Synthesis and Biological Evaluation of a Series of 6,7-dimethoxy-1-(3,4-dimethoxybenzyl)-2-substituted Tetrahydroisoquinoline Derivatives

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Abstract: Multidrug resistance in cancer is a major cause of failure in cancer chemotherapy. In search of new compounds with strong reversal activity and simple molecular structure, we have synthesized a series of compounds in which different substituents were linked to the 2-position of the 6,7-dimethoxy-1-(3,4-dimethoxybenzyl)- tetrahydroisoquinoline system. Compounds were analyzed for their cytotoxicity by MTT in K562 cell line *in vitro*, all of the derivatives exhibited little cytotoxic activity. In the meantime, these compounds were evaluated by MTT in K562/A02 cell line *in vitro*, 6e, 6h and 7c exhibited similar or more potent activities than verapamil with the IC_{50} values at 0.66, 0.65 and 0.96μM, and with the ratio factor of 24.13, 24.50 and 16.59, respectively.

Keywords: Multidrug resistance, anticancer, tetrahydroisoquinoline.

INTRODUCTION

Multidrug resistance (MDR) induced by anti-tumor agents treatment is a significant obstacle to the success of chemotherapy in many cancers [1, 2]. There has been rising interest in the discovery and development of novel small molecules to reverse MDR. Verapamil, the calcium channel blocker, is the most widely used agent to reverse MDR [3]. However, verapamil has been restricted as MDR reversal agent due to its risks in cardiovascular system at effective dose [4]. Consequently, synthesis and screening of novel MDR reversing agents with higher selective activity and low cytotoxicity are essential. Tetrandrine (Fig. **1**), a complex natural alkaloid extracted from *Radix Stephaniae Tetrandrae* root, displayed antioxidant [5], anti-inflammatory [6], anti-allergic [7] and anti-tumor [8] activities. In our previous work [9-12], a series of 6,7-dimethoxy-1-(3,4-dimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline derivatives derived from tetrandrine exhibited potent MDR reversing activities against cancer cells, especially HZ08 (Fig. **1**). HZ08, a well acknowledged P-glycoprotein inhibitor [9,11], possesses superior activity in reversing MDR cell lines induced by various anti-tumor agents. In this paper, the cyano-guanyl group at 2-position of 6,7-dimethoxy-1-(3,4-dimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline was replaced by nitro-vinyl amidino or N-nitro-guanyl groups, to form another class of potent multidrug resistance reversal agents. And their preliminary biological activities were evaluated. We anticipated the designed compounds exhibiting MDR reversal activities and anti-tumor activities.

RESULTS AND DISCUSSION

Synthesis

We designed and synthesized a series of tetrahydroisoquinoline derivatives, including 12 compounds which are the combination of 6,7-dimethoxy-1-(3,4-dimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline with nitro-vinyl amidino or N-nitro-guanyl groups. The synthetic routes of compounds **6a-6h** and **7a-7d** were outlined in Scheme **1**. The compound **1** was treated with formaldehyde by Pictet-Spengler reaction to afford **3a** [13], and **2b-2d** were obtained from compound **1** by Bischler-Napieralski reaction with appropriate substituted acetic acid at 190°C under N_2 environment [14]. Compounds **2b-2d** reacted with $POCl_3$ in toluene under reflux to provide dihydroisoquinoline 1-substituted derivatives **3b-3d**. Compounds **3a-3d** reacted with KBH_4 in methanol to afford 1-substituted derivatives **4a-4d**. Next, the reaction at the N-position of compounds **5a-5d** was achieved with 1,1-dimethylthio-2-nitroethene [15] in toluene, respectively. Reaction of the intermediate **5a-5d** with substituted amines in toluene under reflux gave compounds **6a-6h**. Furthermore, the reaction at the 2-position of compounds **4a-4d** with methyl N'-nitrocarbamimidothioate are compounds **7a-7d** [16] in ethanol under reflux.

