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Synthesis and vasodilator effects of rutaecarpine analogues which might be involved transient receptor potential vanilloid subfamily, member 1 (TRPV1)

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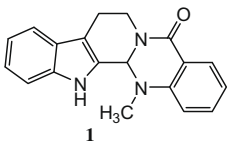
ABSTRACT

Rutaecarpine is the major alkaloid component of Wu-Chu-Yu, a well known Chinese herbal drug. It has been reported that rutaecarpine causes the vasodilator, hypotensive effects by stimulation of CGRP synthesis and release via activation of TRPV1. In present study, 23 rutaecarpine analogues were designed and synthesized. Then, the vasodilator effects of theses compounds were screened by rat aortic ring experiment. The result showed that the 14-N atom of rutaecarpine might be the key site for the activity. The 5-carbonyl might make lower contribution to the effect. And simple substitute in indole-ring or quinazoline-ring would not enhance the vasodilator effect unless in proper position with proper group. One of these compounds, 10-methylrutaecarpine, exhibited similar effect with rutaecarpine. Further functional experiments showed its vasodilator and hypotensive effect were related to the stimulation of CGRP release via activation of TRPV1. The vasodilator effects of these compounds were evaluated and the structure–activity relationship was elucidated for the first time. The results suggested a new direction of valuable TRPV1 agonist as anti-hypertensive drugs.

1. Introduction

Evodiamine (**1**) and rutaecarpine (**2**) are the two major quinazolinocarbolin alkaloidal components in Evodiae Fructus, also known as 'Wu-Chu-Yu', which has been prescribed for the treatment of hypertension in traditional Chinese medicine. Both of them can relax vascular smooth muscle,[1] and markedly decrease the blood pressure.[2]



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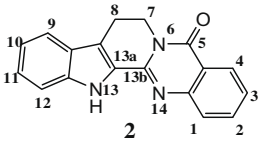

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Previous investigations have demonstrated that the rutaecarpine produces the vasodilator,[3] positive inotropic and chronotropic[4] and myocardium-protective effects[5] via stimulation of CGRP synthesis and release by activation of Transient receptor potential vanilloid subfamily, member 1 (TRPV1). Furthermore, the hypotensive effect of rutaecarpine is mediated by activation of TRPV1, a new anti-hypertensive target.[1]

TRPV1, also known as vanilloid receptor subtype 1(VR1), is now recognized as a molecular integrator of inflammatory mediators.[6] According to molecular mechanisms recently described to play a critical role in pain transmission is the TRPV1 receptor, which provides an opportunity for development of selective agonists or antagonists as agents to treat pathological pain.[7] But only few cardiovascular researches focus on the receptor's agonists or antagonists were reported.

TRPV1 is a nonselective cation channel.[8] Upon TRPV1 activation, both sodium and calcium ions enter the cell through the channel pore, resulting in cell membrane depolarization. TRPV1 activation also leads to release of substance P (SP) and calcitonin gene related peptide (CGRP), which enhance peripheral sensitization of tissue.[6] CGRP is a very potent vasodilator, approximately 100–1000 times more potent than other vasodilators such as adenosine, SP or acetylcholine, and it possesses positive chronotropic and inotropic effects.[9] Several lines of evidence suggest that CGRP plays an important role in regulating vascular resistance and regional organ blood flow, all the more, in the initiation, progression and maintenance of hypertension.[10] Researchers have been pursuing agents that can stimulate the CGRP synthesis and release via activation of TRPV1.

Since modifications of rutaecarpine structure have been investigated for a long time, it has been concluded that rutaecarpine derivates have variety of biological activities such as cytotoxicity,[11] or inhibitory effects on COX-II.[12] But few studies concern the TRPV-1-related vasodilator effects of rutaecarpine derivates. The potential structure–activity relationship remains unclear.

In order to investigate the key region of rutaecarpine which keeps its vasodilator effect and improves its drug-like property,

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several rutaecarpine analogues were designed and synthesized in present study. Then, the vasodilator effect of theses compounds were screened by rat aortic ring experiments. On the other side, in order to explore whether the mechanism of the vasodilator and hypotensive effects of rutaecarpine derivates is related to TRPV1, further aortic ring experiments and anti-hypertensive effect on rat model were conducted by using 10-methyl-rutaecarpine which exerts similar as rutaecarpine. Meanwhile the similarity of structures of rutaecarpine with that of the traditional agonists of TRPV1 is very limited, suggesting that this novel class of enhancers could be a new type of pharmacological activity for TRPV1 function with vasodilator effect.

2. Chemistry

We synthesized 23 rutaecarpine analogues systematically, which included several different skeletons. The synthesis of rutaecarpine and its simple substitutes (**9a**–**m**) are shown in a general sense in Scheme 1. Most of these compounds have been synthesized before in several ways by many others. In this case, anthranilic acid or its substituted derivates were used, they were converted to the activated intermediate (**4** or **5**) by $SOCl_2$, followed reaction with piperdin-2-one to obtain the derivates of 8,9-dihydro-6*H*-pyrido[2,1-*b*]quinazolin-11(7*H*)-one (**6a**–**e**). After the reaction with substituted diazobenzene (**7a–g**) to get hydrazone analogues (**8a**–**n**), the target compounds were obtained by Fischer condensation in high temperature in PPA.[13,14]

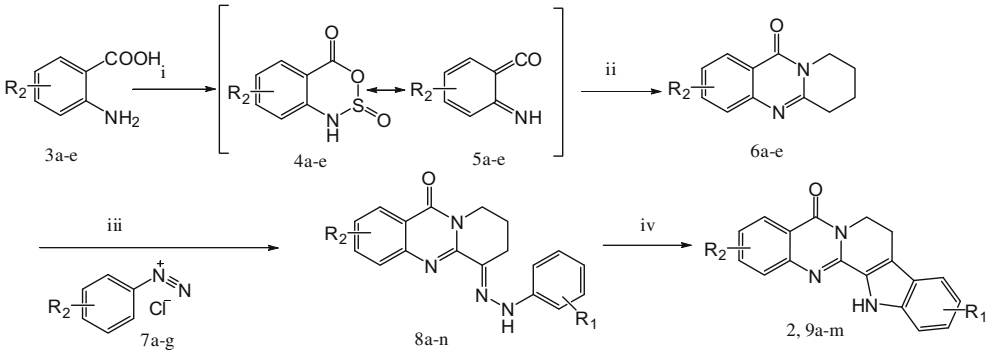
In order to find out whether the 14-N contributes to the activity, we designed and synthesized the 14-O and 14-C analogues of rutaecarpine. The 14-O derivates (**13a**–**d**) could be obtained by reactions of salicyl chloride and substituted dihydrocarbolines (see Scheme 2).[15] Meanwhile, the 14-C derivates (**14a**, **14b**) could be synthesized from tryptamine or its substitute refluxed with ninhydrin (see Scheme 3).[16]

Furthermore, rutaecarpine was reduced by $LiAlH_4$ (see Scheme 4). Two products were obtained (**15a**, **15b**). Finally, we synthesized **18** and **20** to discuss the situation of the connection between indole and quinazoline (see Schemes 5 and 6).[17]

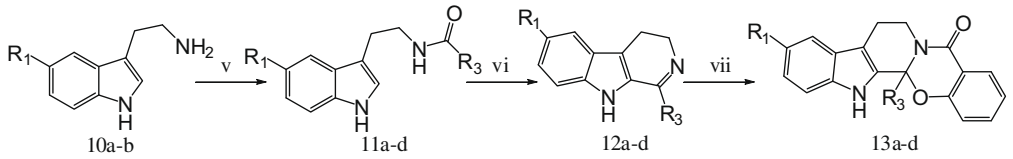
3. Result and discussion

Vasodilator responses to the target compounds were evaluated by the rat aortic ring experiments. The effects caused by the compounds in diverse concentration were measured and the EC_{50} were calculated, respectively. The results were shown in Table 1.

