Synthesis and Biological Evaluation of Nitric Oxide-releasing Derivatives of Capsaicin as Analgesia Drugs

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Abstract: TRPV1 is a ligand-gated non-selective cation channel that is considered to be an important pain integrator. Capsaicin, the prototypical TRPV1 agonist, has a clear therapeutic potential. In this letter, for lowering its pungency, a series of nitric oxide-releasing derivatives of capsaicin were designed and synthesized, including 10 compounds which were the direct combination of capsaicin and dihydro capsaicin with various nitric oxide donors. Preliminary biological tests suggested the compounds had both TRPV1 agonist activity and nitric oxide release activity. Compound B_2, B_5, B_8 had better analgesic activity than capsaicin. Based on these results, Compound B_2, B_5, B_8 can be considered as lead candidates for the further development of analgesic drugs.

Keywords: Analgesia Drugs, Nitric Oxide-Releasing, Capsaicin Derivatives, TRPV1 Agonist.

INTRODUCTION

Pain is a complex perceptual experience that can have a profound impact on the quality of a person's life [1]. Currently, due to its complicated pathological mechanism, all marketed drugs against pain have their deficiencies [2].Transient receptor vanilloid type-1 (TRPV1), formerly known as the vanilloid receptor (VR1), is a ligand-gated non-selective cation channel that is considered to be an important pain integrator [3]. It has been shown to be important in both nociception and the inflammatory response, and hence has rapidly become a significant therapeutic target [4].

Capsaicin, the prototypical TRPV1 agonist, which is responsible for the piquancy of hot-chilli peppers, is a versatile natural compound, the biological use of which is covered by more than 900 patents [5]. Its effect, traditionally referred to as desensitization, has a clear therapeutic potential. In fact, capsaicin-containing creams have been in clinical use for decades to relieve painful conditions such as diabetic neuropathy. Though the highly pungency limited its use, capsaicin has great value for further research [6].

Nitric oxide (NO) is naturally generated from L-arginine by the action of NO synthase (NOS) and a key signaling molecule involved in regulating the numerous physiologic and pathologic processes [7]. NO can increase gastric blood flow and inhibit neutrophil adherence as well as protect the gastric mucosa against damage induced by irritants [8]. In particular, the NO-naproxen derivative has displayed an enhanced analgesic activity [9].

In this letter, according to the molecular structure of capsaicin, intended for lowering its pungency, a series of nitric oxide-releasing derivatives of capsaicin were designed. Capsaicin and dihydro capsaicin were combined with the NO donors, to form a new class of potent TRPV1 agonist. As a result, 10 capsaicin derivatives were synthesized and their activities were evaluated.

METHODS

Evaluation of TRPV1 Agonist Activity

The TRPV1 agonist activity assays were performed using the method reported by the reference [6]. Human embryonic kidney (HEK)-293 cells overexpressing the human TRPV1 were grown as monolayers in minimum essential medium supplemented with nonessential amino acids, 10% fetal calf serum, and 0.2 mM glutamine and maintained under 5% CO_2 at 37°C. The effect of the compounds on $[Ca^{2+}]i$ was determined by using Fura-2, a selective intracellular fluorescent probe for Ca^{2+}. Prior to Ca^{2+} measurements the cells were seeded at a cell density of 20,000 cells/well 3–4 days from measurements to yield 90% confluence in 96-well plates. Acute intracellular increase in Ca^{2+} levels was measured in a fluorescence reader using the cell permeable fluores-cent probe Fura-2/AM, dissolved in DMSO to 2 mM. The cells were incubated in cell medium with 2μM Fura-2/AM for 30 min in 37 ˚C followed by two washings in Krebs–Ringer–Hepes (KRH) buffer, incubation in KRH buffer for 30 min in darkness. The ratio of 340 /380nm excitatory wavelengths was measured without interruption before (basal level) and during the 2 min exposure to the test compounds or capsaicin. All compounds used were diluted to homogenous solutions in KRH buffer in 10 μM. The ratio values after addition of the chemicals was gotten within the two minutes of registration.