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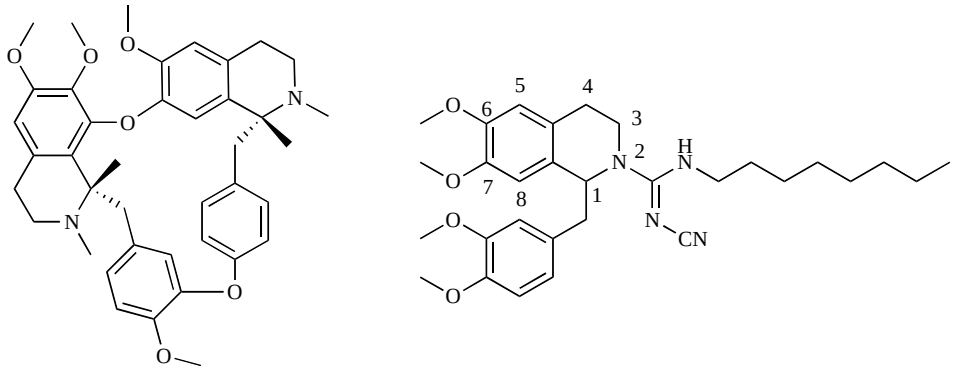
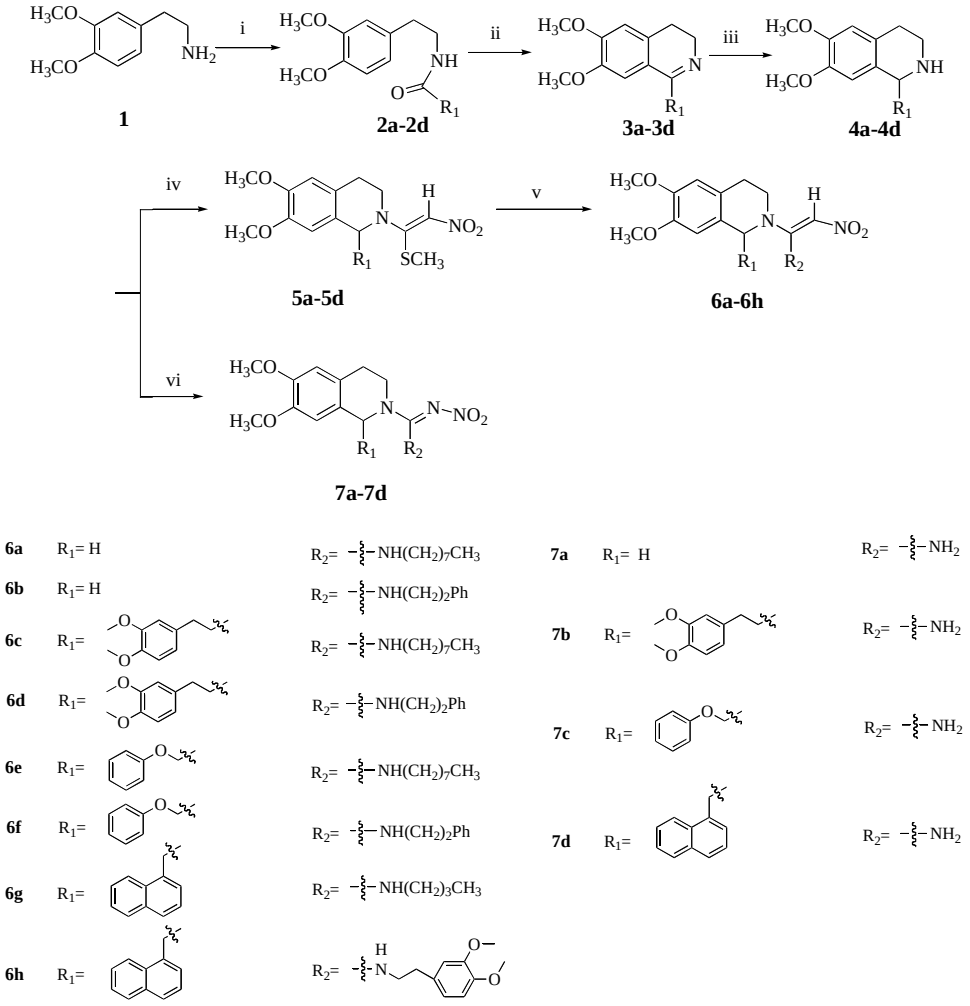


Tetrandrine HZ08

Fig. (1). Structure of Tetrandrine and HZ08.



Scheme 1. Synthesis of compounds **6a-6h** and **7a-7d**. Reagents and conditions: (i) R_1CH_3COOH, N_2, 190°C, 3h; (ii) $POCl_3$, toluene, N_2, 110°C, reflux, 2.5h; (iii) KBH_4, diethylamine, methanol, rt, 24h; (iv) 1, 1 -dimethylthio-2-nitroethene, toluene, reflux, 30-50h; (v) R_2NH_2, toluene, reflux, 14-72h; (vi) methyl N'-nitrocarbamimidothioate, ethanol, reflux, 18-72h.