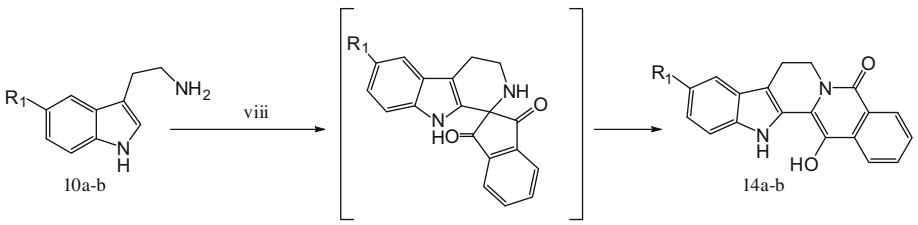
Compounds **9a–f** are substituted on the indole-ring with group of alkyl, alkoxyl, acyl or halide. The vasodilator effect of these compounds had no much difference with rutaecarpine, of which compound **9a** (10-methylrutaecarpine) was the best candidate. Unfortunately, in the cases of compounds **9g–m**, which are halides of rutaecarpine on the quinazoline-ring, showed lower activity of



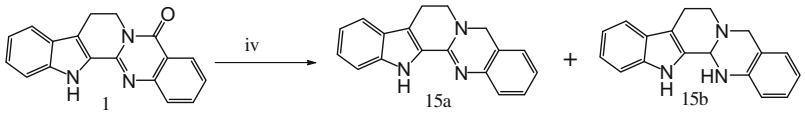
Scheme 1. Synthesis of rutaecarpine and its substitutes. Reagents and conditions: (i) $SOCl_2$, benzene, N_2, reflux, 2 h; (ii) 2-piperidinone, benzene, N_2, rt; (iii) 50% HOAc, −5 °C, 3 h; (iv) PPA, 160 °C, 2 h.



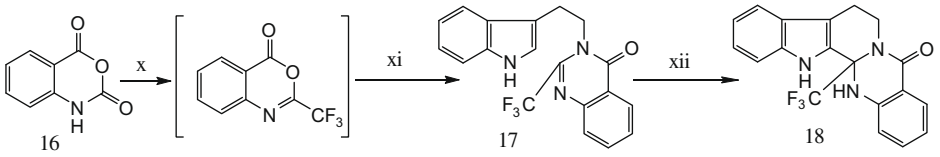
Scheme 2. Synthesis of 14O-rutaecarpine and its derivates. Reagents and conditions: (v) R_3 = H:HCO_2Et, reflux, 5 h; R_3 = Me:Ac_2O, Et_3N, DCM, rt; (vi) R_3 = H:$POCl_3$, DCM, rt; R_3 = Me:P_2O_5, xylene, reflux; (vii) salicyl chloride, benzene rt.



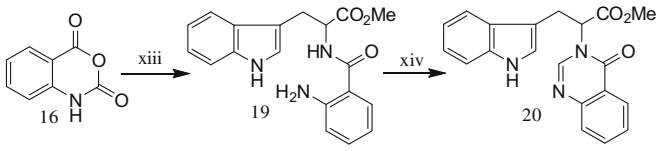
Scheme 3. Synthesis of 14C-rutaecarpine and its derivates. Reagents and conditions: (viii) ninhydrin, EtOH/H_2O/H_2SO_4, 80 °C, 12 h.



Scheme 4. Reduction of rutaecarpin. Reagents: (iv) $LiAlH_4$, THF.



Scheme 5. Synthesis of 13b-trifluororutaecarpine. Reagents and conditions: (x) $(CF_3CO)_2O$, pyridine, 25 °C, 15 min, 115 °C, 5 min; (xi) tryptamine, 115 °C, 30 min; (xii) HCl/HOAc, reflux.



Scheme 6. Synthesis of methyl 3-(1*H*-indol-3-yl)-2-(4-oxoquinazolin-3(4*H*)-yl)propanoate. Reagents and conditions: xiii) tryptophan methyl ester hydrochloride, MeCN, Et_3N, 1 h; (xiv) $HC(OEt)_3$, reflux, 5 h.

vasodilatation. It may be due to their higher lipophilicity which reduces their solubility in water.

In order to test our hypothesis that the 14-N contributes to the activity, compounds of 14-O or 14-C instead of 14-N in rutaecarpine skeleton were prepared. The changes sharply decreased the activity of vasodilatation, especially in the 14-C analogues. It may be the result that the compounds **14a** and 1**4b** are both replaced the 14-N by enol structure which is rather more different to a nitrogen atom than an oxygen atom. All these results suggest that the 14-N is the key structure to keep the vasodilator effect and it is irreplaceable.

Compounds **15a** and **15b** are two 5-carbonyl-reduced products of rutaecarpine. Both of their 5-carbonyls were converted to methylenes. Furthermore, according to compound **15b**, the double bond between 13b and 14-N was also reduced. It is interesting result that they kept the vasodilator effect of rutaecarpine to some extent. It may lead to the conclusion that the absence of 5-carbonyl is acceptable. While in the other hand, the difference between the two compounds, whether 14-N is saturated or not, have no much effect on the activity result. These results further confirmed the key role of 14-N in keeping the vasodilator effect of rutaecarpine.

Compounds **18** and **20** were synthesized in order to investigate the significance of the region around 13a and 13b sites of rutaecarpine. If a group such as trifluoro-methyl was introduced to the 13b site of rutaecarpine (**18**), the dihedral angle between indole-ring and qunazoline-ring would be changed which may be similar with evodiamine (**2**). The result showed that this change decreased the activity somewhat. Because the trifluoromethyl is not a very representative group, the introductions of other more general groups to 13b site are proceeding. Interestingly, the result of compound **20** was significant. Because of the absence of the bond between 13a–13b sites, the flat conformation of rutaecarpine was broken and the vasodilator activity was abolished.

In summary, simple substitution cannot improve the vasodilator activity of rutaecarpine significantly. The active center of rutaecarpine analogues should be the irreplaceable 14-N and its neighbour sites. The connection between 13a and 13b sites and flat-like conformation are vital to the vasodilator effect. The 5-carbonyl might be less important to the activity. Associating with other TRPV1 ligands, quinazoline-ring might be the pharmacore of rutaecarpine.

For the studies on the involvement of TRPV1 and endogenous CGRP in vasodilator responses to the compounds, using the compound **9a** as a representative, the rat aortic rings were exposed to capsaicin, a TRPV1 agonist as the desensitizer, or capsazepin, a competitive TRPV1 antagonists, or CGRP-(8-37) a selective CGRP receptor antagonist, for 10 min, respectively. Then, the vasodilator effect to compound **9a** was tested. The results were shown in Figure 1. The results suggest that the vasodilator effect of the compound **9a** is similar to that of rutaecarpine, it might involve the endogenous CGRP via activation of TRPV1.

In addition, we tested the depressor effect of the compound **9a** and the possible mechanism in the phenol-induced hypertensive rats. Acute administration of the compound **9a** (30, 100 or 300 g/kg, i.v.) caused a depressor effect in a dose-dependent manner (Fig. 2), which was blocked by capsaicin (used to deplete the CGRP from sensory nerves) as shown by an 85% decrease in mean arterial pressure (MAP) (Fig. 3). These results suggest that the hypotensive effect of compound **9a**, just as rutaecarpine, might be mediated by stimulation of CGRP release via activation of TRPV1 in the phenol-induced hypertensive rats.