Evaluation of Nitric Oxide Release Activity

The nitric oxide release activity *in vitro* were determined by Greiss assay. Briefly, MDA-MB-231 cells (5×106/well) were treated in triplicate with 100 μM of one of the compounds for 24h. The cells were harvested lyzed. The cell

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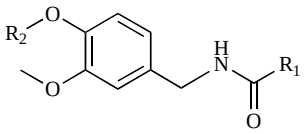
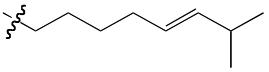
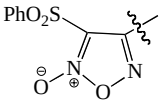
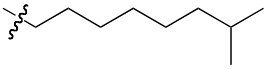
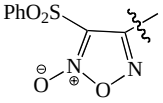
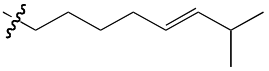
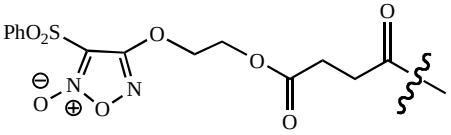
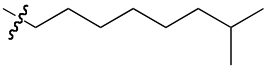
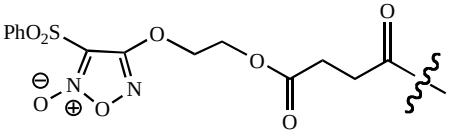
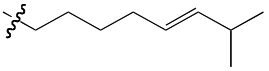
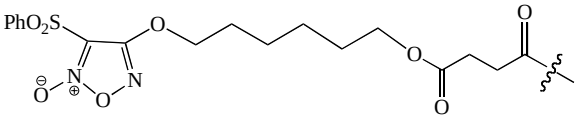
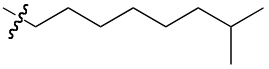
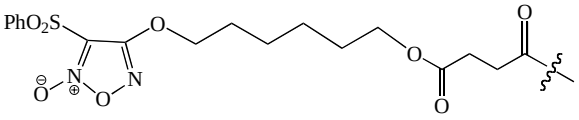
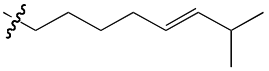
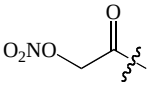
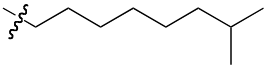
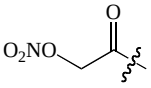
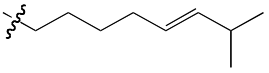
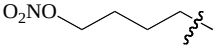
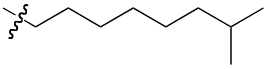
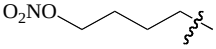
Table 1. The Structures of the Target Compounds B_1-B_{10}



Compound	R_1	R_2
B_1		
B_2		
B_3		
B_4		
B_5		
B_6		
B_7		
B_8		
B_9		
B_{10}		

lysates were mixed with Griess for 30-300 min, followed by measuring at 540 nm. The cells treated with diluent were used as negative controls for the background levels of nitrate/nitrite production, while with sodium nitrate at different concentrations was used as positive controls for the standard curve [10]. According to the determined levels of nitrate/nitrite formed from individual compounds in the cells, the nitric oxide release activity of the compounds was obtained.

Evaluation of Analgesic Activity

According to the method of tail-immersion test [10], analgesic activity was carried out in 14 groups (buprofen group, capsaicin group, dihydro capsaicin group, ten compounds synthesized groups and blank group) with 112 mice (half male and half female mice, 8 mice for each group). Ibuprofen, capsaicin, dihydro capsaicin and ten compounds synthesized were dissolved by 0.5% CMC-Na aqueous solution. The Compounds (30 mg/kg) was orally admin-

istered by gastric intubation once a day for 4 days (ibuprofen, capsaicin, dihydro capsaicin and vehicle administered as control). In the last day, baseline latency of each mouse in each group was determined twice, 5 min apart and averaged to give a single pre-drug latency. The pre-drug latency is presented as the mean (±SE) latency in second. Determine the post-drug latency of pain response of the mice at 30min after administration, according to the method mentioned above. Analgesia data are expressed as the increased percentage of pain threshold.

Increased percentage of pain threshold (%) = [(Post-drug latency- pre-drug latency)/ pre-drug latency]*100.

In the equation, pre-drug latency and post-drug latency are represented the mean value of each group.

RESULTS AND DISCUSSION

Synthesis

We designed and synthesized a series of nitric oxide-releasing derivatives of capsaicin, including 10 compounds which were the direct combination of capsaicin and dihydro capsaicin with various NO donors. The target compounds were shown in the Table **1**.

