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Graphical abstract

Synthesis and study of new paramagnetic and diamagnetic verapamil derivatives

pp xxx–xxx

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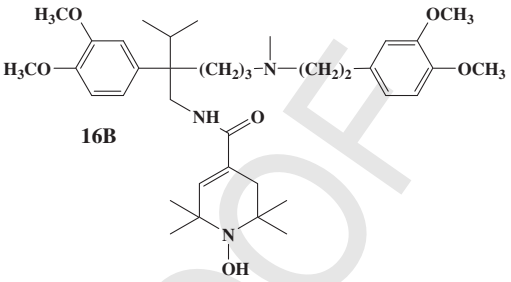

16B

New derivatives of verapamil modified with nitroxides and their precursors were synthesized and screened for reactive oxygen species (ROS)-scavenging activities. Among the new verapamil derivatives compound **16B** proved to be the best ROS scavenger in vitro.

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Synthesis and study of new paramagnetic and diamagnetic verapamil derivatives

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ABSTRACT

New derivatives of verapamil (**1**) modified with nitroxides and their precursors were synthesized and screened for reactive oxygen species (ROS)-scavenging activities. The basic structure was modified by changing the nitrile group to an amide or the methyl substituent on tertiary nitrogen with nitroxides and their reduced forms (hydroxylamine and secondary amines). Among the new verapamil derivatives compound **16B** [Mohan, I. K.; Kahn, M.; Wisel, S.; Selvendiran, K.; Sridhar, A.; Carnes, C.A.; Bognár, B.; Kálai, T.; Hideg, K.; Kuppusamy, P. *Am. J. Physiol. Heart Circ. Physiol.* **2009**, *296*, 140], modified with hydroxylamine salt of 2,2,6,6-tetramethyl-1,2,3,6-tetrahydropyridine-1-yloxyl proved to be the best ROS scavenger in vitro and protected HSMC and CHO cells against H_2O_2 induced damage.

1. Introduction

The five- and six-membered nitrogen heterocyclic nitroxides and their diamagnetic derivatives (sterically hindered amine and hydroxylamine) are known as low-molecular-weight nonenzymatic multifunctional antioxidants as they can participate in one-electron redox processes. Nitroxides have attenuated oxidative damage in various experimental models, such as cultured cells,[2] brain injury,[3] lipid peroxidation in liver microsomes,[4] post-ischemic reperfusion injury in isolated organs,[5] and ionizing irradiation of rats and mice.[6,7] This antioxidant activity is attributed to scavenging of oxygen-, nitrogen-, and carbon-centered radicals,[8,9] and oxidation of reduced transition metal ions (such as Fe^{2+}) pre-empting their participation in Fenton-reaction.[10] The sterically hindered secondary amines oxidized to non-toxic nitroxides by ROS[11] and hydroxylamines can act as a proton-donor antioxidants.[9]

Recently we have synthesized and investigated several biologically active molecules modified with nitroxides and their amine precursors, which showed antioxidant activity owing to the possible scavenging of various free radicals which cause deleterious biological processes such as oxidative stress.[12] The paramagnetic analogues of ebselen,[13] amiodarone,[14,15] trimetazidine[16,17] and mexiletine[5] retained their essential activity and they exhibited increased antioxidant activity.[14,17,18] These studies suggested, that nitroxides and their precursors' antioxidant activity can be exploited when the nitroxide is attached to a drug molecule, for example, this allows the binding of a nitroxide to a certain receptor or its accumulation in a membrane to scavenge the generated free radicals *in statu nascendi*. The superoxide-scavenging is a representative example of how doxorubicin-induced cardiomyopathy was prevented by a pyrroline ring containing experimental drug (H-2545) in a rat heart model.[19]

Verapamil, a known calcium-channel blocker, is used for the treatment of hypertension, angina pectoris, arrhythmia and an effective multi-drug resistance (MDR) reversing agent.[20] Several new verapamil analogues were synthesized and tested[21–27] and these studies concluded that both aromatic ring, nitrile group attached to a quaternary carbon and tertiary amine are important, while isopropyl group and substituents of the aminoethyl aromatic ring are less important. The substituents on the aromatic ring with benzyl cyanide group on carbon influences the calcium antagonistic activity. Therefore, verapamil seemed to be a well established model for modification with nitroxides and their precursors not only as calcium-channel antagonist acting on the L-type channels[28] but as an MDR reversing agent. It was also elucidated that *S*-verapamil is 10-fold more potent calcium antagonist than *R*-verapamil, while this enantioselectivity was not observed in case of MDR

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reversing activity.[29,30] It is interesting to note that Omote and Al-Shawi used a spin-labeled verapamil to study the transport mechanism of P-glycoprotein, which mediates multi-drug resistance through its ability to transport various hydrophobic compounds across the plasma membrane.[31] We now report modification of the racemic calcium antagonist verapamil (**1**) with nitroxides and their precursors on tertiary amine and on primary amine moiety; the latter was achieved by reduction of nitrile group. The new molecules might be important both for structure-cardiovascular activity relationship studies and MDR-reversing investigations, although in this paper only the former topic is outlined.

2. Chemistry

To achieve new verapamil (**1**) derivatives the nitrile group of the parent compound was reduced to primary amine with Red-Al in THF to yield compound **2**[32] which was then acylated with **3–8** paramagnetic acid chlorides[33] in CH_2Cl_2 in the presence of Et_3N to afford **11C–16C** nitroxide amides. Instead of unstable paramagnetic acid chlorides paramagnetic acids (**9, 10**) activated with DCC were used in synthesis of amides **17C–18C.** The more water soluble hydroxylamines **11B–18B** were prepared by refluxing **11C–18C** with EtOH saturated previously with HCl gas[9] and reduction of **11C–18C** with iron powder in glacial acetic acid[34] yielded **11A–18A**. For further structure–activity relationship studies, the basicity of side chain was treasured. Therefore the primary amino group of compound **2** was alkylated with **19** paramagnetic allylic bromide[35] in $CHCl_3$ in the presence of K_2CO_3 to yield compound **20C** which was converted to the more water soluble **20A** and **20B** derivatives as described above (Scheme 1).

An evident procedure for getting further new verapamil derivatives was the alkylation of nor-verapamil (**21**) on the secondary nitrogen atom with allylic bromide **19** and with its six-membered analogue (**22**)[36] in acetonitrile in the presence of K_2CO_3 to afford **23C** and **24C.** These derivatives were further reduced to **23B** and **24B** hydroxylamines by refluxing nitroxides in ethanolic HCl solution and to **23A** and **24A** amines by treatment of nitroxides with iron powder and glacial acetic acid. Methylating the tertiary nitrogen atom of compound **23C** and **24C** with methyl iodide, using methyl iodide as a solvent, in a sealed tube under pressure afforded the quaternary salts **25** and **26** as water-soluble compounds with an intact (non-reduced) nitroxide function (Scheme 2). Carboxylic acids (**29**, **30**) were prepared by oxidation of aldehydes (**27**, **28**) with $H_2O_2/NaClO_2$ system in a buffer solution (Scheme 3).

3. Results and discussion

The compounds synthesized were screened for superoxide and peroxyl radical-scavenging activity and protection of human aortic smooth muscle HSMC and Chinese hamster ovary CHO cells against H_2O_2-mediated cytotoxicity.

The protective effect of verapamil against oxygen free radical damage is well documented. Verapamil preserved myocite viability during exposure to hypoxantine and xantine oxidase.[37]