In conclusion, some compounds synthesized acted similarly as rutaecarpine, while some had negative responses. The functional experiments and anti-hypertensive effects on rat model showed that the vasodilator and hypotensive activity of rutaecarpine analogues might be related to the stimulation of CGRP release via activation of VR1. But the binding site of the compounds with TRPV1's structure is still unknown and the mechanism responsible for the depressor and vasodilator effects of rutaecarpine and its derivates are not clearly elucidated. The molecular pharmacological studies of these compounds are proceeding in order to find the direct evidence of activating TRPV1 and their possible binding sites. The preliminary structure–activity relationships of rutaecarpine analogues described in present this study might be more descriptive than mechanistic. But this suggest that rutaecarpine can be a potential anti-hypertensive leading compound with novel mechanism.

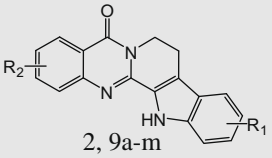
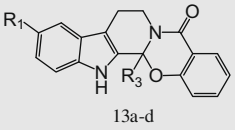
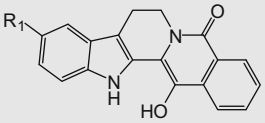
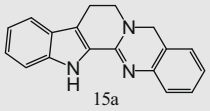
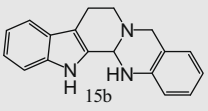
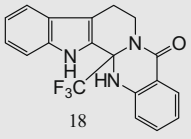
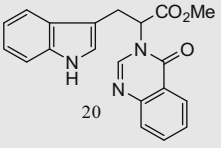
4. Experimental

4.1. Chemical synthesis

4.1.1. Materials and instrumentation

All materials were obtained and used as received from commercial sources unless specially noted. The purity of all compounds were examined by high performance liquid chromatography. ^1H NMR and ^{13}C NMR spectra were obtained at 300 MHz or

Table 1
The vasodilator effects of rutaecarpine and its derivates

Compound	R_1	R_2	R_3	EC_{50} (mol L^{-1})	E_{max}[a] ± REM (%)
			 2, 9a-m		
2	H	H	–	1.33×10^{-6}	79.3 ± 3.5
9a	10-CH_3	H	–	1.24×10^{-6}	85.1 ± 3.2*
9b	12-CH_3	H	–	2.99×10^{-6}	47.0 ± 5.5**
9c	10-$COCH_3$	H	–	3.25×10^{-6}	68.2 ± 2.1*
9d	10-OCH_3	H	–	5.51×10^{-5}	42.6 ± 3.1**
9e	12-Cl	H	–	2.44×10^{-6}	50.1 ± 1.1**
9f	9,10,12-TriCl	H	–	2.31×10^{-6}	51.0 ± 1.3**
9g	H	2-Cl	–	2.11×10^{-5}	36.6 ± 1.5**
9h	H	3-Cl	–	3.23×10^{-5}	40.6 ± 4.6**
9i	H	4-Cl	–	1.68×10^{-5}	39.6 ± 2.6**
9j	10-OCH_3	2-Cl	–	2.25×10^{-5}	33.9 ± 1.7**
9k	10-OCH_3	3-Cl	–	2.11×10^{-6}	55.2 ± 3.2**
9l	10-OCH_3	4-Cl	–	1.97×10^{-5}	37.1 ± 2.1**
9m	10-OCH_3	3-Br	 13a-d	9.40×10^{-6}	40.3 ± 2.8**
13a	H	–	H	2.20×10^{-4}	39.8 ± 4.2**
13b	H	–	CH_3	1.59×10^{-3}	29.9 ± 5.3**
13c	OCH_3	–	H	6.36×10^{-2}	13.0 ± 2.2**
13d	OCH_3	–	CH_3	9.34×10^{-4}	31.6 ± 6.7**
			 14a-b		
14a	H	–	–	1.54×10^{-2}	14.1 ± 3.1**
14b	OCH_3	–	–	5.70×10^{-3}	13.2 ± 2.0**
		 15a	 15b		
15a	–	–	–	2.51×10^{-5}	68.9 ± 2.4*
15b	–	–	–	2.01×10^{-5}	63.6 ± 1.9*
		 18	 20		
18	–	–	–	7.86×10^{-6}	42.5 ± 4.3*
20	–	–	–	5.46×10^{-3}	15.5 ± 2.6**

[a] E_{max} ± REM, $n = 6–8$, *$P < 0.5$ versus rutaecarpine, **$P < 0.01$ versus rutaecarpine.

400 MHz using Me_4Si as an internal standard. Mass spectras were recorded on Qstar LC/MS instrument. Chromatography was performed with commercial silica gel (300–400 mesh).

4.1.2. General procedure for preparation of rutaecarpine substitutes (2, 9a–m)

To a solution of anthranilic acid (or its substituted derivates, **3a**–**e** 0.1 mol) in dry benzene (300 ml) was added $SOCl_2$ (60 g, 0.5 mol) dropwise under N_2. After refluxed for 2 h, the mixture was evaporated under 25 °C. The residue was dissolved in dry benzene (300 ml), followed adding piperdin-2-one (9.9 g, 0.1 mol). The mixture was stirred at rt for 14 h under N_2. The mixture was concentrated in vacuum and then diluted with $CHCl_3$. The solution was basified by 10% Na_2CO_3, then washed by H_2O, brine and dried (Na_2SO_4). The derivates of 8,9-dihydo-6*H*-pyrido [2,1-*b*]quinazolin-11(7*H*)-one (**6a–e**) was obtained by column chromatography (EtOAc/petroleum ether = 1:4) .

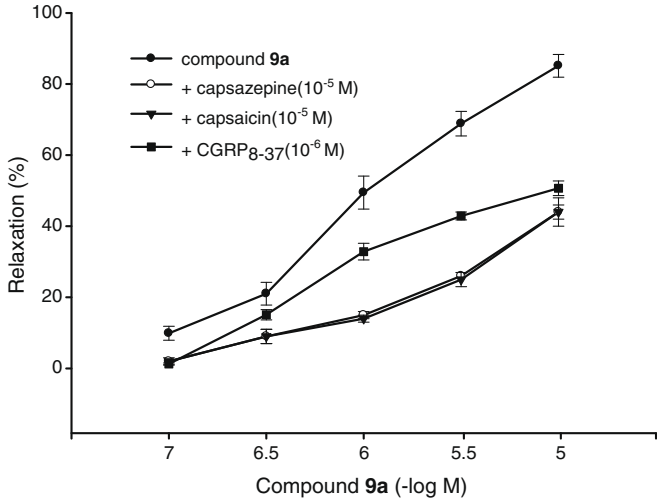


Figure 1. Concentration–response curve of vasodilator effect of compound **9a** to rat aortic rings. Capsaicin was exposed for 20 min, and then washed with fresh Krebs' solution. CGRP-(8-37) or capsazepine was exposed for 10 min before vasodilator response to compound **9a** (value represent mean ± REM, $n = 8$, $^{**}P < 0.01$ vs compound **9a**).