Biological Activity

The new compounds were tested for TRPV1 agonist activity, nitric oxide release activity and analgesic activity. The results of primary pharmacological test are as followed: In the TRPV1 agonist high throughput screening model (Table **2**), all the compounds enhance Ca^{2+} inflow, among which compound show better than capsaicin. The NO release activity *in vitro* was determined by Greiss assay (Table **2**). The result showed that these compounds all have NO release. In the model of mouse tail-immersion test (Table **3**), compound B_2, B_3, B_4, B_5, B_6, B_7, B_8, B_9 can significantly inhibit mouse's tail-immersion reaction and B_2, B_5, B_8 show better than capsaicin, which means that the compounds have potent analgesic activity.

SYNTHESIS EXPERIMENTAL SECTION

All reagents were of commercial quality and were used as received. Solvents were dried and purified by using standard techniques. Reactions were monitored by TLC. Melting points were uncorrected. ^{1}H NMR spectra were recorded on a Brucker ACF-300 MHz spectrometer with TMS as the internal standard in $CDCl_3$. Mass Spectra were made with HP1100 analyzer. Element analyses were performed on Carlo Erba 1106 instrument.

General Procedure for the Preparation of B_1-B_{10}

(a) Capsaicin or dihydro capsaicin 0.50g (1.64mmol), NaOH 0.26g(6.5mmol) and different derivatives of furazan (1.97mmol), dissolved in 35ml dry CH_2Cl_2.The mixture was stirred at 30-35 ˚C for 0.5 h. Added 0.47g (2.3mmol)DCC/5ml CH_2Cl_2 solution under ice bath and the mixture was stirred at 30-35 ˚C. The reaction was monitored by TLC and filtrated. The filtrate was concentrated under reduced pressure to give a residue which was partitioned between ethyl acetate and water. The ethyl acetate extract was washed with saturated brine, dried over Na_2SO_4. After evaporation of solvent, the crude product was subjected to silica gel column, and chromatographed with eluent (petroleum ether /ethyl acetate 1:2) to yield the desired product B1, B2.

(b) Capsaicin or dihydro capsaicin 0.50g (1.64mmol), DMAP 0.02g(0.16mmol) and different derivatives of furazan (1.97mmol), dissolved in 35ml dry CH_2Cl_2.The mixture was stirred at 30-35 ˚C for 0.5 h. The reaction was monitored by TLC and filtrated. The filtrate was concentrated under reduced pressure to give a residue which was partitioned be-

Table 2. The TRPV1 Agonist Activity and the Nitric Oxide Release Activity of the Synthesized Compounds

Compound	R340/R380*	NO(+/-)**
blank	0.999	-
capsaicin	1.885	-
dihydro capsaicin	1.996	-
B_1	1.771	+
B_2	1.683	+
B_3	1.711	+
B_4	1.695	+
B_5	1.546	+
B_6	1.584	+
B_7	1.772	+
B_8	1.554	+
B_9	2.103	+
B_{10}	1.953	+

*The ratio of 340 /380nm excitatory wavelengths which means the intracellular increase in Ca^{2+} levels.
**The "+/ –" means whether there is nitric oxide releasing.

Table 3. The Analgesic Activity of the Synthesized Compounds

Compound	Increased of Pain Threshold (%)	Compound	Increased of Pain Threshold (%)
blank	20.19	B_4	147.93*
ibuprofen	78.66**	B_5	259.97**
capsaicin	258.74**	B_6	162.33*
dihydro capsaicin	94.07**	B_7	195.90**
B_1	66.05	B_8	282.96**
B_2	307.17**	B_9	58.34*
B_3	157.16*	B_{10}	74.47*

*Statistically significant between pre-drug latency and the post-drug latency in the same group by paired t-test, P<0.05.
**Statistically significant between pre-drug latency and the post-drug latency in the same group by paired t-test, P<0.01.

tween ethyl acetate and water. The ethyl acetate extract was washed with saturated brine, dried over Na_2SO_4. After evaporation of solvent, the crude product was subjected to silica gel column, and chromatographed with eluent (petroleum ether /ethyl acetate 1:1) to yield the desired product B3-B6.

