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Discovering selective agonists of endothelial target for acetylcholine (ETA) via diversity-guided pharmacophore simplification and simulation ☆

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Abstract—Two types of lead structures for selective agonists of ETA, a biological target not yet fully elucidated, has first been discovered via diversity-guided pharmacophore simplification and simulation. And it is first demonstrated that potent selective ETA agonists might be useful for protection of endothelium and for prophylaxis and treatment of cardiovascular diseases with endothelial dysfunction such as atherosclerosis and thrombosis.

Considerable information concerning the roles of the endothelium in maintaining the functions of cardiovascular system has been available since it was discovered that acetylcholine (ACh)-induced endothelium-dependent relaxation required an intact endothelium.[1] The loss of vasodilator function in response to ACh testing has been considered as a marker of endothelial dysfunction. In many cardiovascular diseases such as hypertension, heart failure, hypercholesterolemia, and atherosclerosis, the vascular responses to ACh have been shown to be impaired.[2] We envisioned that agonism of endothelial targets for acetylcholine (ETA),[3] which mediates the endothelial-dependent vascular relaxation and is monitored as the relaxation of a drug on the pre-contracted blood vessel with norepinephrine, may be potential strategy for prevention and treatment of endothelial dysfunction and that selective agonists for ETA may be desired drugs for prevention and treatment of diseases involving endothelial dysfunction such as atherosclerosis, myocardial infarction and thrombosis.

In general, target compounds are designed based on the mechanism or structure of the biological target of interest, or on the library upon lead compound(s) identified to bind to the biological target. Unfortunately, for many biological targets, structural, or mechanistic information is not available or does not provide sufficient insight to enable productive library design. Additionally, for many targets, lead compounds have not yet been identified or novel motifs for binding are desired.[4] Under these circumstances, the preparation and screening of libraries has been much less successful. There have recently appeared two new methods for discovering small molecule ligands for such biological targets or processes, namely combinatorial target-guided ligand assembly,[4] and target-oriented and diversity-oriented organic synthesis.[5]

Herein, we report discovering selective lead compounds for ETA, a biological target, which is not fully elucidated, via an approach by combination of two reported-above methods based-on diversity-guided pharmacophore simplification and simulation (Scheme 1).

As the research basis, we merely knew that some classical M agonists such as ACh, arecholine (Arec), carbachol (Carb), and oxotremorine (Oxot) could activate ETA, whereas pilocarpine (Pilo) could not activate ETA, and that there might exist similarities and discrepancies between ETA and M receptors.[3]

So, we first reviewed all reported agonists[6] and abstracted and simplified the pharmacophores, and designed a general structure of B–L–Y as the initial structures for

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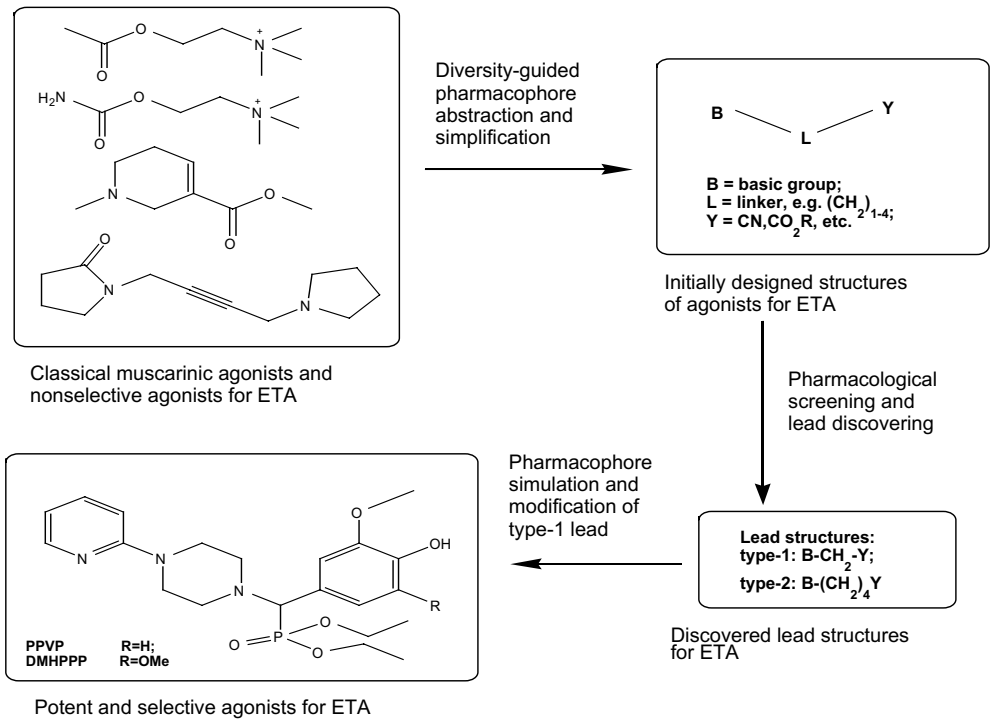