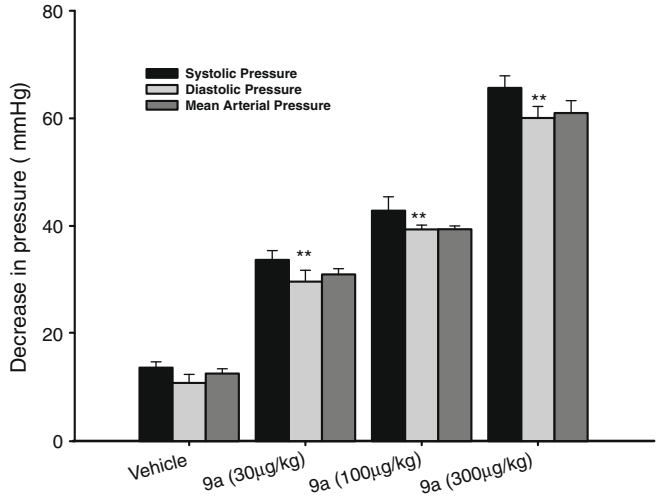


Figure 2. Effect of compound **9a** on blood pressure (value represent mean ± REM, $n = 5$–9, $^{*}P < 0.05$, $^{**}P < 0.01$ vs vehicle).

To a solution of aniline (or its substitutes, 0.01 mol) in 20% HCl was added $NaNO_2$ aqueous solution (6.9 g in 5 ml) dropwise in 0 °C. The diazobenene chloride (**7a–g**) was obtained by continued stirring for further 30 min in this temperature, whereafter the pH of this reaction solution was adjust to pH 4 by adding sodium acetate. The mixture was diluted with glacial acid (5 ml) and a solution of **6a–e** in 50% acetic acid (10 ml) was added dropwise. The reaction mixture was stirred for 3 h at 5 °C. The mixture is then allowed to stand overnight in a refrigerator. The precipitated crystals were collected and washed with water, The obtained 6-aromatic hydrazono-11-oxo-tetrahydro-11*H*-pyrido[2,1-*b*]quinazolines' derivates (**8a–n**) could be used without further purification or recrystallized from n-propanol if necessary.

Compounds **8a–n** (0.01 mol) were heated in 20 g of polyphosphoric acid at 160 °C for 60 min under N_2. After cooling, the mixture was diluted with water and the pH was adjusted to 9 by ammonium hydroxide solution. The precipitated solid was filtered, then recrystallized from DMF. The products (**2** or **9a–m**) were obtained by column chromatography (EtOAc/petroleum ether = 1:5) and recrystallized from EtOAc. The purification of products were determinated by HPLC.

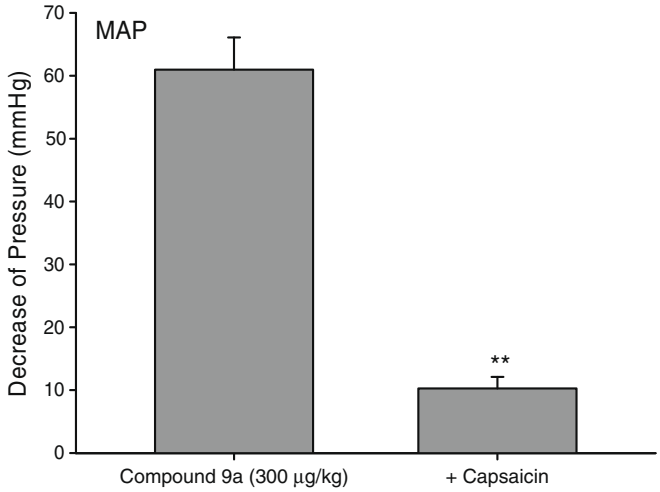


Figure 3. Effect of pre-treating with capsaicin on depressor effect of compound **9a** (values represent mean ± REM, $n = 5$–9, $^{**}P < 0.01$ vs compound **9a**). MAP: mean arterial pressure.

4.1.2.1. Rutaecarpine (2). Starting with anthranilic acid and using aniline. $C_{18}H_{13}N_3O$. Mp: 255–256 °C; MS (*m*/*z*): 287 (M^+); ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.23 (t, $J = 6.8$ Hz, 2H), 4.6 (t, $J = 6.8$ Hz, 2H), 7.17 (t, $J = 7.2$ Hz, 1H), 7.31 (t, $J = 7.2$ Hz, 1H), 7.37 (d, $J = 4.2$ Hz, 1H), 7.42 (m, 1H), 7.62 (d, $J = 4.0$ Hz, 1H), 7.65 (d, $J = 7.2$ Hz, 1H), 7.70 (m, 1H), 8.31 (dd, $J = 0.8$, 8.0 Hz, 1H), 9.56 (1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.7, 41.3, 112.2, 118.6, 120.1, 120.7, 121.1, 125.6, 125.7, 126.3, 126.5, 127.0, 127.3, 134.4, 138.4, 145.1, 147.3, 161.5.

4.1.2.2. 10-Methylrutaecarpine (9a). Starting with anthranilic acid and using *p*-toluidine. $C_{19}H_{15}N_3O$. Mp: 295–297 °C; MS (*m*/*z*): 301 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.39 (s, 3H), 3.14 (t, $J = 6.9$ Hz, 2H), 4.44 (t, $J = 6.9$ Hz, 2H), 7.10 (dd, $J = 1.5$, 8.7 Hz, 1H), 7.39 (d, $J = 4.8$ Hz, 1H), 7.41 (d, $J = 2.1$ Hz, 1H), 7.46 (t, $J = 6.3$ Hz, 1H), 7.67 (d, $J = 7.4$ Hz, 1H), 7.80 (t, $J = 7.8$ Hz, 1H), 8.16 (dd, $J = 1.2$,7.8 Hz, 1H), 11.0 (1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 19.2, 21.3, 41.1, 112.5, 117.6, 119.4, 120.9, 125.4, 126.1, 126.7, 126.8, 126.9, 127.2, 128.8, 134.6, 137.4, 145.6, 147.7, 160.9.

4.1.2.3. 12-Methylrutaecarpine (9b). Starting with anthranilic acid and using *o*-toluidine. $C_{19}H_{15}N_3O$. Mp: 291–293 °C; MS (*m*/*z*): 301 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.38 (s, 3H), 3.11 (t, $J = 6.9$ Hz, 2H), 4.40 (t, $J = 6.9$ Hz, 2H), 7.08 (dd, $J = 1.5$, 8.7 Hz, 1H), 7.36 (m, 1H), 7.40 (d, $J = 8.7$ Hz, 1H), 7.44 (m, 1H), 7.67 (d, $J = 7.4$ Hz, 1H), 7.82 (t, $J = 7.8$ Hz, 1H), 8.15 (dd, $J = 1.2$, 7.8 Hz, 1H), 11.61 (1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 19.3, 21.4, 41.2, 112.6, 117.7, 119.5, 121.1, 125.5, 126.2, 126.8, 126.9, 127.3, 128.8, 134.6, 137.4, 145.6, 145.7, 147.8, 161.0.

4.1.2.4. 12-Acetylrutaecarpine (9c). Starting with anthranilic acid and using *p*-acteyl-aniline. $C_{20}H_{15}N_3O$. Mp: 267–268 °C; ^{1}H NMR (400 MHz, DMSO): δ 2.74 (s, 3H), 3.23 (t, $J = 6.8$ Hz, 2H), 4.58 (t, $J = 6.8$ Hz, 2H), 7.23 (m, 1H), 7.43 (t, $J = 3.6$ Hz, 1H), 7.74 (m, 2H), 7.87 (m, 2H), 8.29 (d, $J = 8.0$ Hz, 1H), 11.00 (1H, NH); ^{13}C NMR (100 MHz, DMSO): δ 19.5, 26.6, 41.0, 117.5, 119.7, 121.1, 121.2, 126.2, 126.4, 126.9, 127.0, 127.1, 128.0, 129.0, 134.4, 136.7, 144.2, 147.5, 161.6, 199.6.