(c) Capsaicin or dihydro capsaicin 1.0g (3.3mmol), chloroacetic acid 0.37g(3.96mmol) and DMAP 0.04g(0.33mmol), dissolved in 50ml dry CH_2Cl_2 .The mixture was stirred at 30-35 ˚C for 0.5 h, added 0.94g DCC/10ml CH_2Cl_2 with the ice bath and reacted over night. The filtrate was concentrated under reduced pressure and dissolved in 30ml dry acetone with KI 0.85g.The reaction was under refluxing and in the darkness for 2 h. Then added ethyl acetate, washed with water, and dried over $MgSO_4$. After evaporation of solvent, the solid dissolved in 25ml dry CH_3CN and reacted with $AgNO_3$ 0.84g under refluxing and in the darkness for 48 h. Added CH_2Cl_2 and 2N HCl after evaporation of CH_3CN. The filtrate was washed with water, dried over Na_2SO_4. After evaporation of solvent, the crude product was subjected to silica gel column, and chromatographed with eluent(petroleum ether / acetone 3:1) to yield the desired product B7, B8.

(d) Capsaicin or dihydro capsaicin 1.0g (3.3mmol), 1,4-dibromo butane 1.16ml(9.9mmol) and K_2CO_3 0.9g(6.6mmol), dissolved in 50ml dry ethyl acetate .The reaction was under refluxing and in the darkness for 24 h. The filtrate was washed with water, dried over Na_2SO_4. After evaporation of solvent, the solid and $AgNO_3$ 0.59g (3.45mmol), dissolved in 25ml dry CH_3CN, under refluxing and in the darkness for 3 h. The reaction was monitored by TLC and filtrated. After evaporation of CH_3CN, added CH_2Cl_2 and filtrated. After evaporation of solvent, the crude product was subjected to silica gel column, and chromatographed with eluent(petroleum ether / acetone 3:1) to yield the desired product B9, B10.

Spectra Data

N-[[3-methoxy-4-(3-benzenesulfonyl-1,2,5-oxadiazole-2-oxide-4-yloxy)-phenyl]-methyl]-8-methyl-non-6-enoyl-amine(B_1)

Yield 41.5%; white crystal 0.36g; m.p. 134-136℃; IR(KBr,ν):3308, 1649, 1624, 1542, 1509, 1452, 1165, 600cm^{-1}; ^{1}HNMR($CDCl_3$,300Hz,*δppm*): 7.64-8.20(m, 5H, Ar-H), 6.87-7.24(m, 3H, Ar-H), 5.75(bs, 1H, N-H), 5.37(m, 2H, CH=CH), 4.46(t, 2H, *J*=5.3Hz, $ArCH_2NH$), 3.70(s, 3H, OCH_3), 2.24(m, 3H, $NHCOCH_2$,$CH(CH_3)_2$), 2.0(m, 2H, CH_2CH=CH), 1.38-1.71(m, 4H, $CH_2CH_2CH_2CH$=CH), 0.96(d, 6H, *J*=6.7Hz, $CH(CH_3)_2$); MS(ESI, m/z) : 552.2(M+Na$^+$, base peak); Anal. Calcd. for $C_{26}H_{31}N_3O_7S$:C 58.96, H 5.90, N 7.93; Found C 58.91, H 5.89, N 7.89.

N-[[3-methoxy-4-(3-benzenesulfonyl-1,2,5-oxadiazole-2-oxide-4-yloxy)-phenyl]-methyl]-8-methyl-nonanoylamine (B_2)

Yield 35.6%; white crystal 0.31g; m.p. 124-126℃;IR(KBr,ν) : 3309, 1649, 1625, 1542, 1510, 1452, 1164, 600cm^{-1}; ^{1}HNMR($CDCl_3$, 300Hz, *δppm*): 7.64-8.19(m, 5H, Ar-H), 6.90-7.25(m, 3H, Ar-H), 5.76(bs, 1H, N-H), 4.47(d, 2H, *J*=6.0Hz, $ArCH_2NH$), 3.70(s, 3H, OCH_3), 2.24(t, 2H, *J*=7.6Hz, $NHCOCH_2$), 1.17-1.70(m, 11H, $CH_2CH_2CH_2$ $CH_2CH_2CH(CH_3)_2$), 0.87(d, 6H, *J*=6.7Hz, $CH(CH_3)_2$); (ESI,m/z): 554.1(M+Na$^+$, base peak); Anal. Calcd. for $C_{26}H_{33}N_3O_7S$:C 58.74, H 6.26, N 7.90; Found C 58.69, H 6.23, N 7.89.