Scheme 1. Outline for the method based-on diversity-guided pharmacophore simplification and simulation.

agonists of ETA, wherein, B is a basic group, L is a linker such as $(CH_2)_n$ ($n = 1$–4), and Y is a hydrogen bond acceptor such as CN, CO_2R, CONHR, etc. Thus, we designed and synthesized the first round of 34 diverse compounds (Table 1), of which, B was most added an electro-negative atom (O and N) as extra-pharmacophore, linker is chain or cyclic (bicycloamine). Of them, most were prepared by substitution of secondary amines with the corresponding halides in presence of triethylamine or produced by Michael addition of the amines to the unsaturated compounds. After screened in vitro according to the reference method,[3,7] three lead compounds were discovered, namely, **1**, **2**, and **34**, which are, respectively, belonged to type-1 and type-2. Wherein, the type-1 structure is a substituted α-aminoacetonitrile, and the type-2 structure is substituted 5-aminopentanate.

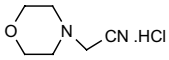
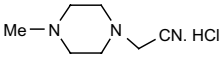
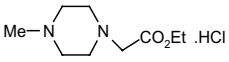
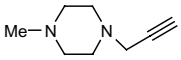
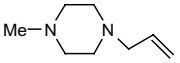
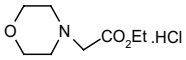
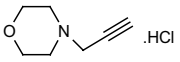
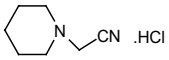
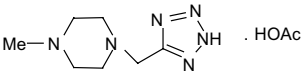
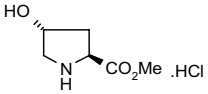
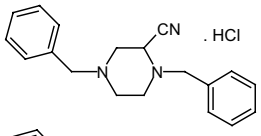
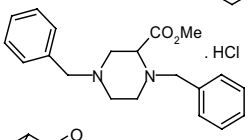
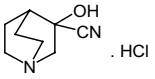
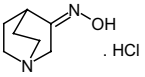
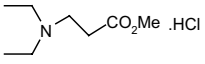
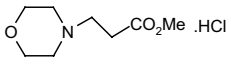
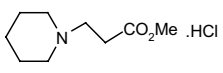
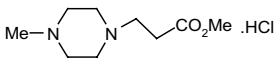
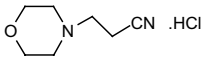
In view of easiness and efficiency of synthesis and diversity, we consequently embarked on optimization of type-1 lead, which was efficiently prepared by a three-component Mannich-type reaction. At first, we proposed the initial structure model of the type-1 lead compounds (Scheme 2) through MM2 calculation[8] (Table 2). It could be perceived that agonism of ETA might require, in addition to a basic group and a hydrogen bond acceptor spacing between 2 and 3 angstrom (Å), another electronegative pharmacophore that lies within 2–4 Å to the basic group and 4–6 Å to the hydrogen bond acceptor such as the alkenyl group of Erec, the alkynyl group of Oxot, the γ-oxygen of compound **1**, and γ-nitrogen of compound **2** while it is not necessary for agonism of M_3. And that is why Pilo and compounds **20** and **23** cannot activate ETA, which accord only two points with the model.