4.1.2.5. 10-Methoxylrutaecarpine (9d). Starting with anthranilic acid and using *p*-methoxyl aniline. $C_{19}H_{15}N_3O_2$. Mp: 252–254 °C; MS (*m*/*z*): 317 (M^+); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.16 (t, $J = 6.8$ Hz, 2H), 3.80 (s, 3H), 4.45 (t, $J = 6.8$ Hz, 2H), 6.92 (dd,

J = 2.0, 8.8 Hz,1H), 7.12 (d, J = 2.0 Hz, 1H), 7.38 (d, J = 8.8 Hz, 1H), 7.47 (t, J = 7.2 Hz, 1H), 7.67 (d, J = 7.6 Hz, 1H), 7.81 (t, J = 7.6 Hz, 1H), 8.16 (d, J = 7.2 Hz, 1H), 11.74 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 19.06, 40.90, 55.37, 100.49, 113.45, 115.99, 117.47, 120.66, 125.11, 125.91, 126.43, 126.63, 127.44, 133.99, 134.45, 145.37, 147.46, 153.82, 160.66.

4.1.2.6. 12-Chlororutaecarpine (9e). Starting with anthranilic acid and using *p*-chloro-aniline. $C_{18}H_{12}N_3OCl$. Mp: 215–216 °C; MS (m/z): 321 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.18 (t, J = 6.9 Hz, 2H), 4.46 (t, J = 6.9 Hz, 2H), 7.11 (t, J = 7.8 Hz, 1H), 7.34 (d, J = 7.5 Hz, 1H), 7.49 (m, 1H), 7.63 (d, J = 8.1 Hz, 1H), 7.75 (d, J = 8.4 Hz, 1H), 7.81 (m, 1H), 8.17 (dd, J = 1.2, 7.8 Hz, 1H), 11.83 (1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 19.3, 41.02, 117.2, 119.3, 119.4, 121.2, 124.8, 126.6, 126.9, 127.1, 127.3, 128.8, 134.7, 135.8, 134.0, 145.1, 147.6, 160.9.

4.1.2.7. 9,10,12-Trichlororutaecarpine (9f). Starting with anthranilic acid and using 2,4,5-tri-chloroaniline. $C_{18}H_{10}N_3OCl_3$. Mp: 260.9–263.7 °C; MS (m/z): 391 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.44 (t, J = 6.9 Hz, 2H), 4.45 (t, J = 6.9 Hz, 2H), 7.48 (m, 1H), 7.54 (s, 1H), 7.74 (d, J = 7.5 Hz, 1H), 7.80 (m, 1H), 8.15 (d, J = 7.2 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 20.5, 40.8, 117.0, 118.7, 121.3, 123.0, 123.3, 124.7, 125.0, 126.8, 126.9, 127.2, 130.9, 134.7, 135.1, 144.3, 147.3, 160.6.

4.1.2.8. 2-Chlororutaecarpine (9g). Starting with 2-amino-4-chlorobenzoicacid and using aniline. $C_{18}H_{12}N_3OCl$. Mp: 296–298 °C; MS (m/z): 321 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.19 (t, J = 6.9 Hz, 2H), 4.44 (t, J = 6.9 Hz, 2H), 7.10 (m, 1H), 7.28 (m, 1H), 7.47 (dd, J = 2.1, 9.0 Hz, 1H), 7.51 (d, J = 8.4 Hz, 1H), 7.63 (m. 2H), 8.14 (d, J = 8.4, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 19.2, 41.2, 112.9, 118.9, 119.8, 120.2, 120.3, 125.2, 125.3, 125.7, 126.3, 126.9, 129.0, 139.1, 139.2, 146.8, 148.9, 160.4.

4.1.2.9. 3-Chlororutaecarpine (9h). Starting with 2-amino-5-chlorobenzoicacid and using aniline. $C_{18}H_{12}N_3OCl$. Mp: 320–322 °C; MS (m/z): 321 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.21 (t, J = 6.9 Hz, 2H), 4.45 (t, J = 6.9 Hz, 2H), 7.10 (m, 1H), 7.28 (m, 1H), 7.49 (m, 1H), 7.64 (m, 1H), 7.69 (s, 1H), 7.81 (dd, J = 1.2,9 Hz, 1H), 8.07 (d, J = 2.7 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 19.4, 41.6, 113.1, 118.8, 120.4, 120.6, 122.4, 125.3, 125.5, 126.1, 127.2, 129.2, 130.6, 135.1, 139.2, 146.2, 146.7, 160.3.

4.1.2.10. 4-Chlororutaecarpine (9i). Starting with 2-amino-6-chlorobenzoicacid and using aniline. $C_{18}H_{12}N_3OCl$. Mp: 313–314 °C; MS (m/z): 321 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.18 (t, J = 6.9 Hz, 2H), 4.39 (t, J = 6.9 Hz, 2H), 7.10 (m, 1H), 7.28 (m, 1H), 7.46 (dd, J = 1.2, 7.2 Hz, 1H), 7.50 (m, 1H), 7.61 (dd, J = 1.2, 8.4 Hz, 1H), 7.65 (m, 1H), 7.71 (t, J = 8.1 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 19.3, 41.3, 113.0, 118.0,118.9, 120.2, 120.4, 125.2, 155.3, 126.5, 126.9, 128.7, 133.3, 134.5, 139.1, 146.2, 150.3, 159.0.

4.1.2.11. 2-Chloro-10-methoxylrutaecarpine (9j). Starting with 2-amino-4-chlorobenzoicacid and using 4-methoxylaniline. $C_{19}H_{14}N_3O_2Cl$. Mp: 333–334 °C; MS (m/z): 351 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.16 (t, J = 6.9 Hz, 2H), 3.80 (s, 3H), 4.43 (t, J = 6.9 Hz, 2H), 6.94 (dd, J = 2.4, 8.7 Hz, 1H), 7.10 (d, J = 2.1 Hz, 1H), 7.40 (dd, J = 0.6, 8.7 Hz, 1H), 7.45 (dd, J = 2.1, 8.1 Hz, 1H), 7.60 (d, J = 0.6, 2.1 Hz, 1H), 8.13 (dd, J = 0.6, 8.7, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 19.3, 41.3, 55.8, 101.1, 113.8, 116.6, 118.4, 119.8, 125.3, 125.6, 126.2, 127.3, 128.9, 134.5, 139.2, 146.9, 148.9, 154.3, 160.4.

4.1.2.12. 3-Chloro-10-methoxylrutaecarpine (9k). Starting with 2-amino-5-chlorobenzoicacid and using 4-methoxylaniline. $C_{19}H_{14}N_3O_2Cl$. Mp: 283–284 °C; MS (m/z): 351 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.15 (t, J = 6.9 Hz, 2H), 3.79 (s, 3H), 4.43 (t, J = 6.9 Hz, 2H), 6.93 (dd, J = 2.4, 9.0 Hz, 1H), 7.09 (d, J = 2.4 Hz, 1H), 7.39 (d, J = 9.0 Hz, 1H), 7.64 (d, J = 8.7 Hz, 1H), 7.77 (dd, J = 2.4, 8.4 Hz, 1H), 8.05 (d, J = 2.4 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 19.3, 41.4, 55.8, 101.1, 113.7, 116.5, 118.1, 122.1, 125.4, 125.8, 127.4, 128.9, 130.2, 134.4, 134.7, 145.9, 146.6, 154.3, 160.0.