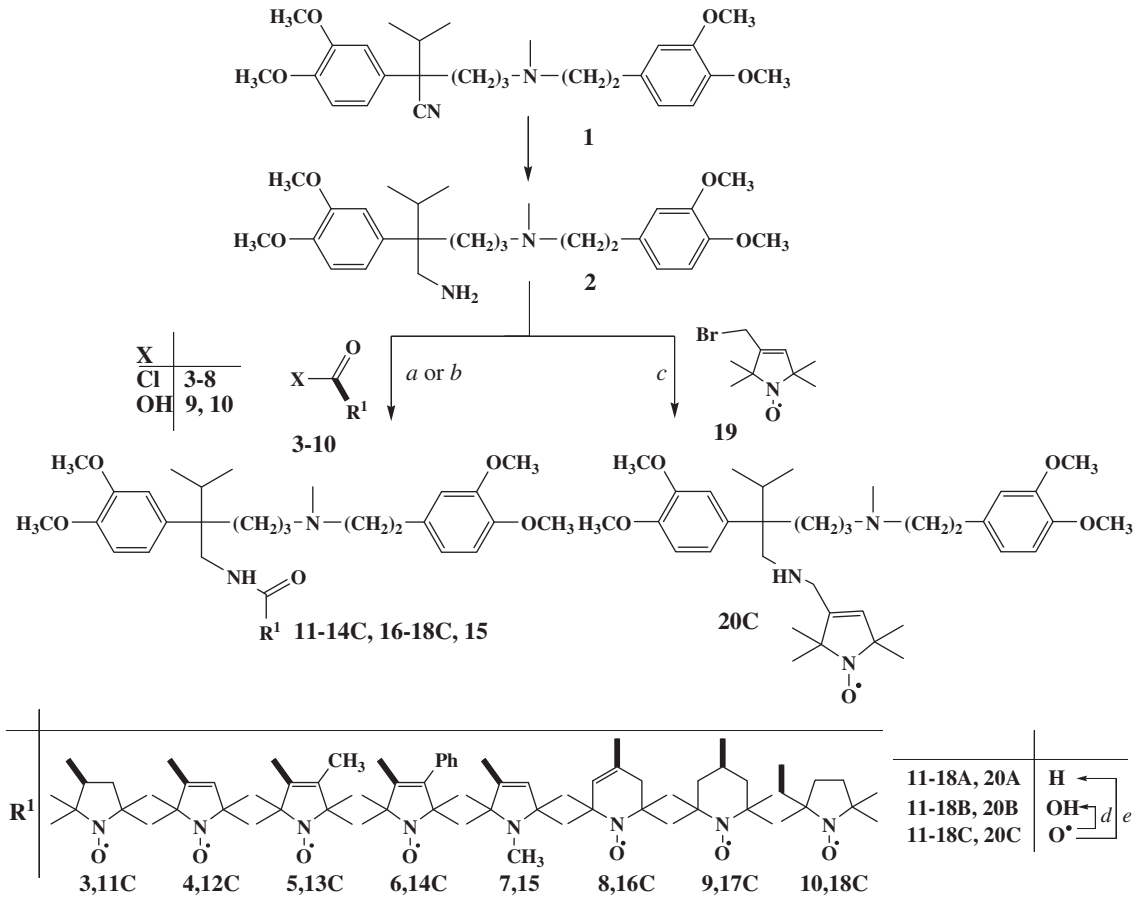
Recently Kovács et al. reported that in a Langendorff-perfused heart model experiment during ischemia and reperfusion verapamil decreased oxidative damage attributed to activation of Akt and ERK1/2 pathways, hence significantly contribute to cardioprotective effects.[38] Villari et al. reported the beneficial effect of Ca^{2+} antagonists to decreased myocardial demand during ischemia.[39]

Verapamil does not exhibit any superoxide-scavenging capability while all the other derivatives exhibit such ability. The primary amine acylated with pyrroline nitroxide **12A** and **12B** exhibits better protection than compound **1**, however the *N*-hydroxylamine (**12B**) proved to be a better superoxide scavenger and was more effective than sterically hindered amine (**12A**); therefore, mainly the hydroxylamines were prepared and tested (Table 1). Among the acylated derivatives containing a saturated five-membered ring **11B,** the six-membered rings **(16B, 17B),** 2-substituted saturated ring (**18B**) and unsaturated ring with 4-methyl or 4-phenyl groups (**13B, 14B**) have proven better superoxide scavenger than **12B.** When *N*-hydroxylamine function is blocked as in case of compound **15** the superoxide-scavenging activity is decreased. When primary amine group is alkylated with five-membered ring (**20A** and **20B**) the superoxide-scavenging activity is better than **12A** or **12B** amide analogues. The derivatives where the tertiary amine of verapamil is modified with five- or six-membered nitroxide reduced form (*N*-hydroxylamine, **23B** and **24B**) are better superoxide scavengers than the amine derivatives (**23A** and **24A**), while the quaternary salts such as compounds **25** and **26** with nitroxide function exhibit more limited superoxide-scavenging capability than compounds **23B** or **24B** (Tables 2 and 3).

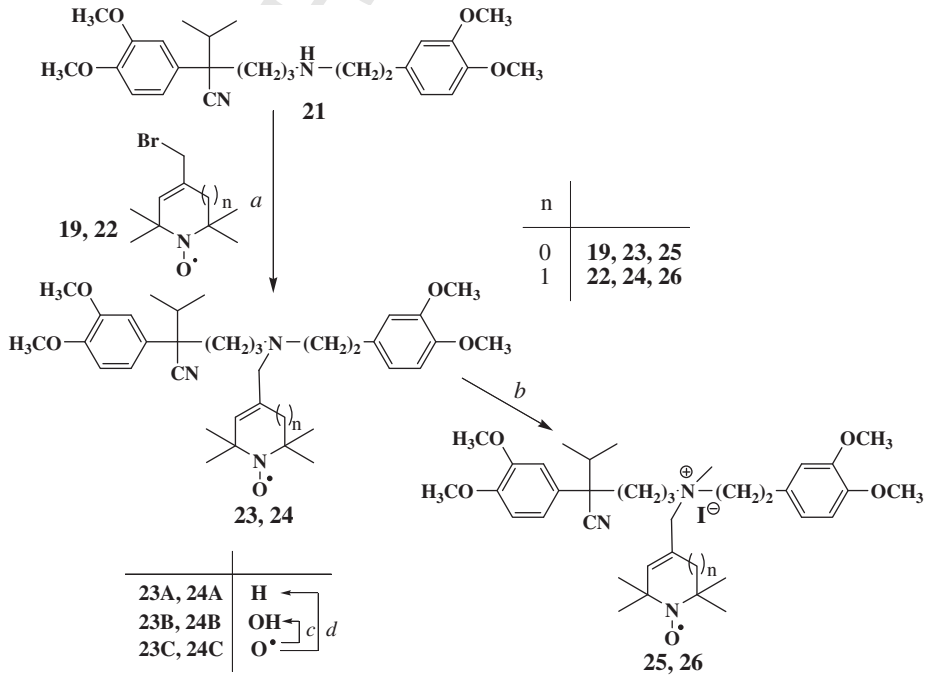
A similar tendency was observed for peroxyl radical-scavenging except that verapamil itself can scavenge peroxyl radicals up to 60% while the quaternary salts **25** and **26** and unexpectedly **12B** are worse scavengers than verapamil itself. The superoxide-scavenging feature of hydroxylamines can be interpreted easily as hydroxylamines are spontaneously deprotonated in a buffer solution and oxidized back to nitroxide. Then nitroxide can dismutate superoxide via oxidation to oxoammonium cation (Fig. 1). This ability is highly influenced by the redox potential depending on the substituent of the nitroxide rings. The in vitro results of superoxide-scavenging capability are in good accordance that saturated five-membered rings (such **11B, 18B**), six-membered rings (such **16B, 17B**) has lower redox potential[40] and an electron-donating group on nitroxide ring also decreases the redox potential[41,42] (**13B, 14B, 20A, 20B, 23A, 23B, 24A, 24B**), however when an electron-withdrawing group is attached—such as quaternary salt with permanent positive charge in case of compounds **25** and **26**—results in higher redox potential and hence worse capability of superoxide scavenging. The superoxide-scavenging abilities of new compounds were lowered by 20–30% by decreasing the concentration of verapamil derivatives from 1 mM to 100 μM, and 10–20% decrease were resulted in peroxyl-scavenging abilities. The competitive reaction with DEPMPO shows that the verapamil and derivatives are in general comparable or even better scavengers of peroxyl radicals than superoxide. This may indicate that the verapamils have a relatively higher reactivity toward peroxyl radicals when compared to superoxide in competing with DEPEMPO. However, a more rigorous evaluation of the reaction kinetics is required for further understanding of the contributing factors to the observed differences in the scavenging abilities.

At first glance this would inspire to accomplish the synthesis only leading to six-membered rings with amine function only, however in case of amines the in vivo protonation and toxicity also should be considered. When comparing the cell-viability data in CHO cells and in vitro superoxide-scavenging capability, an obvious relationship can not be drawn. Compounds **20A, 23A, 24A** behave as sensitizers and worse than the protection of verapamil, while *N*-hydroxy compounds (**16B, 17B, 12B**) and quaternary salt **25** exhibit good protection. HSMC cells are protected by quaternary salts **25**, **26** *N*-hydroxylamines **13B, 14B, 20B, 16B, 17B** and **20A** sterically hindered amine. In the protection HSMC cells against H_2O_2-mediated toxicity, only compounds **23A** and **24A** behave as sensitizers, for example, worse than verapamil.