Cytotoxicity Assay

Human chronic myelogenous leukemia cells: The K562 cell line was obtained from the erythroleukemia type (Institute of Hematology Chinese Academy of Medical Sciences, Tianjin, China). 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 cells/mL, the original medium was removed and the cells were incubated in the 160 mL RPMI 1640 containing no serum for 30 min. With 10 μg/mL tested compounds (adriamycin, compounds **6a-6h** and **7a-7d**) or the same volume PBS as vehicle control were added to incubate at 37°C for 48h. During the next 4 h incubation

Table 1. Cytotoxicity to K562 Cells as Determined by MTT Assay ($\bar{x}$, n=3) Dose: 10 μg/ml

Compound	Survival (%)
verapamil	98.3*
HZ08	93.5*
6a	90.6*
6b	93.9*
6c	97.2*
6d	95.2*
6e	90.0*
6f	87.5*
6g	89.8*
6h	95.0*
7a	95.9*
7b	95.9*
7c	88.0*
7d	99.4*

* P<0.005, determined by X^2 test.

the cells were determined by MTT assay [17]. The cells were exposed to MTT (5 mg/mL, sigma) and the resulting formazan crystals were dissolved in 200 μM DMSO, followed by measuring the absolute fluorescence value at λ=492 nm on a microplate reader (Thermo, USA). Assays were performed in duplicate, with at least three separate experiments. Cytotoxicity rate was expressed as survival, which was calculated using (mean absolute fluorescence value of the treated group/the mean absolute fluorescence value of control) ×100 (%). The results were showed in Table **1**. The cell survival of the tested compounds was over 85%. It indicated that all tested compounds were not toxic to K562 cancer cell lines thus and cannot be regarded as anti-tumor agents.

MDR Reversal Activity

MDR reversal activity of tested compounds (compounds **6a-6h** and **7a-7d**) was reported as IC_{50} values and ratio factor (RF) in the K562/A02 cell line (Institute of Hematology Chinese Academy of Medical Sciences, Tianjin, China). Cells were seeded into 96-well plates at 3×10^4 cells/well. Various concentrations of adriamycin and the tested compounds were gradually added and plates were incubated in 5% humidified incubator at 37°C for 48h. Finally, the MTT assay was performed as mentioned before. IC_{50} values of adriamycin (concentration resulting in 50% inhibition of cell growth) were calculated in the presence of five concentrations of the tested compounds (0.5μM, 1μM, 10μM, 20μM and 50μM). The RF was calculated by the following equation: MDR ratio factor (RF) = IC_{50} (adriamycin alone) / IC_{50} (adriamycin + tested compound).

As shown in Table **2**, compounds **6e**, **6h** and **7c** displayed similar or more potent reversal activities in comparison with

Table 2. MDR Reversal Activity Tested Compounds in K562/A02 Cell Lines by MTT Assay

Compound (10μg/mL)	IC_{50} of Adriamycin and Ratio Factor (RF)	
	IC_{50}*	RF
verapamil	0.88 [a]	18.10
HZ08	0.73 [a]	21.82
6a	1.68 [a]	9.48
6b	23.31 [a]	0.68
6c	1.79 [a]	8.89
6d	12.87 [a]	1.23
6e	0.66 [a]	24.13
6f	1.82 [a]	8.75
6g	3.97 [a]	4.01
6h	0.65 [a]	24.50
7a	18.62 [a]	0.85
7b	1.76 [a]	9.05
7c	0.96 [a]	16.59
7d	1.69 [a]	9.42
-	15.93 [b]	-

[a] IC_{50} of adriamycin in the presence of tested compounds;
[b] IC_{50} of adriamycin alone (control IC_{50});
* IC_{50} of adriamycin (μg/mL), $\bar{x}$, n=3, reversal adriamycin.

verapamil and HZ08. Compound **6h**, with a 1-positioned 1-naphthylmethyl group and 2-positioned 3,4-dimethoxyphenylethyl linked by nitro-vinyl amidino, exhibited the most potency with the IC_{50} values at 0.65μM and the RF of 24.50 in K562/A02 cells. Compounds **6e** and **7c** also exhibited high potent with the IC_{50} value at 0.65μM and 0.96μM, and with the RF of 24.13 and 16.59, respectively. In comparison with HZ08, compounds **6c** and **6e** with 2-positioned n-octyl group showed better anti-MDR activities, indicating that the introduction of a long linear chain of fatty amine at R_2 may benefit to the anti-MDR effect. The activity could be improved when the 3,4-dimethoxybenzyl group was replaced by phenoxymethyl group (compounds **6e** and **7c**). additionally, when the R_1 was substituted by 1-naphthyl-methyl group while R_2 was replaced by 3,4-dimethoxyphenethylamino group at the meantime, the derivative **6h** also exhibited more potent than HZ08. As all the derivatives were not toxic to K562 cancer cell lines under the dosage of 10 μg/ml, it can be attributed to the reversal agents that displayed a sensitizing effect on anti-tumor drugs, but not their cytotoxic effect to tumor cells.