4.1.2.13. 4-Chloro-10-methoxylrutaecarpine (9l). Starting with 2-amino-6-chlorobenzoicacid and using 4-methoxylaniline. $C_{19}H_{14}N_3O_2Cl$. Mp: 300–301 °C; MS (m/z): 351 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.15 (t, J = 6.9 Hz, 2H), 3.80 (s, 3H), 4.38 (t, J = 6.9 Hz, 2H), 6.94 (dd, J = 2.4, 8.7 Hz, 1H), 7.11 (d, J = 2.1 Hz, 1H), 7.40 (dd, J = 0.6, 9 Hz, 1H), 7.44 (dd, J = 1.2, 4.8 Hz, 1H), 7.59 (dd, J = 1.2, 8.1 Hz, 1H), 7.69 (t, J = 8.1, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 19.3, 41.2, 55.9, 101.3, 113.7, 116.5, 117.9, 118.3, 125.4, 126.3, 127.2, 128.5, 133.2, 134.3, 134.5, 146.1, 150.3, 154.3, 159.0.

4.1.2.14. 2-Bromo-10-methoxylrutaecarpine (9m). Starting with 2-amino-4-bromobenzoicacid and using 4-methoxylaniline. $C_{19}H_{14}N_3O_2Br$. Mp: 288–289 °C; MS (m/z): 395 (M−1), 397 (M^++1); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.14 (t, J = 6.9 Hz, 2H), 3.79 (s, 3H), 4.43 (t, J = 6.9 Hz, 2H), 6.93 (dd, J = 2.4, 8.1 Hz, 1H), 7.09 (d, J = 2.1 Hz, 1H), 7.38 (d, J = 9.0 Hz, 1H), 7.57 (d, J = 8.7 Hz, 1H), 7.90 (dd, J = 2.4, 8.7 Hz, 1H), 8.19 (d, J = 2.4 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 19.3, 41.5, 55.8, 101.9, 113.8, 116.6, 118.2, 118.3, 122.5, 125.4, 127.3, 128.9, 129.1, 134.4, 137.4, 146.1, 146.9, 154.3, 159.9.

4.1.3. General procedure for preparation of 14-O rutaecarpines (Method 1) (13a and 13c)

A solution of tryptamine (or 5-methoxyl- tryptamine, 0.125 mol) in ethyl formate (150 ml) was refluxed for 6 h. After evaporating the excess solvent in vacuum, the residue was dissolved in $CHCl_3$, followed washed with saturated $NaHCO_3$ solution, 2% HCl, dried by Na_2SO_4. The *N*-formyltryptamine derivates (**11a** or **11c**) can be used in the next step by evaporating the excess solvent without further purification.

To a solution of **11a** (or 1**1c**, 0.12 mol) in dry CH_2Cl_2 (60 ml) was added $POCl_3$ (10 ml, 0.12 mol) dropwise in ice-bath. The mixture was stirred for 2 h. After evaporating the excess solvent, the residue was dissolved in water, washed with ether. The pH of aqueous layer was adjusted by ammonium hydroxide to pH 10. Then the solution was extracted with CH_2Cl_2, and washed with saline, dried by Na_2SO_4. The crude 4,9-dihydro-carboline derivates (**12a** or **12c**) were obtained by evaporating solvent in vacuum followed recrystallized from ethyl acetate.

To a solution of **12a** (or **12c**, 0.04 mol) and salicyl chloride (prepared from salicyl acid in sulphoxide chloride, 0.04 mol) in dry benzene (200 ml) was stirred in rt for 2 h. The mixture was evaporated under reduced pressure, which followed residue was diluted by CH_2Cl_2. After washed with 10% NaOH, water, saline, the solution was dried by Na_2SO_4. The products (**13a** or **13c**) were obtained by column chromate- graphy ($CHCl_3$) followed recrystallized from ethyl acetate.

4.1.3.1. 6,7,8,13b-Tetrahydro-5-oxo-5*H*-β-carbolin[1,2-*b*] quinazoline-8(6*H*)-one (13a). $C_{18}H_{14}N_2O_2$. Mp: 225–227 °C; MS (m/z): 291.5 (M^++1; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.02 (m, 2H), 3.19 (m, 1H), 4.94 (dd, J = 4.4, 2.8 Hz, 1H), 6.47 (s, 1H), 7.03 (d, J = 8 Hz, 1H), 7.15 (d, J = 7.2, 1H), 7.19 (d, J = 7.2, 1H), 7.29 (t, J = 8 Hz, 1H), 7.43 (d, J = 8 Hz, 1H), 7.49 (t, J = 8 Hz, 1H), 7.61 (d, J = 8 Hz, 1H), 8.06 (d, J = 8 Hz, 1H), 8.34 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.2, 39.1, 81.2, 111.6, 113.7, 116.3, 118.6,

119.4, 120.3, 123., 123.7, 125.9, 127.0, 128.8, 134.2, 137.2, 156.7, 163.0.

4.1.3.2. 6,7,8,13b-Tetrahydro-10-methoxyl-5-oxo-5*H*-β-carbolin[1,2-*b*] quinazoline-8(6*H*)-one (13c). $C_{19}H_{16}N_2O_3$. Mp: 221–223 °C; MS (*m*/*z*): 321.4 (M^++1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.96 (m, 2H), 3.10 (m, 1H), 3.88 (s, 3H), 4.93 (dd, *J* = 5.2, 13.2 Hz, 1H), 6.45 (s, 1H), 6.95 (dd, *J* = 2.4, 8.8 Hz, 1H), 7.02 (s, 1H), 7.03 (d, *J* = 8 Hz, 1H), 7.17 (t, *J* = 7.2 Hz, 1H), 7.32 (d, *J* = 8.8 Hz, 1H), 7.49 (m, 1H), 8.06 (d, *J* = 7.2 Hz, 1H), 8.21 (s,1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.2, 39.1, 55.9, 81.2, 100.9, 112.4, 113.5, 114.1, 116.3, 118.6, 123.0, 126.3, 127.8, 128.8, 132.3, 134.2, 154.6, 156.7, 163.0.

4.1.4. General procedure for preparation of 14-O rutaecarpines (Method 2) (13b and 13d)

To a solution of tryptamine (or 5-methoxyl-tryptamine, 0.050 mol) in CH_2Cl_2 (135 ml) was added a solution of acetic anhydride (9 ml) and triethylamine (16 ml) in CH_2Cl_2 (30 ml) dropwise. The mixture was stirred at rt for 1 h. The mixture was then washed with saturated $NaHCO_3$ aqueous solution, saline and dried by Na_2SO_4. After evaporated the solvent, the crude *N*-acetic-tryptamine derivates (**11b** or **11d**) were obtained and it can be recrystallized in EtOAc–petroleum ether if necessary.

To a solution of **11b** (or **11d**, 0.040 mol) in xylene (150 ml) was added P_2O_5 (24 g, 0.169 mol) at rt with stirring. The mixture was heated to reflux for another 1 h. After cooling, the mixture was diluted with water, washed with ether. The pH of aqueous layer was adjusted by 25% NaOH solution to pH 10. The solution was extracted with CH_2Cl_2. The organic layer was washed with saline, dried by Na_2SO_4. The crude 1-methyl-4,9-dihydro-carboline derivates (**12b** or **12d**) were obtained by evaporating solvent in vacuum followed recrystallized from ethyl acetate.

A solution of **12b** (or **12d**, 0.05 mol) and salicyl chloride (7.8 g, 0.05 mol) in dry CH_2Cl_2 (200 ml) was stirred at rt for 2 h. The mixture was evaporated under reduced pressure, and the residue was diluted by CH_2Cl_2. After washed with 10% NaOH, water, saline, the solution was dried by Na_2SO_4. The products (**13b** or **13d**) were obtained by column chromatography ($CHCl_3$) followed recrystallized from ethyl acetate.