Thus, we designed the second round compounds by application of the proposed structure model (Scheme 2) and bioisostere rule (substitution of CN with $PO(OEt)_2$) and addition of auxiliary binding groups (aromatic R_1 and R_2), and synthesized the second round of 47 compounds (Table 3). Of them, 30 α-substituted α-aminoacetonitriles were efficiently produced through a three-component Mannich-type reaction according to the known methods, 2 substituted acetamides were prepared by substitution of the *N*-substituted chloroacetamide with the corresponding amines, and 15 substituted α-aminomethyl-phosphates were prepared by an auto-catalyzed three-component Mannich-type reaction we discovered.[9] After screened as in first round, six compounds (**35**, **36**, **37**, **65**, **66**, and **72**) of potent vascular relaxation were found.

In order to demonstrate the hypothesis that selective agonists of ETA may be desired drugs for prevention and treatment of diseases concerning endothelial dysfunction and their possible mechanisms, we selected two potent compounds, namely, 35 DMHPPP and 36 PPVP, for further pharmacological studies.

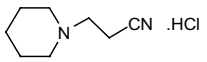
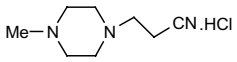
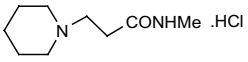
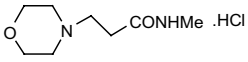
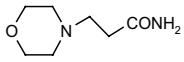
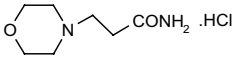
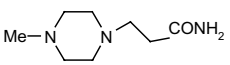
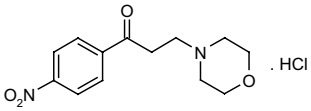
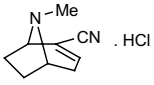
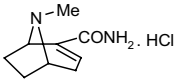
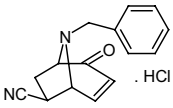
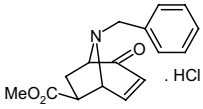
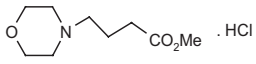
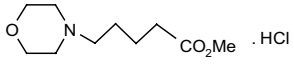
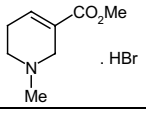
As shown in Table 4, the relaxation of rat aortas induced by DMHPPP and PPVP was endothelium-dependent and could not be blocked by M antagonist atropine, but can be significantly inhibited by indomethacin, a cycloxygenase inhibitor, or L-NAME, a nitrous oxide (NO) synthesase inhibitor. The results suggested that agonism of ETA of novel compounds might be induced via prostacyclin (PGI_2) and NO pathways.

Table 1. Compounds designed and synthesized in the first round and their activities

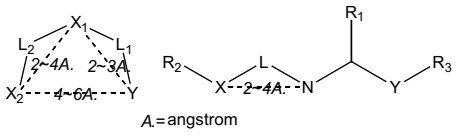
Compound	Formula	Structure	Agonism of ETA[a]	Agonism of M_3[b]
1 MANC	$C_6H_{11}ClN_2O$		+++	–
2 PANCI	$C_7H_{14}ClN_3$		+++	–
3	$C_9H_{19}ClN_2O_2$		++	–
4	$C_8H_{15}BrN_2$		++	–
5	$C_8H_{17}BrN_2$		++	
6	$C_8H_{16}ClNO_3$		+	–
7	$C_7H_{12}ClNO$		–	–
8	$C_7H_{13}ClN_2$		–	–
9	$C_9H_{15}N_6O_2$		++	–
10	$C_6H_{11}ClNO_3$		++	–
11	$C_{19}H_{22}ClN_3$		++	–
12	$C_{20}H_{25}ClN_2O_2$		++	–
13	$C_8H_{14}ClNO$		–	–
14	$C_9H_{15}ClN_2O$		–	–
15	$C_8H_{15}ClN_2O$		–	–
16	$C_{10}H_{18}ClNO_2$		–	–
17	$C_{10}H_{16}ClNO_3$		–	–
18	$C_{11}H_{18}ClNO_2$		+	–
19	$C_9H_{19}ClN_2O_2$		–	–
20	$C_7H_{13}ClN_2O$		–	–

(*continued on next page*)

Table 1 (*continued*)