Based on these results, compound **16B** was chosen as the lead compound which exhibited fairly good superoxide and peroxyl radical scavenging and cell-protection ability. The compound **16B** was recently demonstrated to protect hearts against ischemia-reperfusion (I/R) injury in a Langendorf model. Hearts were subjected to 30 min of global ischemia followed by 45 min of reperfusion. Coro-



Scheme 1. Reagents and conditions: (a) **3–8** (1.1 equiv), Et_3N (1.1 equiv), CH_2Cl_2 0 °C→rt, 1 h; (b) **9, 10** DCC (1.0 equiv), 4-dimethylaminopyridin (0.05 equiv), rt, 24 h; (c) **19** (1.1 equiv), K_2CO_3 (1.1 equiv), 18-crown-6 (cat.), $CHCl_3$, reflux, 8 h; (d) EtOH/HCl, reflux, 20 min; (e) Fe (10 equiv), AcOH, 70 °C, 1 h, then K_2CO_3 to pH 9.



Scheme 2. Reagents and conditions: (a) **19** or **22** (1.1 equiv), K_2CO_3 (1.1 equiv), 18-crown-6 (cat.), acetonitrile, reflux, 3–8 h; (b) **23** or **24,** MeI (5.0 equiv), 50 °C, 48 h; (c) EtOH/HCl, reflux, 20 min; (d) Fe (10 equiv), AcOH, 70 °C, 1 h, then K_2CO_3 to pH 9.

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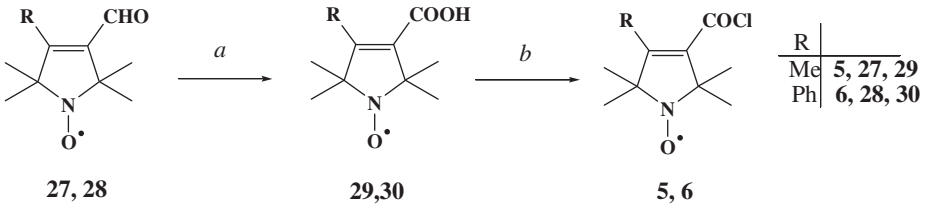


Scheme 3. Reagents and conditions: (a) $NaClO_2$ (1.1 equiv), H_2O_2 (0.9 equiv), H_2O–MeCN, KH_2PO_4, 0 °C, 1 h, rt, then $Na_2S_2O_5$, H^+; (b) $SOCl_2$ (1.23 equiv), pyridine (1.25 equiv), benzene, 0 °C, 10 min, then rt, 40 min.

nary flow (CF), (left ventricular developed pressure (LVDP), and rate pressure product (RPP) were continuously monitored before the induction of global ischemia and during the reperfusion. verapamil or **16B** was administered to the heart via a side arm infusion for 1 min before the onset of ischemia. While hearts pre-treated with the verapamil showed a significant improvement in the recovery of contractile functions compared with the I/R hearts, hearts treated with **16B** showed a significantly better recovery in cardiac function compared to verapamil alone.[1] Similar studies performed using an in vivo model of I/R injury showed that **16B** markedly attenuated superoxide production, increased nitric oxide generation, and enhanced Akt and Bcl-2 levels in the reperfused myocardium.

4. Conclusions

New verapamil derivatives were synthesized by modification on nitrile group and on tertiary nitrogen. The new compounds were tested on superoxide radical and peroxyl radical-scavenging and cell protection assays. Among the synthesized compounds, **16B** compound modified on nitrile group with tetrahydropyridine ring was chosen as lead compound. Overall, the results demonstrated that **16B** significantly protected hearts against I/R-induced cardiac dysfunction and damage through the combined beneficial actions of calcium-channel blocking, antioxidant, and prosurvival signaling activities.

5. Experimental

Melting points were determined with a Boethius micro melting point apparatus and are uncorrected. Elemental analyses (C, H, N, S) were performed on Fisons EA 1110 CHNS elemental analyzer. Mass spectra were recorded on a Thermoquest Automass Multi and VG TRIO-2 instruments in the EI mode and ESI-TOF MS measurements were performed with a BioTOF II instrument (Bruker Daltonics, Billerica, MA). ^1H NMR spectra were recorded with Varian ^{UNITY}*INOVA* 400 WB spectrometer. Chemical shifts are referenced to Me_4Si. Measurements were run at 298 K probe temperature in $CDCl_3$ solution. ESR spectra were taken on Miniscope MS 200 in 10^{-4} M $CHCl_3$ solution and all monoradicals gave triplet line, a_N = 14.7–16.4 G. Flash column chromatography was performed on Merck Kieselgel 60 (0.040–0.063 mm). Qualitative TLC was carried out on commercially prepared plates (20 × 20 × 0.02 cm) coated with Merck Kieselgel GF_{254}. Compounds **2**,[32] **4**,[33] **9**,[43] **10**,[44] **19**,[35] **22**[36] were prepared according to published procedures. Acid chlorides **3, 5, 6, 7, 8** were prepared from the corresponding carboxylic acids[33,36] analogously for the preparation of compound **4** and used immediately in the acylation step without isolation. Compounds **1, 21** and all other reagents were purchased from Aldrich and Sigma or received as a kind donation of Sanofi-Aventis (Budapest, Hungary).

5.1. Acylation of compound 2 with acid chlorides, general procedure (11–16C)

To a solution of compound **2** (917 mg, 2.0 mmol) and Et_3N (222 mg, 2.2 mmol) in CH_2Cl_2 (30 mL) **3–8** acid chlorides (2.22 mmol) dissolved in CH_2Cl_2 (5 mL) were added dropwise at 0 °C. After stirring at rt for 1 h, the solvent was washed with brine (10 mL), the organic phase was separated, dried ($MgSO_4$), filtered and evaporated. The residue was purified by flash column chromatography (hexane/EtOAc) to give the title compounds in 50–69%.

5.1.1. 1-Oxyl-2,2,5,5-tetramethyl-pyrrolidine-3-carboxylic acid-[(2-(3,4-dimethoxyphenyl)-5-[[2-(3,4-dimethoxyphenyl) ethyl]methylamino]-2-isopropyl-pentylamide radical (11C)

Yield 664 mg 53%; brown oil. MS (EI) *m/z* (%): 626 (M^+, 9), 475(16), 594(2), 151(100). Anal. Calcd for $C_{36}H_{56}N_3O_6$: C, 68.98; H, 9.00; N, 6.70. Found: C, 69.18; H, 8.90; N, 6.66.

5.1.2. 1-Oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-carboxylic acid-[(2-(3,4-dimethoxy-phenyl)-5-[[2-(3,4-dimethoxyphenyl)ethyl]methylamino]-2-isopropyl-pentylamide radical (12C)

Yield 850 mg 68%; pale-brown oil. MS (EI) *m/z* (%): 624 (M^+, 3), 594(10), 458(59), 151(100). Anal. Calcd for $C_{36}H_{54}N_3O_6$: C, 69.20; H, 8.71; N, 6.73. Found: C, 69.16;H, 8.80; N, 6.68.

5.1.3. 1-Oxyl-2,2,4,5,5-pentamethyl-2,5-dihydro-1*H*-pyrrol-3-carboxylic acid-[(2-(3,4-dimethoxyphenyl)-5-[[2-(3,4-dimethoxyphenyl)ethyl]methylamino]-2-isopropyl-pentylamide radical (13C)

Yield 690 mg 54%; brownish-yellow oil. MS (EI) *m/z* (%): 638 (M^+, 1), 487(9), 303(54), 151(66), 43(100). Anal. Calcd for $C_{37}H_{56}N_3O_6$: C, 69.56; H, 8.84; N, 6.58. Found: C, 69.55;H, 8.61; N, 6.51

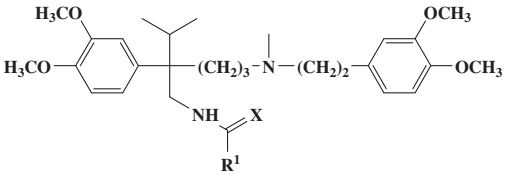
5.1.4. 1-Oxyl-2,2,5,5-tetramethyl-4-phenyl-2,5-dihydro-1*H*-pyrrol-3-carboxylic acid-[(2-(3,4-dimethoxyphenyl)-5-[[2-(3,4-dimethoxyphenyl)ethyl]methylamino]-2-isopropyl-pentylamide radical (14C)

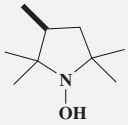
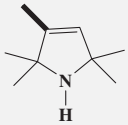
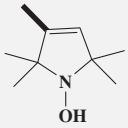
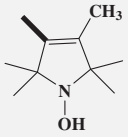
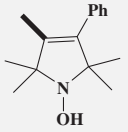
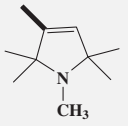
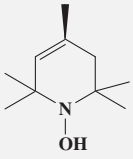
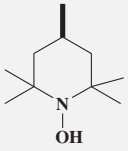
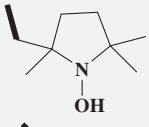
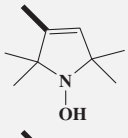
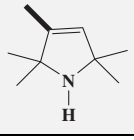
Yield 700 mg 50%; brown solid; mp 75–77 °C. MS (ESI): 701 $[M+1]^+$. Anal. Calcd for $C_{42}H_{58}N_3O_6$: C, 71.97; H, 8.34; N, 5.99. Found: C,71.89; H, 8.11; N, 5.71.