4.1.4.1. 6,7,8,13b-Tetrahydro-13b-methyl-5-oxo-5*H*-β-carbolin-[1,2-*b*] quinazoline-8(6*H*)-one (13b). $C_{19}H_{16}N_2O_2$. Mp:161–163 °C; MS (*m*/*z*): 305.4 (M^++1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.90 (s, 3H), 2.91 (m, 1H), 2.99 (m, 1H), 3.19 (m, 1H), 5.18 (m, 1H), 6.97 (d, *J* = 7.6 Hz, 1H), 7.12 (m, 1H), 7.17 (m, 1H), 7.27 (m, 1H), 7.43 (d, *J* = 8 Hz, 1H), 7.46 (m, 1H), 7.57 (d, *J* = 8 Hz, 1H),8.04 (dd, *J* = 1.6,6.4 Hz, 1H), 8.23 (1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.6, 23.5, 38.1, 87.8, 111.5, 111,5, 116.8, 117.6, 119.3, 120.2, 122.5, 123.4, 126.1, 128.5, 131.9, 134.3, 136.8, 154.5, 160.6.

4.1.4.2. 6,7,8,13b-Tetrahydro-10-methoxyl-13b-methyl-5-oxo-5*H*-β-carbolin[1,2-*b*] quinazoline-8(6*H*)-one (13d). $C_{20}H_{18}N_2O_3$. Mp: 223–225 °C; MS (*m*/*z*): 335.4 (M^++1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.89 (s, 3H), 2.86 (m, 1H), 2.97 (m, 1H), 3.17 (m, 1H), 3.87 (s, 3H), 5.18 (dd, *J* = 4.4,8.8 Hz, 1H), 6.92 (m, 2H), 6.99 (m, 1H), 7.12 (t, *J* = 7.6 Hz, 1H), 7.31 (d, *J* = 8.8 Hz, 1H), 7.46 (m, 1H), 8.04 (dd, *J* = 1.2,6.8 Hz, 1H), 8.16 (1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.6, 23.5, 38.1, 55.9, 87.9, 101.0, 111.2, 112.3, 113.7, 116.8, 117.6, 122.4, 126.5, 128.5, 131.8, 132.6, 134.3, 154.5, 154.5, 160.6.

4.1.5. General procedure for preparation of 14-C rutaecarpine (14a and 14b)

To a solution of tryptamine (or 5-methoxyl-tryptamine, 0.010 mol) in a mixture of ethanol (25 ml), water (25 ml) and 1 M H_2SO_4 (10 ml) was added a aqueous solution of ninhydrin (1.78 g, 0.010 mol) in 50% ethanol dropwise under N_2. The mixture was heated to 80 °C with stirring under N_2 for 18 h. The mixture was filtered and washed with ethanol. The product (**14a** or **14b**) was obtained by column chromatography ($CHCl_3$) followed recrystallized from methanol.

4.1.5.1. 14-Hydroxy-(3,14,15,16,17,18,19,20)-octade-hydro-21-yohimbanone (14a). $C_{19}H_{14}N_2O_2$. Mp: 238–240 °C; MS (*m*/*z*): 302 (M^+); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.05 (t, *J* = 6.4 Hz, 2H), 4.41 (t, *J* = 6.4 Hz, 2H), 7.04 (t, *J* = 7.6 Hz, 1H), 7.15 (t, *J* = 7.6 Hz, 1H), 7.53 (m, 2H), 7.62 (d, *J* = 8.4 Hz, 1H), 7.79 (t, *J* = 7.2 Hz, 1H), 7.98 (d, *J* = 8.0 Hz, 1H), 8.27 (d, *J* = 7.6 Hz, 1H), 9.25 (s, 1H), 11.09 (s, 1H); ^{13}C NMR(100 MHz, DMSO-d_6): δ 19.8, 41.07, 110.9, 112.7, 118.5, 119.4, 120.1, 121.8, 122.7, 124.8, 125.3, 126.9, 127.6, 128.0, 130.5, 132.4, 133.5, 137.6, 159.6.

4.1.5.2. 10-Methoxyl-14-hydroxy-(3,14,15,16,17,18,19,20)-octade-hydro-21-yohimbanone (14b). $C_{20}H_{16}N_2O_3$. Mp: 237–239 °C; MS (*m*/*z*): 332 (M^+); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.05 (t, *J* = 6.4 Hz, 2H), 3.78 (s, 3H), 4.41 (t, *J* = 6.4 Hz, 2H), 6.81 (dd, *J* = 2.0, 6.4 Hz, 1H), 7.04 (s, 1H), 7.51 (m, 2H), 7.78 (t, *J* = 7.2 Hz, 1H), 7.97 (d, *J* = 8.0 Hz, 1H), 8.27 (d, *J* = 7.6 Hz, 1H), 9.21 (s, 1H), 10.95 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 19.9, 41.1, 55.6, 99.7, 110.7, 113.3, 113.5, 120.2, 121.7, 124.7, 125. 3, 126.8, 128.0, 130.3, 132.3, 132.8, 133.6, 153.8, 159.6.

4.1.6. Preparation of rutaecarpene (15a) and rutaecarpane (15b)

To a solution of rutaecarpine (prepared followed the general procedure described above, 2.87 g, 0.01 mol) in dry tetrahydrofuran (100 ml) was added lithium aluminium hydride (1.52 g, 0.04 mol) in portion. The mixture was stirred at rt for about 6 h. The mixture was quenched with 0.1 N HCl. After evaporating the great mass of solvent in vacuum, the residue was extracted with CH_2Cl_2, washed with saline and dried by Na_2SO_4. The two products were obtained by column chromatography (EtOAc/petroleum ether = 1:4) followed recrystallized from ethyl acetate.

4.1.6.1. Rutaecarpene (15a). $C_{18}H_{15}N_3$. Mp: 165–168 °C; MS (*m*/*z*): 272 (M^+–1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.15 (t, *J* = 6.8 Hz, 2H), 4.57 (t, *J* = 6.8 Hz, 2H), 4.59 (s, 2H), 6.98 (d, *J* = 6.8 Hz, 1H), 7.04 (t, *J* = 7.6 Hz, 1H), 7.11 (t, *J* = 7.6 Hz, 1H), 7.18 (t, *J* = 7.6 Hz, 1H), 7.23 (m, 2H), 7.34 (d, *J* = 8.4, 1H), 7.54 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.2, 49.9, 50.5, 112.3, 116.4, 119.5, 120.0, 123.1, 124.8 125.4, 125.5, 128.7, 137.8, 149.4.

4.1.6.2. Rutaecarpane (15b). $C_{18}H_{17}N_3$. Mp: 238–240 °C; MS (*m*/*z*): 274 (M^+–1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.81 (m, 2H), 2.92 (m, 1H), 3.22 (m, 2H), 3.93 (m, 2H), 4.14 (m, 1H), 4.68 (s, 1H), 6.78 (d, *J* = 3.6 Hz, 1H), 6.89 (t, *J* = 7.2, 1H), 7.02 (d, *J* = 7.2 Hz, 1H), 7.10 (t, *J* = 7.2 Hz, 1H), 7.17 (t, *J* = 8.0 Hz, 1H), 7.23 (d, *J* = 8 Hz, 2H), 7.49 (d, *J* = 8 Hz, 1H), 8.59 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.45, 48.8, 55.5, 68.2, 109.8, 110.2, 111.2, 118.7, 119.2, 119.6, 121.0, 122.0, 123.6, 126.7, 127.3, 127.4, 131.6, 136.3, 141.4.