CONCLUSION

We have synthesized a series of nitro-vinyl amidino and nitro-guanyl substituted 6,7-dimethoxy-1-(3,4-dimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline derivatives. The biological evaluation included two tumor cell lines, K562 and

drug-resistant K562/A02 cell lines. All synthesized compounds displayed little cytotoxic effect in K562 cell line. Some of these compounds (compounds **6e**, **6h** and **7c**) exhibited similar or more potent MDR reversal activities than verapamil with IC_{50} values at 0.66μM, 0.65μM and 0.96μM, and with the RF of 24.13, 24.50 and 16.59 in K562/A02 cell line, respectively. So these three compounds could be chosen as candidates for MDR reversal agents. Further studies on separating the chiral enantiomers and the mechanism of action of these three compounds are currently in progress and the results will be reported in due course.

EXPERIMENTAL

The melting points were determined with an x4-type micro melting point apparatus (Beijing Optical Instrument Factory III) and were uncorrected. The ^{1}H NMR spectra were recorded on an ACF-300 Bruker spectrometer in d_1-chloroform. Tetramethylsilane (TMS) was used as the internal standard. The mass spectra were obtained with a HP1100LC/MSD spectrometer with a direct inlet system at 70 eV. The IR spectra were recorded on a Nicolet Impact 410 infrared spectrometer (KBr disk). The elemental analysis were obtained with a Carlo Erba 1106-type analyzer. Reactions were monitored by TLC on Silica Gel 60 F254 (Qingdao Ocean Chemical Company, China) plates. Column chromatography was performed with Silica Gel 100-200 (Qingdao Ocean Chemical Company, China).

6,7-dimethoxy-2-(1-methylthio-2-nitro)vinyl-1,2,3,4-tetrahydroisoquinoline (5a)

A mixture of compound **4a** (9.7g, 50mmol), 1,1-dimethylthio-2-nitroethene (11.6g, 70mmol) in acetone (100mL) was refluxed for 18h. Then the resultant mixture was concentrated under reduced pressure and the residue was re-dissolved in hot ethanol (50mL). The solvent was cooled and filtered. The mother solution was concentrated under pressure and the residue was purified by column chromatography on silica gel (EtOAc-petroleum ether 2-1 v/v), and compound **5a** was obtained as a yellow solid 10.9g, yield: 70%, mp 130-132℃. ^{1}HNMR ($CDCl_3$, 300M, δppm) δ: 2.51 (s, 3H, SCH_3), 2.94 (t, 2H, C_4-H), 3.84, 3.86 (s, 6H, 2×OCH_3), 3.96 (t, 2H, C_3-H), 4.58 (s, 2H, C_1-H), 6.55 (s, 1H, C＝CH(NO_2)), 6.64, 6.72 (each s, 2H, C_5-H, C_8-H); IR (KBr, ν): 3460, 2932, 2837, 1660, 1559, 1455, 1381 cm^{-1}; MS (ESI, m/z): 311.1 (M+H, base peak).

6,7-dimethoxy-1-(3,4-dimethoxy)benzyl-2-(1-methylthio-2-nitro)vinyl-1,2,3,4-tetrahydroisoquinoline (5b)

Following the procedure described for **5a** from **4b**, compound **5b** was obtained as a yellow solid, 55%, mp 152-154℃. ^{1}HNMR ($CDCl_3$, 300M, δppm) δ: 2.50 (s, 3H, SCH_3), 2.85-3.53 (m, 6H, C_3-H, C_4-H, $ArCH_2$), 3.76-3.85 (s, 12H, 4×OCH_3), 4.71 (t, 1H, C_1-H), 6.34 (s, 1H, C＝CH(NO_2)), 6.41-7.26(m, 5H, Ar-H); IR (KBr, ν): 3444, 2951, 2933, 1592, 1540, 1514, 1463, 1450 1366 cm^{-1}; MS (ESI, m/z): 461.1 (M+H, base peak).

6,7-dimethoxy-1-phenoxymethyl-2-(1-methylthio-2-nitro)vinyl-1,2,3,4-tetrahydroisoquinoline (5c)

Following the procedure described for **5a** from **4c**, compound **5c** was obtained as a yellow solid, 50%, mp 195-197℃. ^{1}HNMR ($CDCl_3$, 300M, δppm) δ: 2.50 (s, 3H, SCH_3), 2.72-3.87 (m, 4H, C_3-H, C_4-H), 3.87 (s, 6H, 2×OCH_3), 4.17 (m, 2H, Ar-OCH_2), 4.89 (t, 1H, C_1-H), 6.52 (s, 1H, C＝CH(NO_2)), 6.63, 6.66 (each s, 2H, C_5-H, C_8-H), 6.83-7.30 (m, 5H, Ar-H); IR (KBr, ν): 3451, 3034, 2972, 2935, 2874, 1665, 1611, 1599, 1555, 1519, 1432, 1356 cm^{-1}; MS (ESI, m/z): 417.1 (M+H, base peak).