5.1.5. 1,2,2,5,5-Pentamethyl-2,5-dihydro-1*H*-pyrrol-3-carboxylic acid-[(2-(3,4-dimethoxy-phenyl)-5-[[2-(3,4-dimethoxyphenyl)ethyl]methylamino]-2-isopropyl-pentylamide (15)

Yield 711 mg 57%; orange oil. MS (EI) *m/z* (%): 623 (M^+, <1), 608(1), 472(20), 151(45), 43(100). Anal. Calcd for $C_{37}H_{57}N_3O_5$: C, 71.23; H, 9.21; N, 6.74. Found: C, 71.25; H, 8.90; N, 6.63. ^1H NMR (D_2O): 6.91–6.87 (d, ArH, 2H); 6.85–6.79 (d, ArH, 2H); 6.70 (d, ArH, 2H); 6.16 (s, CH, 1H); 3.75, 3.74, 3.73, 3.71 (4s, $4OCH_3$, 12H); 3.65–3.55 (t, NCH_2, 2H); 3.14–3.0 (m, $2NCH_2$, 4H); 2.85–2.80 (t, CH_2, 2H); 2.79 (s, NCH_3, 3H); 2.5 (s, NCH_3, 3H); 2.0–1.85 (m, CH_2, 2H); 1.82–1.70 (t, CH_2, 2H); 1.50, 1.43, 1.38, 1.31 (4s, $4CH_3$, 12H); 1.25–1.19 (m, CH, 1H); 0.73 (d, J = 6,6 Hz, CH_3, 3H); 0.70 (d, J = 6,2 Hz, CH_3, 3H).

Table 1
Biological activity of new verapamil derivatives



Compound	R^1	X	Superoxide scavenging (%)	Peroxyl scavenging (%)	Cytotoxicity CHO (%)	Cytotoxicity HSMC (%)
1 Verapamil	—	—	2.1 ± 4.2[a]	60.0 ± 3.5[a]	61.03 ± 6.2	55.95 ± 7.87
11B HO-4149		O	100[a] 84.2 ± 4.4[b]	100[a] 98.0 ± 0.5[b]	62.1 ± 9.1	40.2 ± 3.8
12A H-3009		O	34.9 ± 2.5[a]	84.1 ± 3.8[a]	53.5 ± 11.0	53.7 ± 4.5
12B H-3010		O	55.2 ± 3.2[a]	14.9 ± 17.8[a]	48.9 ± 4.8	59.1 ± 4.5
13B HO-4148		O	100[a] 78.2 ± 4.9[b]	100[a] 100[b]	60.0 ± 3.6	39.7 ± 4.2
14B HO-4099		O	100[a] 79.3 ± 1.2[b]	100[a] 86.1 ± 0.1[b]	68.3 ± 7.4	47.5 ± 3.0
15 HO-4087		O	60.7 ± 0.2[a]	89.8 ± 1.8[a]	55.5 ± 8.0	48.7 ± 5.2
16B HO-4038		O	100[a] 66.5 ± 1.2[b]	100[a] 91.4 ± 0.3[b]	46.1 ± 12.4	33.1 ± 6.9
17B HO-4218		O	100[a] 76.5 ± 4.3[b]	90.1 ± 3.4[a]	50.3 ± 10.4	40.2 ± 3.5
18B HO-4152		O	100[a] 70.7 ± 4.3[b]	100[a] 89.2 ± 0.1[b]	54.0 ± 6.4	44.0 ± 6.2
20B HO-3886		H_2	100[a] 88.5 ± 4.2[b]	100[a] 92.9 ± 1.0[b]	63.6 ± 6.5	43.3 ± 7.3
20A HO-3887		H_2	100[a] 73.8 ± 1.0[b]	100[a] 89.8 ± 3.9[b]	71.2 ± 4.1	39.1 ± 4.6

[a] Measured using 1-mM compound in presence of 1-mM DEPMPO.
[b] Measured using 100-μM compound in presence of 1-mM DEPMPO.

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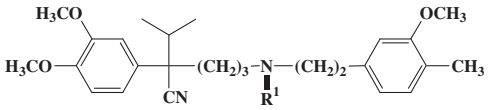
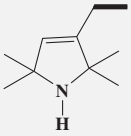
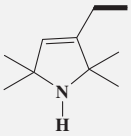
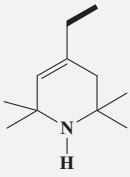
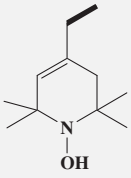
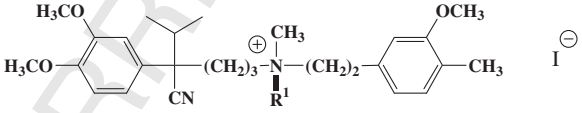
Table 2
The biological activity of *N*-alkyl derivatives achieved from nor-verapamil

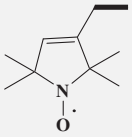
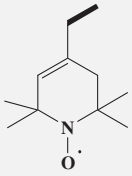


Compound	R^1	Superoxide scavenging (%)	Peroxyl scavenging (%)	Cytotoxicity CHO (%)	Cytotoxicity HSMC (%)
23A HO-3940		70.6 ± 3.3[a]	92.2 ± 0.5[a]	74.0 ± 3.4	64.8 ± 7.1
23B HO-3939		100[a] 78.1 ± 2.6[b]	100[a] 66.5 ± 9.2[b]	66.7 ± 3.0	58.6 ± 4.8
24A HO-3945		68.2 ± 7.6[a]	90.6 ± 1.5[a]	95.3 ± 0.5	91.1 ± 0.5
24B HO-3944		100[a] 82.5 ± 2.7[b]	100[a] 75.2 ± 1.3[b]	64.8 ± 5.6	57.2 ± 5.4

[a] Measured using 1-mM compound in presence of 1-mM DEPMPO.
[b] Measured using 100-μM compound in presence of 1-mM DEPMPO.

Table 3
The biological activity of quaternary salts of verapamil



Compound	R^1	Superoxide scavenging (%)	Peroxyl scavenging (%)	Cytotoxicity CHO (%)	Cytotoxicity HSMC (%)
25 HO-3983		73.8 ± 3.0[a]	47.9 ± 5.3[a]	48.3 ± 11.0	41.9 ± 5.1
26 HO-3975		48.1 ± 2.7[a]	40.4 ± 1.8[a]	61.9 ± 5.7	33.4 ± 6.6

[a] Measured using 1-mM compound in presence of 1-mM DEPMPO.

5.1.6. 1-Oxyl-2,2,6,6-tetramethyl-1,2,3,6-tetrahydro-pyridine-4-carboxylic acid-[(2-(3,4-dimethoxyphenyl)-5-[[2-(3,4-dimethoxyphenyl)ethyl]methylamino]-2-isopropyl-pentylamide radical (16C)

Yield 638 mg 50%; brownish solid; mp 106–108 °C. MS (EI) *m/z* (%): 638 (M^+, <1), 487(7) 472(2), 156(62), 43(100). Anal. Calcd for $C_{37}H_{56}N_3O_6$: C, 69.56; H, 8.84; N, 6.58. Found: C, 69.48; H, 8.90; N, 6.46.

5.2. Acylation of compound 2 with acids, general procedure (17C, 18C)

The solution of the acids (2.0 mmol), **2** amine (917 mg, 2.0 mmol) and 4-dimethylamino-pyridine (12 mg, 0.1 mmol) in dry ethyl-acetate (20 mL) was stirred for 10 min at room temperature, then DCC (412 mg, 2.0 mmol) dissolved in EtOAc (10 mL) was

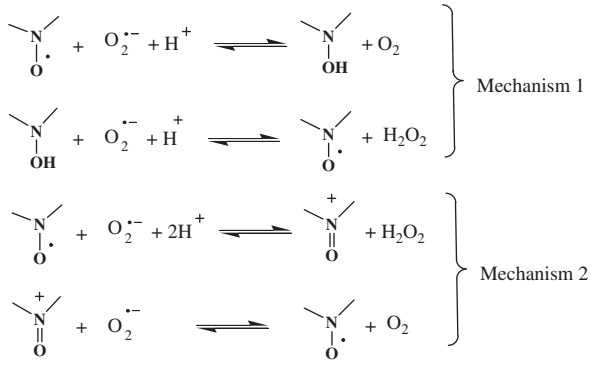


Figure 1. SOD mimetic mechanisms of cyclic nitroxides.

added, and the mixture was stirred at rt for 24 h. The mixture was filtered, the filtrate was evaporated, the residue was dissolved in $CHCl_3$ (30 mL), washed with brine (20 mL) and separated. The organic phase was dried ($MgSO_4$), filtered and evaporated. The residue was purified by flash column chromatography ($CHCl_3/Et_2O$) to give the compounds as yellow or red oils.

