is well known that some L-type Ca^{2+} channel antagonists (verapamil, diltiazem, and nifedipine) show little inhibitory effect on the proliferation of vascular smooth cells, which is related to the development of vascular diseases [29]. While, it was reported that 16c, a novel channel blocker, had anti-proliferation activity [30]. Ca^{2+}, as a second messenger, stimulates nuclear transcription factors Egr-1 expression which involves cell proliferation [30]. We previously showed that Egr-1 overexpression induced cell proliferation by using antisense Egr-1 ODNs, and demonstrated that 16c attenuated the Ang II-induced extracellular Ca^{2+} influx and inhibited Egr-1 overexpression. In this study, we demonstrated further that 16c had vasodilatory activity, although the vasodilator effect of 16c was not more potent than other L-type Ca^{2+} antagonists (nifedipine, 1–10 nM; verapamil, 0.1–1 μM; and diltiazem, 0.5–1 μM; concentrations for 50% inhibition of vascular contractions in rat or rabbit aorta) [31].

However, 16c, as a novel channel blocker, had vasodilatory activity and anti-proliferation activity that provides a basis for further studies of novel calcium channel blockers on intervention of vascular remodeling and atherosclerosis [30].

**Methods**

**Chemistry**

**General methods for preparation of the haloperidol derivatives.** The preparation of the title compounds was very simple. Haloperidol was dissolved in chloroform and was then reacted with benzyl halide under reflux conditions, or halohydrocarbon (iodomethane, ethyl iodide, iodopropane) were used as a solvent and were reacted with haloperidol directly. All melting points were taken on a Buchi apparatus and were uncorrected. IR spectra were recorded with a Nicolet-Impact 410 system as KBr pellets. 1H NMR spectra were recorded on a Bruker AM-400 spectrometer (400 MHz). The 1H NMR internal standard used was tetramethylsilane (TMS). Chromatographic separations were performed on a silica gel column by gravity chromatography (Kieselgel 40, 0.01–0.04 mm; Merck). Yields are given after purification, unless otherwise stated. Where analyses are indicated by symbols, the analytical results are within ±1% of the theoretical values. All spectral and elemental analysis data (shown in the Supporting Information) were in agreement with the assigned structures. All compounds were estimated to be >95% pure according to analytical reversed-phase high performance liquid chromatography (RP-HPLC) and detection with MS (see Supporting Information for details) for details. No UV-active impurities were observed with analytical RP-HPLC and UV detection at 245 nm. The data for 16c was displayed as represented in Figure 2.

**Synthesis of compounds 1–3.** Haloperidol (10 mM) was dissolved in the corresponding halohydrocarbon (4 mL). The mixture was heated in an oil bath for 24 h to maintain reflux and was then cooled to room temperature. The filtered solid was recrystallized from an ethanol-water mixed solvent to a crystalline product.

**Synthesis of some benzyl halide (compounds I–V).** The corresponding benzaldehyde compounds (100 mM) were dissolved in methanol (20 mL) [for I–III] or THF (20 mL) [for IV–V]. NaBH₄ (200 mM) was then added gradually in ice-bath. The mixtures were elevated to room temperature and stirred for 5 min (for I–III) or 1 h (for IV–V). Then water (20 mL) was added to end the reaction and the mixtures were evaporated to dryness. The products were again dissolved in ethylacetate (40 mL), washed with water (20 mL) and ethylacetate extracts were concentrated to dryness. The products of benzyl alcohol derivatives were obtained in this step. These products were then dissolved in THF (20 mL without water, contained DMF 1–2 d), SOCl₂ (3 mL) was added gradually in ice-bath, and then the mixtures were stirred at 0°C for 20 min, reactions were terminated and the pH value of the mixture was adjusted to about 7 with a 1 N NaOH solution. The oil layers were separated from the mixture with a separatory funnel and the products (benzyl halide) were obtained by distillation. The 1H NMR data of compounds I–V were compared with literature [32,33], and reported in supporting information (Table S5).

Others benzyl halide were purchased from Alfa Aesar, A Thunson Matthey Company and some Chinese Companies. These compounds have been confirmed with literature data in HNMR.

**Synthesis of compounds 4–16.** Haloperidol (10 mM) was dissolved in chloroform (4 mL) and the corresponding benzyl halide (30 mM) was then added. The mixture was heated to maintain reflux for 18–24 h, and was then cooled to room temperature. The white solid was filtered off, and was washed with chloroform. The crude product was recrystallized in an ethanol–water mixed solvent to provide a crystalline product.

**Pharmacology**

**Vasoactivity determination.** The vasoactivity of compounds 1–16 was evaluated in isolated rat thoracic aortic rings according to the methods of Towitz and Polster et al [34,35]. Thoracic aortas were isolated from male Wistar rats weighing 200–250 g. The vessels were cut into ring segments, 2–3 mm wide, and 5 rings were obtained from each aorta. Rings were then mounted in standard 10-mL organ baths filled with Krebs bicarbonate solution (PSS), containing (in mM): 118.0 NaCl, 4.7 KCl, 1.2 KH₂PO₄, 1.2 MgSO₄·7H₂O, 5.0 glucose, 25.0 NaHCO₃, and 2.5 CaCl₂·7H₂O at 37°C and were gassed with 95% O₂–5% CO₂ (pH = 7.4). The aortic rings were allowed to equilibrate for 90 min at a testing tension of 1 g, and were then depolarized by the addition of a solution of KCl to a final concentration of 80 mM. The preparations were then extensively washed with PSS, and a second contraction was evoked by K⁺ depolarization (80 mM). When the amplitude of the contraction reached a plateau, cumulative concentrations of the tested compounds in a vehicle of water and DMSO (1000:1) were added every 45 min. Development of antagonism occurred so slowly that doses had to be increased at established times, without waiting for complete equilibrium to be reached. Cumulative concentration-response cures to all compounds were determined in the absence and presence of endothelium. According to experimental protocol, when required, the endothelium was mechanically removed from some rings by gently rubbing the lumen with the tips of a forceps. Acetylcholine was used to confirm that the endothelium had effectively been removed from the aortic rings [36].

**Inhibitory action of active compound (16c) on the contraction caused by influx of extracellular Ca²⁺.** To determine the effect of 16c on the response to the influx of extracellular Ca²⁺, the contractile response to the exogenous application of Ca²⁺ was examined in the presence of 16c in Ca²⁺-free high-K⁺ solution (80 mM, K⁺-HBSS) as previously described [37]. Aortic rings were initially equilibrated at a resting tension of 1 g in normal Ca²⁺-free HBSS for 60 min. The bath medium was then replaced with Ca²⁺-free high-K⁺ HBSS for 30 min to
Haloperidol Derivatives as Novel Calcium Blockers

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