3- [[2-(3- Benzenesulfonyl-1,2,5-oxadiazole-2-oxide-4-yloxy)ethyl]oxyformyl] propionic acid 2-methoxy-4-[(8-methyl-non-6-enoylamino)methyl] phenyl ester(B_3)

Yield 90.9%; white crystal 1.0 g; m.p. 88-89℃;IR(KBr,ν) : 3263, 1762, 1748, 1641, 1619, 1555, 1510, 1450, 1360, 1140, 600cm^{-1}; ^{1}HNMR($CDCl_3$,300Hz,*δppm*): 7.59-8.05(m, 5H, Ar-H), 6.81-6.97(m, 3H, Ar-H), 5.70(bs, 1H, N-H), 5.35(m, 2H, CH=CH), 4.54-4.63(m, 4H, OCH_2CH_2O), 4.41(d, 2H, *J*=5.5Hz, $ArCH_2NH$), 3.81(s, 3H, OCH_3), 2.96(t, 2H, *J*=6.7Hz, CH_2COOAr), 2.81(t, 2H, *J*=6.7Hz, CH_2COO), 2.22(m, 3H, CH_2CONH,$CH(CH_3)_2$), 1.99(m, 2H, CH_2CH=CH), 1.40-1.69(m, 4H, $CH_2CH_2CH_2$ CH=CH), 0.90(d, 6H, *J*=6.7Hz, $CH(CH_3)_2$); MS(ESI,m/z): 696.1(M+Na$^+$, base peak);Anal. Calcd. for $C_{32}H_{39}N_3SO_{11}$:C 57.06, H 5.79, N 6.24 ; Found C 56.97, H 5.79, N 6.28.

3-[[2-(3-Benzenesulfonyl-1,2,5-oxadiazole-2-oxide-4-yloxy)ethyl]oxyformyl] propionic acid 2-methoxy-4-[(8-methyl-nonanoylamino)methyl] phenyl ester(B_4)

Yield 80.9%; white crystal 0.89g; m.p. 91-92℃;IR(KBr,ν):3314, 1754, 1734, 1626, 1556, 1512, 1464, 1143, 601cm^{-1}; ^{1}HNMR($CDCl_3$,300Hz,*δppm*): 7.73-8.07(m,5H,Ar-H), 6.81-6.97(m, 3H, Ar-H), 5.74(bs, 1H, N-H), 4.63(t, 2H, *J*=3.1Hz, OCH_2), 4.54(t, 2H, *J*=4.6Hz,

CH_2O), 4.41(d, 2H, *J*=5.6Hz, $ArCH_2NH$), 3.81(s, 3H, OCH_3), 2.96(t, 2H, *J*=6.7Hz, CH_2COOAr), 2.80(t, 2H, *J*=6.7Hz, CH_2COO), 2.21(t,2H, *J*=7.6Hz, CH_2CONH), 1.13-1.69(m, 11H, $CH_2CH_2CH_2CH_2CH_2CH(CH_3)_2$), 0.85(d, 6H, *J*=6.7Hz, $CH(CH_3)_2$); MS(ESI,m/z): 698.2($M+Na^+$, basepeak); Anal.Calcd. for $C_{32}H_{41}N_3SO_{11}$:C 56.89, H 6.07, N 6.22 ; Found C 56.70, H 6.22, N 6.17.

3-[[6-(3-benzenesulfonyl-1,2,5-oxadiazole-2-oxide-4-yloxy)hexyl]oxyformyl] propionic acid 2-methoxy-4-[(8-methyl-non-6-enoylamino)methyl] phenyl ester (B_5)

Yield 74.6%; white crystal 0.89g; m.p. 73-75℃;IR(KBr,ν):3316, 1765, 1759, 1638, 1621, 1556, 1451, 1377, 1145, 605cm^{-1}; ^{1}HNMR($CDCl_3$,300Hz,*δppm*): 7.60-8.06(m,5H,Ar-H), 6.82-7.0(m,3H, Ar-H), 5.74(bs,1H,N-H), 5.35(m,2H,CH=CH), 4.40(m,4H,OCH2$C_4H_8CH_2O$), 4.13(t,2H, *J*=3.3Hz, $ArCH_2NH$), 3.80(s,3H,OCH_3), 2.92(t,2H, *J*=6.9Hz, CH_2COOAr), 2.76(t, 2H, *J*=6.9Hz, CH_2COO), 2.21(m, 3H, CH_2CONH, $CH(CH_3)_2$), 2.05(m, 2H, CH_2CH=CH), 1.26-1.69(m, 12H, $CH_2CH_2CH_2$ CH=CH, $OCH_2CH_2CH_2CH_2CH_2CH_2O$), 0.89(d,6H, *J*=6.6Hz, $CH(CH_3)_2$); MS(ESI,m/z): 752.3($M+Na^+$, base peak); Anal. $C_{36}H_{47}N_3SO_{11}$: C 59.26, H 6.45, N 5.76; Found C 59.34, H 6.71, N 5.55