4.1.7. Preparation of 13b-trifluoromethyl-13b,14-dihydrorutaecarpine (18)

To a solution of isatoic anhydride (4.0 g, 0.025 mol) in dry pyridine (80 ml) was added trifluoroacetic anhydride (3.7 g, 0.025 mol) during 15 min with well-stirred at rt. Then the mixture was refluxed for 15 min, whereupon tryptamine (4.0 g, 0.025 mol) was added and refluxed for continued 2 h. After cooling, the mixture was poured into water, and collect the solid formed. The inter-

mediate (**17**), 3-[2-(3-indolyl)-ethyl]-2-(trifluoromethy1)-4(3*H*)-quinazolinone was recrystallized from methanol without further treatment.

Compound **17** was refluxed in a mixture of acetic acid (30 ml) and HCl (5 ml) for 2 h. The mixture was diluted with water (50 ml) and collected the solid formed. The product was obtained by recrystallized from ethyl acetate. $C_{19}H_{14}F_3N_3O$. Mp: 300–301 °C; MS (*m*/*z*): 357 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.80 (m, 1H), 2.96 (m, 1H), 3.30 (m, 1H), 5.15 (dd, J = 6.8, 18 Hz, 1H), 6.87 (m, 2H), 7.10 (m, 1H), 7.24 (m, 1H), 7.39 (m, 1H), 7.54 (m, 1H), 7.59 (m, 1H), 7.79 (dd, J = 2, 10.8 Hz, 1H), 10.84 (1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.1, 37.5, 112.5(split), 114.8(split), 115.3, 119.3(split), 119.9, 123.5, 123.9, 125.2, 125.3(split), 125.4(split), 127.9, 128.1, 131.9, 134.2, 137.2(split), 144.2(split), 161.8.

4.1.8. Preparation of methyl 3-[2-(3-Indolyl)ethyl]-2-(4-quinazolinone-3(4*H*)-yl)-propanoate (20)

A mixture of tryptophan methyl ester hydrochloride (2.54 g, 0.01 mol), isatoic anhydride (1.63 g, 0.01 mol) and triethylamine (1.4 ml, 0.01 mol) was refluxed in acetonitrile (20 ml) for 2 h. The crystals formed were collected after cooling. The product (**19**) *N*-(2′-aminobenzoyl)tryptophan methyl ester could be used to the next step without further purification.

A mixture of *N*-(2′-aminobenzoyl)tryptophan methyl ester (1.69 g, 0.005 mmol) and triethyl orthoformate (20 ml) was refluxed for 10 h. After evaporated excess reagent, the product was obtained by column chromatography (CH_2Cl_2). $C_{20}H_{17}N_3O_3$. Mp: 139–140 °C; MS (*m*/*z*): 348 (M^++1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.63 (m, 1H), 3.76 (m, 1H), 3.80 (s, 3H), 5.37 (dd, J = 4.8,9.6 Hz, 1H), 6.83 (d, J = 2.4 Hz, 1H), 7.07 (m, 1H), 7.15 (m, 1H), 7.29 (d, J = 8.4 Hz, 1H), 7.49 (m, 1H), 7.53 (d, J = 7.6 Hz, 1H), 7.62 (m, 2H), 7.73 (m, 1H), 8.18 (1H, NH), 8.28 (dd, J = 1.2, 8.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.0, 52.9, 59.1, 109.7, 111.5, 118.0, 119.9, 121.7, 122.5, 123.1, 126.6, 126.8, 127.3, 134.5, 136.2, 145.6, 147.4, 160.6, 169.5.

4.2. Biological methods

4.2.1. Animals and reagents

All animals received humane care in compliance with Guide for the *Care and Use of Laboratory Animals* published by the National Institutes of Health. For the development of phenol-induced hypertensive rats, male Sprague-Dawley rats weighing 250–300 g were used in the present study. After anesthesia with sodium pentobarbital (60 mg/kg, i.p.), the left kidney was exposed and injected with 50 μl of 10% phenol in the parenchyma of the lower pole of the kidney. Control rats received 50 μl of 0.9% NaCl in the lower pole of the kidney as a normaotensive control for the hypertensive rats. The rats were kept in cages in a room on a 12-h light: 12-h dark cycle and with free access to tap water and standard rat chow.

Capsaicin, capsazepine, CGRP-(8-37) and dimethyl sulfoxide were purchased from Sigma chemical company (St. Louis, USA). In the study of vasodilator effects, the candidate compounds, capsaicin, and capsazepine were initially dissolved in dimethyl sulfoxide, and further diluted in Kreb's solution to the proper final concentration. The final concentration of dimethyl sulfoxide did not exceed 0.1%, which had no effect on vascular tension. CGRP-(8-37) was dissolved in distilled water. In the case of the hypotensive effect of the compound **9a**, compound **9a** wad dissolved in a mixture of dimethyl sulfoxide and ethanol (2:8, v/v), and capsaicin was dissolved in a vehicle containing 10% Tween 80, 10% ethanol and 80% saline.

4.2.2. Vasodilator responses to the candidate compounds

Male Sprague-Dawley rats weighing 250–300 g were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and the thoracic aorta were rapidly isolated cleaned of fat and connective tissues, and then cut into rings of 4 mm length. The rings were suspended horizontally between two stainless steel wires and mounted in a 5-ml organ chamber filled with warmed (37 °C) and oxygenated (95% O_2 and 5% CO_2) Krebs' solution, which had the following composition (mM): NaCl, 119.0; $NaHCO_3$, 25.0; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 2.5; and glucose, 11.0. Each of the rings' end was connected to a force transducer. The aortic rings were stretched with 2 g resting force, equilibrated for 60 min, and then precontracted with KCl (60 mM). After a maximal response to KCl was observed, the rings were washed with Krebs' solution and equilibrated for another 30 min. The rings were contracted with phenylephrine (2 μM). After the contraction had stabilized, a cumulative concentration–response curve to rutaecarpine and other candidates (10^{-7}–10^{-5} M) was observed.

For the study on the involvement of TRPV1 and endogenous CGRP in vasodilator effect of compound **9a**, the preparations were divided into four groups. After the contraction caused by phenylephrine (2 μM) had stabilized, three groups were exposed to capsaicin (10^{-5} M), a TRPV1 agonist as the desensitizer, for 20 min; capsazepin (10^{-5} M), a competitive TRPV1 antagonists, for 10 min; CGRP-(8-37) (10^{-6} M), a selective CGRP receptor antagonist, for 10 min, respectively. Then, the cumulative vasodilator response to compound **9a** was recorded.

4.2.3. Hypotensive effect of compound 9a

Fifteen days after phenol treatment, rats were divided randomly into six groups and subjected to the following experimental protocols: (1) hypertension group; (2) vehicle group, rats were intravenously treated with 0.1 ml vehicle (a mixture of dimethyl sulfoxide and ethanol, 2:8, v/v); (3) compound **9a** (30 μg/kg) group; (4) compound **9a** (100 μg/kg) group; (5) compound **9a** (300 μg/kg) group, rats of these three groups received a intravenous injection of compound **9a** at the dose of 30, 100 or 300 μg/kg, respectively; (6) capsaicin plus compound **9a** (300 μg/kg) group, which was given capsaicin (50 mg/kg, s.c.) 4 days before compound **9a** administration under anesthesia with sodium pentobarbital.

After anesthesia with sodium pentobarbital, the right jugular vein and carotid artery of rat were cannulated for administration of drugs or for monitoring of blood pressure with a pressure transducer coupled to computerized recorder (BL-NewCentury 410, Chengdu, China).

4.3. Statistical analysis

Data are expressed as means ± SEM (n = 8–12). All values were analyzed using ANOVA and multiple comparison test (the Student–Newman–Keuls t-test), using SPSS 10.0 (SPSS Inc., Chicago, Illinois, USA). The acceptable value of significance was $P < 0.05$.

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