5.2.1. 1-Oxyl-2,2,6,6-tetramethyl-piperidine-4-carboxylic acid-[(2-(3,4-dimethoxyphenyl)-5-[[2-(3,4-dimethoxyphenyl)-ethyl]methylamino]-2-isopropyl-pentylamide radical (17C)

Yield 600 mg 46%; orange solid, mp 52–64 °C. (Turbo Spray) *m/z* (%): 641 ([M+H]$^+$, 100). Anal. Calcd for $C_{37}H_{58}N_3O_6$: C, 69.34; H, 9.12; N, 6.56. Found: C, 69.54; H, 9.56; N, 6.54.

5.2.2. *N*-(2-(3,4-Dimethoxyphenyl)-5-[[2-(3,4-dimethoxyphenyl) ethyl]methylamino]-2-isopropyl-pentyl-2-(1-oxyl-2,5,5-trimethyl-pyrrolidin-2-yl)-acetamide radical (18C)

Yield 538 mg 42%; yellow oil. (EI) *m/z* (%): 626 (M$^+$, 1), 475(5) 151(100). Anal. Calcd for $C_{36}H_{56}N_3O_6$: C, 68.98; H, 9.00; N, 6.70. Found: C, 68.74; H, 8.96; N, 6.53.

5.2.3. 3-[(2-(3,4-Dimethoxyphenyl)-5-[[2-(3,4-dimethoxyphenyl)ethyl]-methylamino]-2-isopropyl-pentylamino)methyl]-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-1-yloxyl radical (20C)

A solution of compound **2** (917 mg, 2.0 mmol), **19** allylic bromide (513 mg, 2.2 mmol), K_2CO_3 (303 mg, 2.2 mmol), 18-crown-6 (50 mg) in $CHCl_3$ (30 mL) was stirred and refluxed for 8 h, the inorganic salt was filtered off, the filtrate was washed with water (10 mL), the organic phase was separated, dried ($MgSO_4$), filtered and evaporated. The residue was purified by flash column chromatography ($CHCl_3/Et_2O$) to give the title compound 450 mg (36%). Pale-yellow solid, mp 65–67 °C. (EI) *m/z* (%): 610 (M$^+$, 6), 459(2) 278(33), 181(38), 151(100). Anal. Calcd for $C_{36}H_{56}N_3O_5$: C, 70.78; H, 9.24; N, 6.88. Found: C, 70.75; H, 9.20; N, 6.71.

5.3. Alkylation of nor-verapamil (21), general procedure (23C, 24C)

A mixture of nor-verapamil **21** (880 mg, 2.0 mmol), finely powdered K_2CO_3 (203 mg, 2.2 mmol) and 18-crown-6 (50 mg) in dry acetonitrile (40 mL) was stirred for 10 min, then paramagnetic alkyl-halogenide **19** or **22** (2.2 mmol) dissolved in acetonitrile (15 mL) was added in one portion, the mixture was stirred and refluxed for 8 h. After cooling the inorganic salts were filtered off, the acetonitrile was evaporated, the residue was dissolved in $CHCl_3$ (40 mL), the organic phase was washed with brine (20 mL), separated, dried ($MgSO_4$), filtered and evaporated. The remaining oil was purified by flash column chromatography (hexane/EtOAc) to produce the title compounds in 48–61% yield.

5.3.1. 2-(3,4-Dimethoxyphenyl)-5-[[2-(3,4-dimethoxyphenyl) ethyl]-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-ylmethyl)-amino]-2-isopropyl-pentanenitrile radical (23C)

Yield 723 mg 61%; orange oil. (EI) *m/z* (%): 592 (M$^+$, 1), 441(22), 426(19), 151(67), 43(100). Anal. Calcd for $C_{35}H_{50}N_3O_5$: C, 70.91; H, 8.50; N, 7.09. Found: C, 70.87; H, 8.43; N, 7.22.

5.3.2. 2-(3,4-Dimethoxyphenyl)-5-[[2-(3,4-dimethoxyphenyl) ethyl]-(1-oxyl-2,2,6,6-tetramethyl-1,2,3,6-tetrahydropyridin-4-ylmethyl)amino]-2-isopropyl-pentanenitrile radical (24C)

Yield 580 mg 48%; brown oil. (EI) *m/z* (%): 606 (M$^+$, 1), 455(33) 289(85), 151(100). Anal. Calcd for $C_{36}H_{52}N_3O_5$. C, 71.25; H, 8.64; N, 6.92. Found: C, 71.29; H, 8.54; N, 7.09.

5.4. Synthesis of quaternary salts, general procedure (25, 26)

The solution of compounds **23C** or **24C** (2.0 mmol) dissolved in iodomethane (5 mL) in sealed tube was heated at 50 °C for 48 h. After cooling, iodomethane was removed by evaporation, then the residue was triturated with Et_2O, the salt was filtered off, to give yellow crystals in 60–70% yield.

5.4.1. [4-Cyano-4-(3,4-dimethoxyphenyl)-5-methylhexyl]-[2-(3,4-dimethoxyphenyl)ethyl]-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-ylmethyl)methylammonium Iodide salt radical (25)

Yield 1.028 g 70%; pale-yellow solid; mp 110–112 °C. Anal. Calcd for $C_{36}H_{53}IN_3O_5$: C, 58.85; H, 7.27; N, 5.72. Found: C, 58.56; H, 7.17; N, 5.67.

5.4.2. [4-Cyano-4-(3,4-dimethoxyphenyl)-5-methylhexyl]-[2-(3,4-dimethoxyphenyl)ethyl]-(1-oxyl-2,2,6,6-tetramethyl-1,2,3,6-tetrahydropyridin-4-ylmethyl)methylammonium iodide salt radical (26)

Yield 0.928 g 62%; yellow solid; mp 167–170 °C. Anal. Calcd for $C_{37}H_{55}IN_3O_5$: C, 59.35; H, 7.40; N, 5.61. Found: C, 59.15; H, 7.25; N, 5.69.

5.5. General procedure for *N*-hydroxylamine salt formation (11B, 12B, 13B, 14B, 16B, 17B, 18B, 20B, 23B, 24B)

A solution of radicals **11C** or **12C** or **13C** or **14C** or **16C** or **17C** or **18C** or **20C** or **23C** or **24C** (1.0 mmol) was heated under reflux for 20 min in EtOH (10 mL) saturated with HCl previously. The solvent was evaporated off; the residue was crystallized with acetone or Et_2O to give white or pale-yellow solids in 60–78% yield.

5.5.1. 1-Hydroxy-2,2,5,5-tetramethyl-pyrrolidine-3-carboxylic acid-[(2-(3,4-dimethoxyphenyl)-5-[[2-(3,4-dimethoxyphenyl)-ethyl]methylamino]-2-isopropyl-pentylamide HCl salt (11B)

Yield 470 mg 67%; white solid, mp 122–124 °C. ^{1}H NMR (D_2O): 6.90 (d, *J* = 8 Hz, ArH, 2H); 6.87 (s, ArH, 2H); 6.73 (d, *J* = 8.7 Hz, ArH, 2H); 4.79–4.71 (t, CH, 1H); 3.78, 3.76, 3.75, 3.72 (4s, 4OCH$_3$, 12H); 3.52–3.46 (t, NCH$_2$, 2H); 3.31–3.19 (t, NCH$_2$, 2H); 3.12 (s, NCH$_2$, 2H); 2.86–2.81 (t, CH$_2$, 2H); 2.79 (s, NCH$_3$, 3H); 2.09 (t, CH$_2$, 2H); 2.14 (d, *J* = 7.5 Hz, CH$_2$, 2H); 1.99–1.92 (m, CH$_2$, 2H); 1.78–1.69 (m, CH$_2$, 2H); 1.37 (s, CH$_3$, 3H); 1.335 (d, *J* = 6 Hz, CH$_3$, 3H); 1.27 (s, CH$_3$, 3H); 1.15 (d, *J* = 6.5 Hz, CH$_3$, 3H); 1.10–1.02 (m, CH, 1H); 0.77 (d, *J* = 6 Hz, CH$_3$, 3H); 0.70 (d, *J* = 6.6 Hz, CH$_3$, 3H). Anal. Calcd for $C_{36}H_{58}Cl_2N_3O_6$: C, 61.79; H, 8.35; N, 6.00. Found: C, 61.58; H, 8.23; N, 5.87.