3-[[6-(3-benzenesulfonyl-1,2,5-oxadiazole-2-oxide-4-yloxy)hexyl]oxyformyl] propionic acid 2-methoxy-4-[(8-methyl-nonanoylamino)methyl] phenyl ester (B_6)

Yield 77.3%; white solid 0.92g; m.p. 45-47℃;IR(KBr, ν):3311, 1761, 1733, 1644, 1618, 1557, 1511, 1455, 1167, 605cm^{-1}; ^{1}HNMR($CDCl_3$,300Hz,*δppm*): 7.60-8.06(m, 5H, Ar-H), 6.82-7.0(m, 3H, Ar-H), 5.75(bs, 1H, N-H), 4.40(m, 4H, $OCH_2CH_2CH_2CH_2CH_2CH_2O$), 4.14(t, 2H, *J*=6.6Hz, $ArCH_2NH$), 3.81(s,3H,OCH_3), 2.93(t, 2H, *J*=6.6Hz, CH_2COOAr), 2.76(t, 2H, *J*=6.9Hz, CH_2COO), 2.20(t, 2H, *J*=7.5Hz, CH_2CONH), 1.15-1.87(m, 19H, $CH_2CH_2CH_2CH_2CH_2CH(CH_3)_2$, OCH_2CH_2 $CH_2CH_2CH_2CH_2O$), 0.85(d, 6H, *J*=6.6Hz, $CH(CH_3)_2$); MS(ESI, m/z): 732.3($M+H^+$); Anal. Calcd. for $C_{36}H_{49}N_3SO_{11}$:C 59.08, H 6.75, N 5.74; Found C 59.04, H 6.52, N 5.66.

1-Nitroxy-acetic acid 2-methoxy-4-[(8-methyl-non-6-enoylamino)–methyl] phenyl ester(B_7)

Yield 44.7%; white crystal 0.6g; m.p.76-78℃; IR(KBr,ν): 3312, 1779, 1641, 1514, 1464, 1278, 1212, 848cm^{-1}; ^{1}HNMR($CDCl_3$,300Hz,*δppm*):6.84-7.27(m, 3H, Ar-H), 5.75(bs, 1H, N-H), 5.33(m, 2H, CH=CH), 5.17(s, 2H, $OCOCH_2ONO_2$), 4.43(d, 2H, *J*=5.7Hz, $ArCH_2NH$), 3.81(s, 3H, OCH_3), 2.22(m, 3H, CH_2CONH, $CH(CH_3)_2$), 1.98(m, 2H, CH_2CH=CH), 1.37-1.69(m, 4H, $CH_2CH_2CH_2CH$=CH), 0.95(d, 6H, *J*=6.7Hz, $CH(CH_3)_2$); MS(ESI, m/z): 447.1($M+K^+$, base peak); Anal. Calcd. for $C_{20}H_{28}N_2O_7$:C 58.82, H 6.86, N 6.86; Found C 58.65, H 7.13, N 6.52.

1-Nitroxy-acetic acid 2-methoxy-4-[(8-methyl-nonanoyl-amino)-methyl] phenyl ester(B_8)

Yield 32.3%; white crystal 0.43g; m.p. 78-80℃; IR(KBr,ν): 3311, 1777, 1640, 1544, 1470, 1288, 1210, 845cm^{-1}; ^{1}HNMR($CDCl_3$,300Hz,*δppm*):6.86-7.05(m, 3H, Ar-H), 5.73(bs, 1H, NH), 5.18(s, 2H, $OCOCH_2ONO_2$), 4.44(d, 2H, *J*=6.0Hz, $ArCH_2NH$), 3.84(s, 3H, OCH_3), 2.22(t, 2H, *J*=7.6Hz, CH_2CONH), 1.17-1.70(m, 11H, $CH_2CH_2CH_2$ $CH_2CH_2CH(CH_3)_2$), 0.89(d, 6H, *J*=6.7Hz, $CH(CH_3)_2$); MS(ESI,m/z) :411.3($M+H^+$); Anal. Calcd. for $C_{20}H_{30}N_2O_7$:C 58.52, H 7.37, N 6.82; Found C 58.47, H 7.29, N 6.77.