6,7-dimethoxy-1-(1-naphthylmethyl)-2-(1-methylthio-2-nitro)vinyl-1,2,3,4-tetrahydroisoquinoline (5d)

Following the procedure described for **5a** from **4d**, compounds **5d** was obtained as a yellow solid, 70%, mp 148-150℃. ^{1}HNMR ($CDCl_3$, 300M, δppm) δ: 1.96 (s, 3H, SCH_3), 2.95 (m, 2H, C_4-H), 3.43 (s, 3H, C_7-OCH_3), 3.84 (s, 3H, C_6-OCH_3), 3.66,4.08 (m, 4H, C_3-H, Ar-CH_2), 5.73 (t, 1H, C_1-H), 5.93 (s, 1H, C＝CH(NO_2)), 6.62, 6.71 (each s, 2H, C_5-H, C_8-H), 7.22-8.02 (m, 7H, Ar-H); IR (KBr, ν): 3444, 3112, 2931, 2837, 1729, 1607, 1521, 1468, 1391, 1348 cm^{-1}; MS (ESI, m/z): 451.1 (M+H, base peak).

6,7-dimethoxy-2-(1-*n*-octylamino-2-nitro)vinyl-1,2,3,4-tetrahydroisoquinoline (6a)

A mixture of compound **5a** (0.78g, 2.5mmol), *n*-octyl amine (2.6g, 20mmol) and anhydrous toluene (15mL) was stirred for 16h at 60-70℃. The resultant solvent was concentrated under pressure. The residue was purified by column chromatography on silica gel (EtOAc-petroleum ether 1-1 v/v), and compound **6a** was obtained as a white solid 0.52g, yield: 53%, mp 100-102°C. ^{1}HNMR ($CDCl_3$, 300M, δppm) δ: 0.91 (t, 3H, *J*=6.0Hz, $CH_2\underline{CH_3}$), 1.31-1.73 (m, 13H, $N\underline{H}CH_2$, $\underline{(CH_2)_6}CH_3$), 2.92 (t, 2H, *J*=6.0Hz, C_4-H), 3.37，(m, 2H, $NH\underline{CH_2}$), 3.55 (t, 2H, *J*=6.0Hz, C_3-H), 3.89, 3.90 (s, 6H, 2×OCH_3), 4.35 (s, 2H, C_1-H), 6.58 (s, 1H, C＝CH(NO_2)), 6.62, 6.67 (each s, 2H, C_5-H, C_8-H)；IR (KBr, ν): 3451, 2952, 2924, 2854, 1598, 1534, 1518, 1462, 1306 cm^{-1}; MS (ESI, m/z): 392.1 (M+H, base peak); Anal. Calcd. for $C_{21}H_{33}N_3O_4$：C 64.42, H 8.50, N 10.73; Found: C 64.45, H 8.27, N 10.86.

6,7-dimethoxy-2-(1-phenethylamino-2-nitro)vinyl-1,2,3,4-tetrahydroisoquinoline (6b)

Following the procedure (**6a**) for the condensation between **5a** and phenethylamine, the crude residue was purified by column chromatography on silica gel (EtOAc-petroleum ether 1-1 v/v), and compound **6b** was obtained as a white solid, yield: 56%, mp 150-152℃. ^{1}HNMR ($CDCl_3$, 300M, δppm) δ: ^{1}HNMR ($CDCl_3$,300M) δ：3.82-2.84 (m, 3H, C_4-H, $N\underline{H}CH_2$), 2.97 (t, 2H, $ArCH_2$), 3.43 (t, 2H, *J*=6.0Hz, C_3-H), 3.60 (m, 2H, $NH\underline{CH_2}$), 3.85 (s, 6H, 2×OCH_3), 4.21 (s, 2H, C_1-H), 6.49-6.61 (each s, 3H, C＝CH(NO_2), C_5-H, C_8-H), 7.26 (m, 5H, Ar-H)；IR (KBr, ν): 3415, 3240, 3152, 3027, 1612, 1582, 1518, 1450, 1374, 1357 cm^{-1}；MS (ESI, m/z): 384.2 (M+H, base peak); Anal. Calcd. for $C_{21}H_{25}N_3O_4$：C 65.78, H 6.57, N 10.96; Found：C 66.62, H 6.84, N 1.70.