5.5.2. 1-Hydroxy-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-carboxylic acid-[(2-(3,4-dimeth-oxyphenyl)-5-[[2-(3,4-dimethoxyphenyl)ethyl]methylamino]-2-isopropyl-pentylamide HCl salt (12B)

Yield 530 mg 76%; white solid, mp 120–121 °C. ^{1}H NMR (DMSO-d_6): 6.93 (d, *J* = 11 Hz, ArH, 2H); 6.85 (d, *J* = 8.3 Hz, ArH, 2H); 6.68,

6.70 (2s, ArH, 2H); 6.15 (d, J = 5.5 Hz, CH, 1H); 3.77, 3.73, 3.71, 6.70 (4s, 4OCH$_3$,12H); 3.28 (s, NCH$_2$, 2H); 3.20–3.01 (m, NCH$_2$, 4H); 2.88–2.84 (t, CH$_2$, 2H); 2.63 (s, NCH$_3$, 3H); 2.20–2.12 (m, CH$_2$, 4H); 2.06 (s, CH$_3$, 6H); 1.60 (s, CH$_3$, 3H); 1.51 (s, CH$_3$, 3H); 1.32–1.20 (m, CH, 1H); 1.09 (d, J = 6.5 Hz, CH$_3$, 3H); 0.68 (d, J = 6.8 Hz, CH$_3$, 3H). Anal. Calcd for $C_{36}H_{56}Cl_2N_3O_6$: C, 61.97; H, 8.09; N, 6.02. Found: C, 61.86; H, 8.01; N, 5.81.

5.5.3. 1-Hydroxy-2,2,4,5,5-pentamethyl-2,5-dihydro-1*H*-pyrrol-3-carboxylic acid-[(2-(3,4-di-methoxyphenyl)-5-[[2-(3,4-dimethoxyphenyl)ethyl]methylamino]-2-isopropyl-pentylamide HCl salt (13B)

Yield 505 mg 71%; white solid, mp 123–125 °C. ^{1}H NMR (D$_2$O): 6.92 (s, ArH, 2H); 6.86 (d, J = 8.9 Hz, ArH, 2H); 6.76 (d, J = 9.1 Hz, ArH, 2H); 3.77 (s, 2OCH$_3$, 6H); 3.75 (s, 2OCH$_3$, 6H); 3.65–3.55 (t, NCH$_2$, 2H); 3.30–3.25 (m, NCH$_2$, 2H); 3.09–3.06 (t, NCH$_2$, 2H); 2.92–2.88 (m, CH$_2$, 2H); 2.83 (s, NCH$_3$, 3H); 2.78 (s, CH$_3$, 3H); 2.0–1.92 (m, CH$_2$, 2H); 1.71–1.64 (t, CH$_2$, 2H); 1.55, 1.42, 1.37, 1.34 (4s, 4CH$_3$, 12H); 0.96–0.92 (m, CH, 1H); 0.76 (d, J = 6.6 Hz, CH$_3$, 3H); 0.72 (d, J = 5.5 Hz, CH$_3$, 3H). Anal. Calcd for $C_{37}H_{58}Cl_2N_3O_6$: C, 62.44; H, 8.21; N, 5.90. Found: C, 62.25;H, 8.08; N, 5.74.

5.5.4. 1-Hydroxy-2,2,5,5-tetramethyl-4-phenyl-2,5-dihydro-1*H*-pyrrol-3-carboxylic acid-[(2-(3,4-dimethoxyphenyl)-5-[[2-(3,4-dimethoxyphenyl)ethyl]methylamino]-2-isopropyl-pentylamide HCl salt (14B)

Yield 503 mg 65%; white solid, mp 121–123 °C. ^{1}H NMR (D$_2$O): 7.40 (d, J = 7 Hz, ArH, 2H); 7.33–7.30 (m, ArH, 2H); 7.10 (d, J = 6 Hz, ArH, 2H); 6.94–6.86 (m, ArH, 4H); 6.63–6.56 (t, ArH, 1H); 3.78 (s, OCH$_3$, 6H); 3.77, 3.75 (2s, 2OCH$_3$, 6H); 3.63–3.56 (t, NCH$_2$, 2H); 3.49–3.42 (t, NCH$_2$, 2H); 3.25 (s, NCH$_2$, 2H); 2.96–2.89 (m, CH$_2$, 2H); 2.80 (s, NCH$_3$, 3H); 1.67–1.60 (m, CH$_2$, 4H); 1.58, 1.48, 1.46, 1.40 (4s, 4CH$_3$, 12H); 0.86–0.75 (m, CH, 1H); 0.59 (d, J = 7.2 Hz, CH$_3$, 6H). Anal. Calcd for $C_{42}H_{60}Cl_2N_3O_6$: C, 65.19; H, 7.81; N, 5.43. Found: C, 64.99; H, 7.66; N, 5.29.

5.5.5. 1-Hydroxy-2,2,6,6-tetramethyl-1,2,3,6-tetrahydro-pyridine-4-carboxylic acid-[(2-(3,4-dimethoxyphenyl)-5-[[2-(3,4-dimethoxyphenyl)ethyl]methylamino]-2-isopropyl-pentylamide HCl salt (16B)

Yield 448 mg 63%; brownish white solid, mp 125–127 °C. ^{1}H NMR (D$_2$O): 6.94 (d, J = 9 Hz, ArH, 2H); 6.87 (d, J = 8.6 Hz, ArH, 2H); 6.81, 6.73 (2s, ArH, 6H); 6.14 (s, CH, 1H); 3.76, 3.74 (2s, 4OCH$_3$, 12H); 3.67 (s, NCH$_2$, 2H); 3.28–3.16 (t, NCH$_2$, 2H); 3.11–2.98 (t, NCH$_2$, 2H); 2.93–2.85 (m, CH$_2$, 2H); 2.79 (s, NCH$_3$, 3H); 1.96 (s, CH$_2$, 2H); 1.76–1.65 (t, CH$_2$, 2H); 1.60–1.54 (m, CH$_2$, 2H); 1.50–1.40 (m, CH$_3$, 12H); 1.20–1.11 (m, CH, 1H); 0.75 (d, J = 6.6 Hz, CH$_3$, 6H). Anal. Calcd for $C_{37}H_{58}Cl_2N_3O_6$: C, 62.44; H, 8.21; N, 5.90. Found: C, 62.31; H, 8.15; N, 5.75.

5.5.6. 1-Hydroxy-2,2,6,6-tetramethyl-piperidine-4-carboxylic acid-[(2-(3,4-dimethoxyphenyl)-5-[[2-(3,4-dimethoxy-phenyl) ethyl]-methyl-amino]-2-isopropyl-pentyl-amide HCl salt (17B)

Yield 428 mg 60%; pale yellow solid, mp 143–145 °C. ^{1}H NMR (D$_2$O): 6.90 (d, J = 6.6 Hz, ArH, 2H); 6.84 (d, J = 6.9 Hz, ArH, 2H); 6.74 (s, ArH, 2H); 4.75–4.60 (m, CH, 1H); 3.76–3.73 (2s, 4OCH$_3$, 12H); 3.65–3.55 (t, NCH$_2$, 3H); 3.29–3.21 (t, NCH$_2$, 2H); 3.10 (s, NCH$_2$, 2H); 2.90–2.83 (m, CH$_2$, 2H); 2.81 (s, NCH$_3$, 3H); 2.19–2.13 (t, CH$_2$, 2H); 1.99–1.91 (d, CH$_2$, 2H); 1.76–1.69 (m, CH$_2$, 4H); 1.50, 1.39, 1.30, 1.28 (4s, 4CH$_3$, 12H); 1.20–1.11 (m, CH, 1H); 0.99 (d, J = 6.8 Hz, CH$_3$, 3H); 0.72 (d, J = 6.6 Hz, CH$_3$, 3H). Anal. Calcd for $C_{37}H_{60}Cl_2N_3O_6$: C, 62.26; H, 8.47; N, 5.89. Found: C, 62.13; H, 8.38; N, 5.71.