Compound	Formula	Structure	Agonism of ETA[a]	Agonism of M_3[b]
21	$C_8H_{15}ClN_2$		–	–
22	$C_8H_{15}ClN_3$		–	–
23	$C_9H_{19}ClN_2O$		–	–
24	$C_8H_{17}ClN_2O_2$		–	–
25	$C_7H_{14}N_2O_2$		–	–
26	$C_7H_{15}ClN_2O_2$		–	–
27	$C_8H_{17}N_3O$		–	–
28	$C_{13}H_{17}ClN_2O_4$		++	–
29	$C_8H_{13}ClN_2$		+	–
30	$C_8H_{17}ClN_2O$		++	–
31	$C_{15}H_{15}ClN_2O$		–	–
32	$C_{16}H_{18}ClNO_3$		+	–
33	$C_9H_{18}ClNO_3$		–	–
34 MOPMC	$C_{10}H_{20}ClNO_3$		+++	–
Arec	$C_8H_{14}BrN_2O_2$		++++	++++

[a] Agonism of ETA, maximal rate of vascular endothelium-dependent relaxation.
[b] Agonism of M_3, maximal contraction rate of isolated guinea pig ileum; + 0–10%; ++ 20–30%; +++ 30–50%; ++++ >50%; – no activity.



Scheme 2. The initial structure model of the type-1 lead compounds and the general structure of the second round compounds.

As shown in Table 5, PPVP 5 mg/kg could normalize the lower function of ETA of rabbit impaired by high lipid diet.

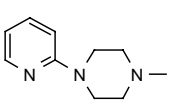
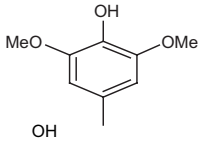
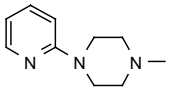
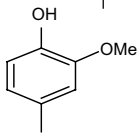
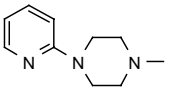
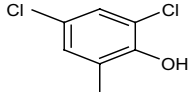
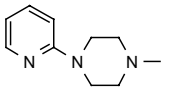
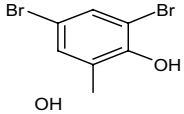
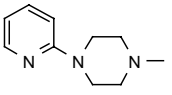
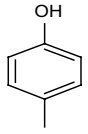
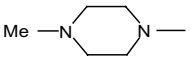
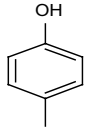
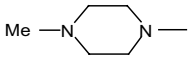
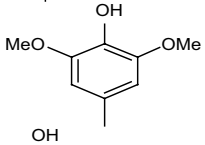
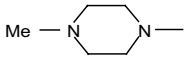
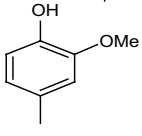
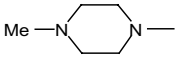
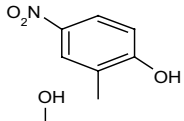
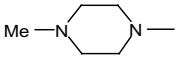
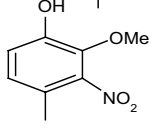
In summary, by combination of 'target-guided ligand assembly' with ' diversity-oriented organic synthesis', we proposed a method termed 'diversity-guided

Table 2. Partial MM2 calculation results for some representative compounds involved

Compound	$d(\delta_1^- - \delta_N^+)$ (Å)	$d(\delta_2^- - \delta_N^+)$ (Å)	$d(\delta_1^- - \delta_2^-)$ (Å)	$d(\delta_3^- - \delta_N^+)$ (Å)	Agonism of ETA[c]	Agonism of M_3[d]
ACh	3.761	5.106	2.235		++++	+++
Carb	3.761	5.096	2.268	6.044	++++	+++
Arec	2.485[a]	4.155	2.386	4.903	++++	+++
Oxot	2.449[b]	5.792	2.404	6.116	++++	+++
Pilo	4.846	6.565	2.223	7.921	−	+++
1	2.907	4.866	5.850		+++	−
2	2.949	4.873	5.861		+++	−
3	2.946	3.421	5.356	2.236	+	−
6	2.907	3.424	5.329	2.237	+	−
20	2.908	6.108	7.046		−	−
23	4.186	4.936	2.288		−	−

[a] Distance between nitrogen and the electronegative carbon atom of alkenyl group of Erec.
[b] Distance between the nitrogen atom and alkynyl group.
[c] Agonism of ETA, maximal rate of vascular endothelium-dependent relaxation.
[d] Agonism of M_3, maximal contraction rate of isolated guinea pig ileum; + 0–10%; ++ 20–30%; +++ 30–50%; ++++ >50%; − no activity.