6,7-dimethoxy-1-(3,4-dimethoxy)benzyl-2-(1-*n*-octylamino-2-nitro)vinyl-1,2,3,4-tetrahydroisoquinoline (6c)

Following the procedure (**6a**) for the condensation between **5b** and *n*-octyl amine, the crude residue was purified

by column chromatography on silica gel (EtOAc-petroleum ether 1-1 v/v), and compound **6c** was obtained as a white solid, yield: 62%, mp 156-158℃. ^{1}HNMR ($CDCl_3$, 300M, δppm) δ: 0.88 (t, 3H, *J*=6.0Hz, $(CH_2)_2\underline{CH_3}$), 1.24-1.55 (m, 15H, $\underline{(CH_2)_6}CH_3$), 2.69-3.56 (m, 8H, C_3-H, C_4-H, NH$\underline{CH_2}$, Ar$\underline{CH_2}$), 3.75-3.88 (s, 12H, 4×OCH_3), 4.70 (t, 1H, *J*=7.5Hz, C_1-H), 6.31 (s, 1H, C=CH(NO_2)), 6.41-7.26 (m, 5H, Ar-H); IR (KBr, ν): 3444, 2996, 2923, 2844, 1592, 1544, 1515, 1464, 1448, 1356 cm^{-1}; MS (ESI, m/z) : 542.3 (M+H, base peak); Anal. Calcd. for $C_{30}H_{43}N_3O_6$: C 66.52, H 8.00, N 7.76; Found: C 66.48, H 8.11, N 7.74.

6,7-dimethoxy-1-(3, 4-dimethoxy)benzyl-2-(1-phenethyl-amino-2-nitro)vinyl-1,2,3,4-tetra hydroisoquinoline (6d)

Following the procedure (**6a**) for the condensation between **5b** and phenethylamine, the crude residue was purified by column chromatography on silica gel (EtOAc-petroleum ether 1-1 v/v), and compound **6d** was obtained as a white solid, yield: 65%, mp 180-181℃. ^{1}HNMR ($CDCl_3$, 300M, δppm) δ: 2.64-3.49 (m, 10H, C_3-H, C_4-H, NH$\underline{CH_2}$, $2ArCH_2$), 3.73-3.85 (s, 12H, 4×OCH_3), 4.65 (t, 1H, *J*=7.5Hz, C_1-H), 6.26 (s, 1H, C=CH(NO_2)), 6.26, 6.37 (each s, 2H, C_5-H, C_8-H), 6.57-7.33 (m, 8H, Ar-H); IR (KBr, ν): 3436,2996, 2924, 2830, 1590, 1514, 1498, 1465, 1451, 1352 cm^{-1}; MS (ESI, m/z): 534.2 (M+H, base peak); Anal. Calcd. for $C_{30}H_{35}N_3O_6$: C 67.52, H 6.61, N 7.87; Found: C 67.30, H 6.77, N 7.92.

6,7-dimethoxy-1-phenoxymethyl-2-(1-*n*-octylamino-2-nitro)vinyl-1,2,3,4-tetrahydroisoquinoline (6e)

Following the procedure (**6a**) for the condensation between **5c** and *n*-octyl amine, the crude residue was purified by column chromatography on silica gel (EtOAc-petroleum ether 1-1 v/v), and compound **6e** was obtained as a white solid, yield: 60%, mp 112-114℃. ^{1}HNMR ($CDCl_3$, 300M, δppm) δ: 0.83 (t, 3H, *J*=6.0Hz, $CH_2\underline{CH_3}$), 1.24-1.68 (m, 12H, $\underline{(CH_2)_6}CH_3$), 2.71-2.77(m. 2H, NH$\underline{CH_2}$), 3.29-3.33(dd, 2H, *J*=13.2Hz, *J*=6.7Hz, C_4-H), 3.59-3.62 (t, 2H, *J*=5.8Hz, C_3-H), 3.87 (s, 6H),3.87 (s, 6H, 2×OCH_3), 4.17 (dd, 2H, *J*=18.0Hz, *J*=9.0Hz, Ar-OCH_2), 4.89 (t, 1H, *J*=9.0Hz, C_1-H), 6.52 (s, 1H, C=CH(NO_2)), 6.62, 6.65 (each s, 2H, C_5-H, C_8-H), 6.81-7.29 (m, 5H, Ar-H); IR (KBr, ν): 3429, 2987, 2922, 2853, 1599, 2587, 1536, 1516, 1432, 1358 cm^{-1}; MS (ESI, m/z): 498.1 (M+H, base peak); Anal. Calcd. for $C_{28}H_{39}N_3O_5$: C 67.58, H 7.90, N 8.44; Found: C 67.63, H 7.98, N 8.41.