5.5.7. *N*-(2-(3,4-Dimethoxyphenyl)-5-[[2-(3,4-dimethoxyphenyl) ethyl]methylamino]-2-isopropyl-pentyl-2-(1-hydroxy-2,5,5-trimethyl-pyrrolidin-2-yl)-acetamide HCl salt (18B)

Yield 440 mg 63%; white solid, mp 108–110 °C. ^{1}H NMR (D$_2$O): 6.98 (d, J = 8.3 Hz, ArH, 2H); 6.88 (d, J = 11.0 Hz, ArH, 2H); 6.82, 6.73, (2s, ArH, 2H); 3.80, (d, J = 2.3 Hz, OCH$_3$, 3H); 3.75, (d, J = 3.1 Hz, OCH$_3$, 3H); 3.72, 3.67 (2s, 2OCH$_3$, 6H); 3.50–3.47 (m, CH$_2$CO, 2H); 3.45 (s, NCH$_2$, 2H); 3.33–3.19 (m, NCH$_2$, 2H); 3.18–3.04 (m, NCH$_2$, 2H); 2.83–2.86 (t, CH$_2$, 2H); 2.69 (s, NCH$_3$, 3H); 2.05–1.98 (m, CH$_2$, 2H); 1.85–1.72 (m, CH$_2$, 2H); 1.42, 1.39, 1.30 (3CH$_3$, 9H); 1.26–1.21 (m, CH, 1H); 1.20 (t, J = 7 Hz, CH$_2$, 2H); 1.10 (t, J = 7,0 Hz, CH$_2$, 2H); 0.78 (d, J = 6.8 Hz, CH$_3$, 3H); 0.74 (d, J = 5.6 Hz, CH$_3$, 3H). Anal. Calcd for $C_{36}H_{58}$ $I_2N_3O_6$: C, 61.79; H, 8.35; N, 6.00. Found: C, 61.68; H, 8.20; N, 5.82.

5.5.8. 1-Hydroxy-3-[(2-(3,4-dimethoxyphenyl)-5-[[2-(3,4-dimethoxy-phenyl)ethyl]-methylamino]-2-isopropyl-pentylamino) methyl]-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol, HCl salt (20B)

Yield 530 mg 73%; white solid, mp 122–124 °C. ^{1}H NMR (D$_2$O): 6.93 (d, J = 9.7 Hz, ArH, 2H); 6.82 (d, J = 6.1 Hz, ArH, 2H); 6.76 (s, ArH, 2H); 5.20 (s, CH, 1H); 3.80, 3.78, 3.74, 3.73 (4s, 4OCH$_3$, 12H); 3.69 (s, NCH$_2$, 2H); 3.40 (s, NCH$_2$, 2H); 3.38–3.30 (m, NCH$_2$, 2H); 3.20–3.10 (m, NCH$_2$, 2H); 2.84 (NCH$_3$, 3H); 2.78 (t, CH$_2$, 2H); 2.28–2.18 (q, CH$_2$, 2H); 1.87–1.80 (t, CH$_2$, 2H); 1.55, 1.42, 1.48, 1.42 (4s, 4CH3, 12H); 1.28–1.20 (m, CH, 1H); 0.90 (d, J = 6.0 Hz, CH$_3$, 3H); 0.79 (d, J = 5.9 Hz, CH$_3$, 3H). Anal. Calcd for $C_{36}H_{59}Cl_3N_3O_5$: C, 60.03; H, 8.26; N, 5.83. Found: C, 59.94; H, 8.19; N, 5.73.

5.5.9. 2-(3,4-Dimethoxyphenyl)-5-[[2-(3,4-dimethoxyphenyl) ethyl]-(1-hydroxy-2,2,5,5-tetramethyl-2,5-dihydro-*1H*-pyrrol-3-ylmethyl)-amino]-2-isopropyl-pentanenitrile HCl salt (23B)

Yield 525 mg 79%; white solid, mp 122–124 °C. ^{1}H NMR (D$_2$O): 6.95 (d, J = 7.2 Hz, ArH, 2H); 6.85 (d, J = 9.1 Hz, ArH, 2H); 6.72 (s, ArH, 2H); 5.85 (s, CH, 1H); 3.77 (s, 2OCH$_3$, 6H); 3.75, 3.74 (2s, 2OCH$_3$, 6H); 3.38 (s, NCH$_2$, 2H); 3.29–3.20 (t, NCH$_2$, 2H); 3.17–3.09 (t, NCH$_2$, 2H); 2.90–2.80 (t, CH$_2$, 2H); 2.22–2.10 (q, CH$_2$, 2H); 1.98–1.90 (m, CH$_2$, 2H); 1.43, 1.40, 1.37, 1.32 (4s, 4CH$_3$, 12H); 1.30–1.20 (m, CH, 1H); 1.06 (d, J = 6.5 Hz, CH$_3$, 3H); 0.63 (d, J = 5.9 Hz, CH$_3$, 3H). Anal. Calcd for $C_{35}H_{52}Cl_2N_3O_5$: C, 63.15; H, 7.87; N, 6.31. Found: C, 63.07; H, 7.74; N, 6.15.

5.5.10. 2-(3,4-Dimethoxyphenyl)-5-[[2-(3,4-dimethoxyphenyl) ethyl]-(1-hydroxy-2,2,6,6-tetramethyl-1,2,3,6-tetrahydro pyridin-4-ylmethyl)amino]-2-isopropyl-pentanenitrile HCl salt (24B)

Yield 468 mg 69%; white solid, mp 164–166 °C. ^{1}H NMR (D$_2$O): 6.93 (d, J = 9.5 Hz, ArH, 2H); 6.85 (d, J = 8.9 Hz, ArH, 2H); 6.72 (s, ArH, 2H); 5.79 (s, CH, 1H); 3.77 (s, 2 OCH$_3$,6H); 3.75, 3.74 (2s, 2 OCH$_3$, 6H); 3.40 (s, NCH$_2$, 2H); 3.27–3.20 (t, NCH$_2$, 2H); 3.19–3.14 (t, NCH$_2$, 2H); 2.89–2.80 (t, CH$_2$, 2H); 2.23 (s, CH$_2$, 2H), 2.20–2.10 (q, CH$_2$, 2H); 1.96–1.90 (m, CH$_2$, 2H); 1.45, 1.40, 1.39, 1.35 (4s, 4CH$_3$, 12H); 1.24–1.16 (m, CH, 1H); 1.06 (d, J = 6.2 Hz, CH$_3$, 3H); 0.66 (s, J = 6.0 Hz, CH$_3$, 3H). Anal. Calcd for $C_{36}H_{54}Cl_2N_3O_5$. C, 63.61; H, 8.01; N, 6.18. Found: C, 63.44; H, 7.82; N, 6.02.

5.6. Reduction of nitroxides to amines; general procedure (12A, 20A, 23A, 24A)

To a solution of nitroxide **12C** or **20C** or **23C** or **24C** (2.0 mmol) in AcOH (10 mL) Fe powder (1.12 g, 20.0 mmol) was added and the mixture was warmed up to 70 °C until the reaction started. The mixture was stirred at room temperature for 1 h, diluted with water (30 mL), decanted and the decanted aq solution made alkaline with solid K_2CO_3 (intensive foaming!). The mixture was extracted with CHCl$_3$/MeOH (9:1) (3 × 15 mL), dried (MgSO$_4$),

filtered, evaporated and chromatographic purification ($CHCl_3$/MeOH) offered the title amines in 38–51% yield.

5.6.1. 2,2,5,5-Tetramethyl-2,5-dihydro-1*H*-pyrrol-3-carboxylic acid-[(2-(3,4-dimethoxyphenyl) -5-[[2-(3,4-dimethoxyphenyl) ethyl]methylamino]-2-isopropyl-pentylamide (12A)

Yield 620 mg 51%; white solid, mp 198–190 °C (2 HCl salt). (EI) *m/z* (%): 609 (M^+, 4), 594(10), 458(59), 151(100). ^1H NMR ($CDCl_3$): 6.81 (d, J = 8.7 Hz, ArH, 2H); 6.78 (d, J = 7.9 Hz, ArH, 2H); 6.69 (s, ArH, 2H); 6.38 (s, CH, 1H); 3.86, 3.85, 3.84, 6.83 (4s, 4 OCH_3,12H); 3.56–3.52 (s, NCH_2, 2H); 3.15–3.10 (t, NCH_2, 2H); 3.04–2.98 (t, NCH_2, 2H); 2.97–2.90 (t, CH_2, 2H); 2.65 (s, NCH_3, 3H); 2.25–2.16 (m, CH_2, 2H); 1.81–1.78 (m, CH_2, 2H); 1.48 (d, J = 6.0 Hz, CH_3, 6H); 1.62 (s, CH_3, 3H); 1.57 (s, CH_3, 3H); 1.21–1.17 (m, CH, 1H); 0.80 (d, J = 6.2 Hz, CH_3, 3H); 0.77 (d, J = 6.0 Hz, CH_3, 3H). Anal. Calcd for $C_{36}H_{55}N_3O_5$: C, 70.90; H, 9.09; N, 6.89. Found: C, 70.84; H, 9.12; N, 6.93.