N-(3-methoxy-4-nitroxy-butyoxy-benzyl)-8-methyl-non-6-enoylanine(B_9)

Yield 52%; white crystal 0.5g; m.p. 69-71℃;IR(KBr, ν):3305, 1635, 1546, 1518, 1464, 1282, 1238, 1138cm^{-1}; ^{1}HNMR($CDCl_3$,300Hz,*δppm*):6.81(m, 3H, Ar-H), 5.66(bs, 1H, NH), 5.34(m, 2H, CH=CH), 4.56(t, 2H, *J*=6.0Hz, OCH_2CH_2), 4.38(t, 2H, *J*=5.7Hz, $CH_2CH_2ONO_2$), 4.04(t, 2H, *J*=5.7Hz, $ArCH_2NH$), 3.84(s, 3H, OCH_3), 2.21(m, 3H, CH_2CONH,$CH(CH_3)_2$), 1.94(m, 4H, $OCH_2CH_2CH_2CH_2$ ONO_2), 1.39-1.69(m, 6H, $CH_2CH_2CH_2CH$=CH), 0.95(d, 6H, *J*=6.7Hz, $CH(CH_3)_2$); MS(ESI,m/z) : 445.2($M+Na^+$, base peak);Anal. Calcd. for $C_{22}H_{34}N_2O_6$:C 62.56, H 8.06, N 6.64 ; Found C 62.40, H8.43, N 6.46.

N-(3-methoxy-4-nitroxy-butyoxy-benzyl)-8-methyl-nonanoylanine(B_{10})

Yield 69%; white crystal 0.72g; m.p. 73-74℃;IR(KBr,ν):3307, 1635, 1544, 1518, 1466, 1297, 1237, 1138cm^{-1}; ^{1}HNMR($CDCl_3$,300Hz,*δppm*):6.81(m, 3H, Ar-H), 5.65(bs, 1H, NH), 4.57(t, 2H, *J*=6.0Hz, OCH_2CH_2), 4.38(t, 2H, *J*=6.0Hz, $CH_2CH_2ONO_2$), 4.04(t, 2H, *J*=5.7Hz, $ArCH_2NH$), 3.85(s, 3H, OCH3), 2.21(t, 2H, *J*=7.6Hz, CH_2CONH), 1.94(m, 4H, $OCH_2CH_2CH_2CH_2ONO_2$), 1.13-1.66(m, 11H, $CH_2CH_2CH_2CH_2CH_2CH(CH_3)_2$), 0.86(d, 6H, J=6.7Hz, $CH(CH_3)_2$); MS(ESI,m/z):425.3($M+H^+$, base peak);Anal. Calcd. for $C_{22}H_{36}N_2O_6$:C 62.26,H 8.49,N 6.60;Found C 62.10, H8.84, N 6.41.

CONCLUSION

In conclusion, we have synthesized a new series of nitric oxide-releasing derivatives of capsaicin and evaluated them for their TRPV1 agonist activity, nitric oxide release activity and analgesic activity. These compounds showed TRPV1 agonist activity as compared with capsaicin and their nitric oxide release activity was identified. Compare the analgesic activity of compounds, and we found that Among the compounds with furazan ring (B_3, B_4, B_5, B_6), the length of the linker between furan ring and capsaicin core is related to the analgesic activity. The length of linker in B_5 or B_6 is longer than it in B_3 or B_4 .And the analgesic activity of B_5 or B_6 is better than B_3 or B_4. As a NO donor, 2-oxopropyl nitrate is better than butyl nitrate, because among the compounds with nitric acid ester portion (B_7, B_8, B_9, B_{10}), the analgesic activity of compounds(B_7, B_8) with 2-oxopropyl nitrate portion is better than the compounds (B_9, B_{10}) with butyl nitrate portion. B_2, B_5, B_8 **s**how better potent analgesic activity than capsaicin. Based on these results, some of these molecules and NO donors can be considered as lead candidates for the further development of nitric oxide-releasing derivatives of capsaicin as analgesic drugs.

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