6,7-dimethoxy-1-phenoxymethyl-2-(1-phenethylamino-2-nitro)vinyl-1,2,3,4-tetrahydroisoquinoline (6f)

Following the procedure (**6a**) for the condensation between **5c** and phenethylamine, the crude residue was purified by column chromatography on silica gel (EtOAc-petroleum ether 1-1 v/v), and compound **6f** was obtained as a white solid, yield: 65%, mp 180-181℃. ^{1}HNMR ($CDCl_3$, 300M, δppm) δ: 2.35-2.97 (m, 8H, C_3-H, C_4-H, $ArCH_2$ NH$\underline{CH_2}$), 3.87 (s, 6H, 2×OCH_3), 4.11 (dd, 2H, *J*=18.0Hz, *J*=9.1Hz, Ar-OCH_2), 4.76 (t, 1H, *J*=9.1Hz, C_1-H), 6.48 (s, 1H, C=CH(NO_2)), 6.58, 6.57 (each s, 2H, C_5-H, C_8-H), 6.79-7.29 (m, 10H, Ar-H); IR (KBr, ν): 3465, 3126, 2916, 2830, 1594, 1536, 1518, 1466, 1433, 1355 cm^{-1}; MS (ESI, m/z): 490.2 (M+H, base peak); Anal.Calcd. for $C_{28}H_{31}N_3O_5$: C 68.69, H 6.38, N 8.58; Found: C, 68.55, H 6.46, N 8.54.

6,7-dimethoxy-1-(α-naphthylmethyl)-2-(1-*n*-butylamino-2-nitro)vinyl-1,2,3,4-tetrahydroisoquinoline (6g)

Following the procedure (**6a**) for the condensation between **5d** and *n*-butylamine, the crude residue was purified by column chromatography on silica gel (EtOAc-petroleum ether 1-1 v/v), and compound **6g** was obtained as a white solid, yield: 60%, mp 221-223℃. ^{1}HNMR ($CDCl_3$, 300M, δppm) δ: 0.83 (t, 3H, *J*=6.0Hz, $(CH_2)_2\underline{CH_3}$), 1.12-1.35 (m, 4H, $\underline{(CH_2)_2}CH_3$), 1.58 (d, 1H, *J*=6.0Hz, N$\underline{H}$CH), 2.76-3.00 (m, 4H, C_4-H, NH$\underline{CH_2}$), 3.51-3.75 (m, 4H, C_3-H, Ar'CH_2,), 3.53 (s, 3H, C_7-OCH_3), 3.86 (s, 3H, C_6-OCH_3), 4.87 (t, 1H, *J*=7.5Hz, C_1-H), 6.03 (s, 1H, C=CH(NO_2)), 6.37, 6.61 (each s, 2H, C_5-H, C_8-H), 7.17-7.96(m, 7H, Ar-H); IR (KBr, ν): 3451, 2957, 2933, 2866, 1588, 1518, 1464, 1452, 1352 cm^{-1}; MS (ESI, m/z): 476.3 (M+H, base peak); Anal.Calcd. for $C_{28}H_{33}N_3O_4 \cdot 0.3H_2O$: C 69.92, H, 7.04, N 8.74; Found: C 70.17, H 7.01, N 8.75.

6,7-dimethoxy-1-(α-naphthylmethyl)-2-(1-(3,4-dimethoxy)-phenethylamino-2-nitro)vinyl-1,2,3,4-tetrahydroisoquinoline (6h)

Following the procedure (**6a**) for the condensation between **5d** and 3,4-dimethoxyphenethylamine, the crude residue was purified by column chromatography on silica gel (EtOAc-petroleum ether 1-1 v/v), and compound **6h** was obtained as a white solid, yield: 60%, mp 221-223℃. ^{1}HNMR ($CDCl_3$, 300M, δppm) δ: 2.57-2.73 (m, 4H, C_4-H, Naphthyl-CH_2), 3.16-3.70 (m, 6H, C_3-H, NH$\underline{CH_2}$, Ar'CH_2,), 3.49, 3.83 (s, 12H, 4×OCH_3), 4.81 (t, 1H, *J*=7.5Hz, C_1-H), 5.86 (s, 1H, C=CH(NO_2)), 6.34, 6.58 (each s, 2H, C_5-H, C_8-H), 6.60-7.88(m, 10H, Ar-H); IR (KBr, ν): 3429, 2924, 2830, 1607, 1589, 1515, 1462, 1353 cm^{-1}; MS (ESI, m/z): 584.3 (M+H, base peak); Anal. Calcd. for $C_{34}H_{37}N_3O_6$: C 69.96, H 6.39, N 7.20; Found: C 69.97, H 6.41, N 7.17.

6,7-dimethoxy-2-(N-nitro)guanyl-1,2,3,4-tetrahydroisoquinoline (7a)

A mixture of **5a** (0.97g, 5.0mmol), anhydrous ethanol (30mL) and S-methyl-N-nitroisothiourea (0.68g, 5.0mmol) was refluxed for 12h. The mixture was cooled to room temperature and stood overnight. Then compound **7a** was collected, washed with cooled anhydrous ethanol and obtained a white, crystalline powder, 0.77g, yield: 55%, mp 145-147℃. ^{1}HNMR ($CDCl_3$, 300M, δppm) δ: 2.88 (t, 2H, *J*=6.0Hz, C_4-H), 3.75 (t, 2H, *J*=6.0Hz, C_3-H), 3.85 (s, 6H, 2×OCH_3), 4.64 (s, 2H, C_1-H), 6.67(s, 2H, Ar-H), 7.86 (s, 2H, NH_2); IR (KBr, ν): 3380, 3281, 3198, 2953, 2830, 1744, 1602, 1577, 1519, 1484, 1467, 1380, 1300 cm^{-1}; MS (ESI, m/z): 281.1 (M+H, base peak); Anal. Calcd. for $C_{12}H_{16}N_4O_4$: C 51.42, H 5.75, N 19.99; Found: C 51.49, H 5.65, N 20.06.