5.6.2. 3-[(2-(3,4-Dimethoxyphenyl)-5-[[2-(3,4-dimethoxyphenyl) ethyl]methylamino]-2-isopropyl-pentylamino)methyl]-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol (20A)

Yield 548 mg 46%; yellowish oil. (EI) *m/z* (%): 590 (M^+, <1), 580 (2), 444 (40), 278 (94), 151 (100). ^1H NMR (D_2O) for HCl salt: 6.95 (d, J = 8.7 Hz, ArH, 2H); 6.82 (d, J = 7.1 Hz, ArH, 2H); 6.79 (s, ArH, 2H); 5.32 (s, CH, 1H); 3.80, 3.78, 3.74, 3.73 (4s, 4OCH_3,12H); 3.65 (s, NCH_2, 2H); 3.42 (s, NCH_2, 2H); 3.40–3.30 (m, NCH_2, 2H); 3.25–3.14 (m, NCH_2, 2H); 2.86 (NCH_3, 3H); 2.82 (t, CH_2, 2H); 2.28–2.19 (m, CH_2, 2H); 1.88–1.82 (t, CH_2, 2H); 1.50, 1.42, 1.48, 1.42 (4s, 4CH_3, 12H); 1.26–1.14 (m, CH, 1H); 0.90 (d, J = 6.2 Hz, CH_3, 3H); 0.79 (d, J = 5.9 Hz, CH_3, 3H). Anal. Calcd for $C_{36}H_{57}N_3O_4$: C, 72.57; H, 9.64; N, 7.05. Found: C, 72.45; H, 9.53; N, 7.13.

5.6.3. 2-(3,4-Dimethoxyphenyl)-5-[[2-(3,4-dimethoxyphenyl) ethyl]-(2,2,5,5-tetramethyl-2,5-dihydro-*1H*-pyrrol-3-ylmethyl)-amino]-2-isopropyl-pentanenitrile (23A)

Yield 462 mg 40%; brown oil. (EI) *m/z* (%): 577 (M^+, <1), 562(1), 426(58), 151(63), 138(100). ^1H NMR (D_2O): 6.86 (d, J = 7.0 Hz, ArH, 2H); 6.77 (d, J = 6.6 Hz, ArH, 2H); 6.63 (s, ArH, 2H); 5.30 (s, CH, 1H); 3.88, 3.87 (2s, OCH_3, 6H); 3.84 (s, OCH_3, 6H); 2.92 (s, NCH_2, 2H); 2.59–2.57 (t, 2NCH_2, 4H); 2.46–2.42 (m, CH_2, 2H); 2.07–2.03 (k, CH_2, 2H); 2.05–1.99 (t, CH_2, 2H); 1.50–1.49 (2s, CH_3, 12H); 1.24–1.20, (m, CH, 1H); 1.18, (d, J = 7 Hz, CH_3, 3H); 0.79 (d, J = 6.8 Hz, CH_3, 3H). Anal. Calcd for $C_{35}H_{51}N_3O_4$: C, 72.75; H, 8.90; N, 7.27. Found: C, 72.77; H, 8.83; N, 7.22.

5.6.4. 2-(3,4-Dimethoxyphenyl)-5-[[2-(3,4-dimethoxypheny lethyl]-(2,2,6,6-tetramethyl-1,2,3,6-tetrahydropyridin-4-ylmethyl)amino]-2-isopropyl-pentanenitrile (24A)

Yield 450 mg 38%; pale-brown oil. (EI) *m/z* (%): 591 (M^+, <1), 576(1), 440(30), 151(100). ^1H NMR (D_2O): 6.84 (d, J = 8.7 Hz, ArH, 2H); 6.76 (d, J = 6.9 Hz, ArH, 2H); 6.64, 6.61 (2s, ArH, 2H) 5.35 (s, CH, 1H); 3.87, 3.86 (2s, OCH_3,6H); 3.83 (s, OCH_3, 6H); 2.90 (s, NCH_2, 2H); 2.59–2.54 (t, NCH_2, 2H); 2.53–2.49 (t, NCH_2, 2H); 2.48–2.38 (m, CH_2, 2H); 2.21 (s, CH_2, 2H); 2.07–2.03 (k, CH_2, 2H); 2.02–1.96 (t, CH_2, 2H); 1.52 (s, CH_3, 6H); 1.45, 1.42 (2s, CH_3, 6H); 1.26–1.22 (m, CH, 1H); 1.16 (d, J = 6.5 Hz, CH_3, 3H). 0.78 (d, J = 7 Hz, CH_3, 3H). Anal. Calcd for $C_{36}H_{53}N_3O_4$: C, 73.06; H, 9.03; N, 7.10. Found: C, 72.96; H, 9.04; N, 7.08.

5.7. Synthesis of carboxylic acids (29, 30); general procedure

To the stirred solution of aldehydes **27**, **28** (2.0 mmol), KH_2PO_4 (140 mg, 1.03 mmol) in MeCN–H_2O (5:3, 16 mL) and aq H_2O_2 (30%, 0.2 mL, 1.76 mmol), $NaClO_2$ (400 mg, 4.4 mmol) dissolved in H_2O (10 mL) at 0 °C was added dropwise over 30 min. After 1 h stirring at rt $Na_2S_2O_5$ (200 mg, 1.05 mmol) was added and the solution was acidified with aq HCl (1.0 M). The solution was extracted with EtOAc (2 × 30 mL), the organic phase was separated, dried ($MgSO_4$) and evaporated, the residue was purified by flash column chromatography (hexane–EtOAc) to give the title acids (45–55%).

5.7.1. 3-Carboxy-4-methyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-1-yloxyl radical (29)

Yield 217 mg 55%; yellow solid; mp 205 °C (subl.) MS (EI) *m/z* (%): 198 (M^+, 88), 183 (100), 168 (46), 153 (21). Anal. Calcd for $C_{10}H_{16}NO_3$: C, 60.59; H, 8.14; N, 7.07. Found: C, 60.7; H 7.98; N 7.27.

5.7.2. 3-Carboxy-4-phenyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-1-yloxyl radical (30)

Yield 234 mg 45%; pale-yellow solid; mp 190–192 °C. MS (EI) *m/z* (%): 260 (M^+, 100), 245 (70), 230 (17), 91 (42), 77 (36). Anal. Calcd for $C_{15}H_{18}NO_3$: C, 69.21; H, 6.97; N, 5.38. Found: C, 69.4; H, 6.8; N, 5.18.

5.8. Biology

Dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), and TritonX were obtained from Sigma. Cell-culture medium (RPMI 1640), smooth-muscle-cell basal medium, fetal bovine serum, antibiotics, sodium pyruvate, trypsin, and phosphate-buffered saline (PBS) were purchased from Invitrogen. All other reagents and compounds were analytical grades.

5.9. Cell lines and cultures

Chinese hamster ovary (CHO) cells and human aortic vascular smooth muscle cells (HSMCs) were obtained at passage 3 (AoSMC; Lonza Walkersville, Inc., Walkersville, MD) were used. Cells were grown in RPMI 1640 and SMC basal medium supplemented with 10% fetal bovine serum, 2% sodium pyruvate, 1% penicillin, and streptomycin. Cells were grown in a 100-mm dish or T-75 Flask to 80% confluence at 37 °C in an atmosphere of 5% CO_2 and 95% air. Cells were routinely trypsinized (0.05% trypsin/EDTA) and counted using an automated cell counter (NucleoCounter, New Brunswick Scientific, Edison, NJ).

5.10. Cell viability assay

Cell viability was determined using the conversion of MTT to formazan via mitochondrial oxidation. CHO and HSMC cells were grown in T-75 flasks to 80% confluence. They were then trypsinized, counted, and seeded in 96-well plates with an average of 7000 cells/well. Cells were incubated for 24–48 h and then treated with 50 μM verapamil derivatives for 2 h and then treated with 1 mM H_2O_2 for 1 h. All experiments were repeated at least six times.

5.11. Measurement of superoxide and alkylperoxyl radicals by EPR spectroscopy

The superoxide and alkylperoxyl radical-scavenging ability of verapamil derivatives were determined by using EPR spectroscopy. A mixture of xanthine (0.2 mM) and xanthine oxidase (0.02 U/ml) in PBS (pH 7.4) was used to generate superoxide radicals. Alkylperoxyl radicals were generated through the thermolytic fission of 2,2-azobis-2-amidonopropane dihydrochloride (25 mM) in aerobic PBS solution at 37 °C. All EPR measurements

were performed in PBS (pH 7.4) containing DEPMPO (1 mM) and diethylenediaminepentaacetate (0.1 mM) in the presence or absence of verapamil derivatives (1 mM or 100 μM). The superoxide and peroxyl radicals were detected as DEPMPO-OOH and DEPMPO-OOR adducts, respectively. The attenuation of DEPMPO adduct generation was quantified by double-integration and expressed as percentage of untreated (without verapamil derivatives) levels.

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