Table 3. Compounds  designed and synthesized in the second round and their activities

Compound	R	R′	Y	Agonism of ETA[a]	Agonism of M[b]
35 DMHPPP			$PO(OEt)_2$	++++	−
36 PPVP			$PO(OEt)_2$	++++	−
37 DCSPPP			$PO(OEt)_2$	++++	−
38			$PO(OEt)_2$	++	−
39			$PO(OEt)_2$	+	−
40			$PO(OEt)_2$	++	−
41			$PO(OEt)_2$	++	−
42			$PO(OEt)_2$	++	−
43			$PO(OEt)_2$	++	−
44			$PO(OEt)_2$	+	−

(*continued on next page*)

Table 3 (*continued*)

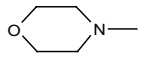
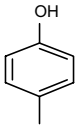
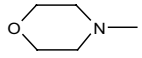
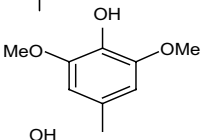
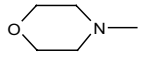
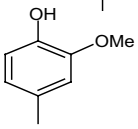
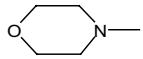
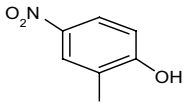
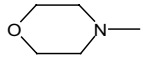
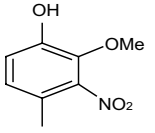
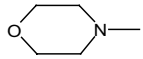
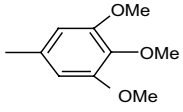
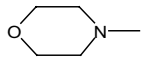
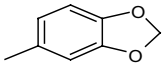
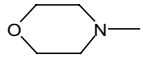
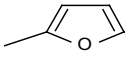
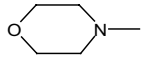
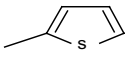
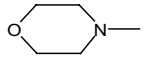
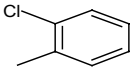
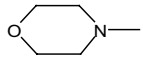
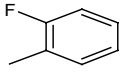
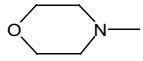
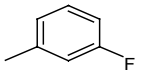
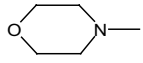
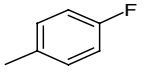
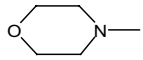
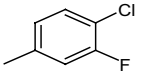
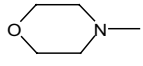
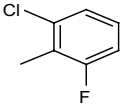
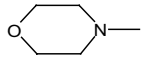
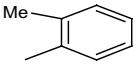
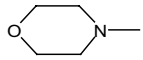
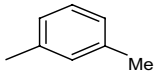
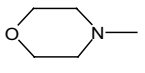
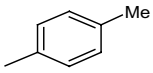
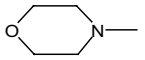
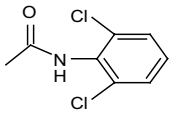
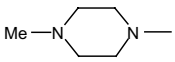
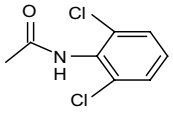
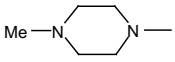
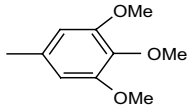
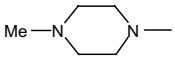
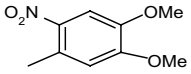
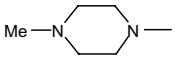
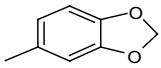
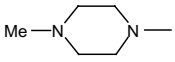
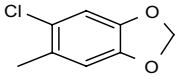
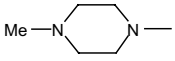
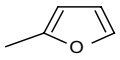
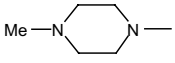
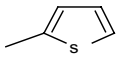
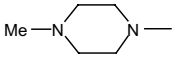
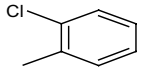
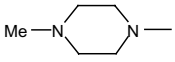
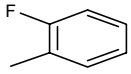
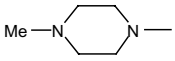
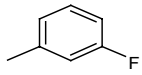
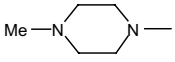
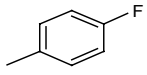
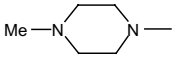
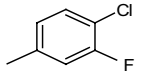
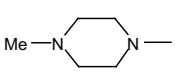
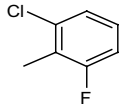
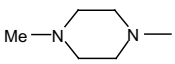
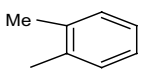
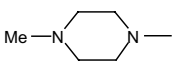
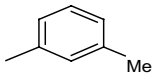
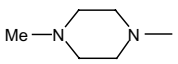
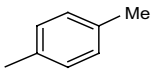
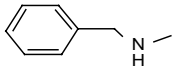
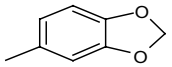
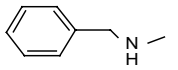
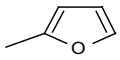
Compound	R	R′	Y	Agonism of ETA[a]	Agonism of M[b]
45			$PO(OEt)_2$	+	–
46			$PO(OEt)_2$	+++	–
47			$PO(OEt)_2$	++	–
48			$PO(OEt)_2$	++	–
49			$PO(OEt)_2$	+	–
50			CN	+++	–
51			CN	++	–
52			CN	+	–
53			CN	+	–
54			CN	+++	–
55			CN	++	–
56			CN	+++	–
57			CN	++	–
58			CN	–	–
59			CN	+	–
60			CN	++	–
61			CN	++	–