6,7-dimethoxy-1-(3,4-dimethoxy)benzyl-2-(N-nitro)guanyl-1,2,3,4-tetrahydroisoquinoline (7b)

A mixture of **5b** (1.7g, 5.1mmol), anhydrous ethanol (30mL) and S-methyl-N-nitroisothiourea (0.68g, 5.0mmol) was refluxed for 48h. The resultant solvent was concentrated under pressure. The residue was purified by column chroma-

tography on silica gel (EtOAc-petroleum ether 1-2 v/v), and compound **7b** was obtained as a white solid 1.2g, yield: 53%, mp 151-152℃. ^{1}HNMR ($CDCl_3$, 300M, δppm) δ: 2.67-3.51 (m, 6H, C_3-H, C_4-H, $ArCH_2$), 3.73, 3.85 (s, 12H, 4×OCH_3), 5.25 (t, 1H, *J*=7.5Hz, C_1-H), 6.37-6.77(m, 5H, Ar-H), 7.32 (s, 2H, NH_2); IR (KBr, ν): 3429, 3310, 2993, 2958, 2937, 2830, 1740, 1615, 1564, 1517, 1446, 1382, 1296 cm^{-1}; MS (ESI, m/z) : 431.1 (M+H, base peak); Anal. Calcd. for $C_{21}H_{26}N_4O_6$: C 58.59, H 6.09, N 13.02; Found: C 58.19, H 6.04, N 12.70.

6,7-dimethoxy-1-phenoxymethyl-2-(N-nitro)guanyl-1,2,3,4-tetrahydroisoquinoline (7c)

Following the procedure (**7b**) for the condensation between **5c** and S-methyl-N-nitroisothiourea, the crude residue was purified by column chromatography on silica gel (EtOAc-petroleum ether 1-2 v/v), and compound **7c** was obtained as a white solid, yield: 53%, mp 122-124℃. ^{1}HNMR ($CDCl_3$, 300M, δppm) δ: 2.80-3.46 (m, 4H, C_3-H, C_4-H), 3.86 (s, 6H, 2×OCH_3), 4.25-4.37 (m, 3H, C_1-H, Ar-OCH_2), 6.67, 6.69 (each s, 2H, C_5-H, C_8-H), 6.87-7.32 (m, 5H, Ar-H), 7.99 (s, 2H, NH_2); IR (KBr, ν): 3373, 3274, 3198, 2966, 2935, 2837, 1747, 1615,1599, 1550, 1516, 1448, 1462, 1394, 1372 cm^{-1}; MS (ESI, m/z) : 387.1 (M+H, base peak); Anal.Calcd. for $C_{19}H_{22}N_4O_5$: C 59.06, H 5.74, N 14.50; Found: C 58.96,H 5.69, N 14.25.

6,7-dimethoxy-1-(α-naphthylmethyl)-2-(N-nitro)guanyl-1,2,3,4-tetrahydroisoquinoline (7d)

Following the procedure (**7b**) for the condensation between **5d** and S-methyl-N-nitroisothiourea, the crude residue was purified by column chromatography on silica gel (EtOAc-petroleum ether 1-2 v/v), and compound **7d** was obtained as a white solid, yield: 59%, mp 192-194℃. ^{1}HNMR ($CDCl_3$, 300M, δppm) δ: 2.93 (t, 2H, *J*=6.5Hz, C_4-H), 2.95-3.66 (m, 4H, C_3-H, $ArCH_2$), 3.79, 3.86 (s, 6H, 2×OCH_3), 5.43 (t, 1H, *J*=7.5Hz, C_1-H,), 6.64, 6.90 (each s, 2H, C_5-H, C_8-H), 7.06-7.87 (m, 7H, Ar-H), 8.49 (s, 2H, NH_2); IR (KBr, ν): 3395, 3306, 2953, 2830, 1740, 1614, 1548, 1518, 1479, 1452, 1396, 1293 cm^{-1}; MS (ESI, m/z) : 421.1 (M+H, base peak); Anal. Calcd. for $C_{23}H_{24}N_4O_4$: C 65.70, H 5.75, N 13.33; Found: C 65.35, H 5.70, N 13.04.

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