Table 3 (*continued*)

Compound	R	R′	Y	Agonism of ETA[a]	Agonism of M[b]
62			CN	++	–
63		H		++	–
64		H		+	–
65 MPTMBC			CN	++++	–
66 MPNVC			CN	++++	–
67			CN	++	–
68			CN	+	–
69			CN	+	–
70			CN	++	–
71			CN	+	–
72 2FBMPC			CN	+++	–
73			CN	+++	–
74			CN	+++	–
75			CN	–	–
76			CN	++	–
77			CN	+	–
78			CN	+	–

(*continued on next page*)

Table 3 (*continued*)

Compound	R	R′	Y	Agonism of ETA[a]	Agonism of M[b]
79			CN	++	−
80			CN	+	−
81			CN	+−	−
Arec				++++	++++

[a] Agonism of ETA, maximal rate of vascular endothelium-dependent relaxation.
[b] Agonism of M_3, maximal contraction rate of isolated guinea pig ileum; + 0–10%; ++ 20–30%; +++ 30–50%; ++++ >50%; − no activity.

Table 4. Effects of different kinds of antagonists on the rate of relaxation of isolated rat aortas of the two candidate compounds

Compound	Rate of relaxation of rat aorta (%)				
	Endothelium intact	Endothelium denuded	Atropine	Indomethacin	L-NAME[a]
DMHPPP	84.0 ± 25.3	5.7 ± 5.8**	82.6 ± 24.8	14.2 ± 6.8**	26.2 ± 28.0**
PPVP	81.4 ± 19.3	13.9 ± 6.7**	85.7 ± 12.7	23.8 ± 13.3**	28.8 ± 22.5**

** $P < 0.01$ versus endothelium intact.
[a] L-NAME: N^G-nitrao-Larginine methylester.

Table 5. Vascular relaxation of acetylcholine of 0.1 μmol L^{-1} on rabbit carotid in control (normal lipid diet), model (high lipid diet) and treatment (with 5 mg/kg PPVP) groups

Rate of vascular relaxation (%)		
Control	Model	Treatment
79.7 ± 3.2	32.2 ± 5.6**	65.9 ± 12.1##

** $P < 0.01$ versus control; ## $P < 0.01$ versus model.

pharmacophore simplification and simulation' for discovering selective agonists of ETA, a biological target not fully elucidated. The proposed method involves Ellman's method in that the desired lead is the optimized ligand assembly of the units of highest affinity and Schreiber's method in that molecular diversity and complexity and synthetic efficacy afford efficient ligands for the target screened. Whereas, Ellman's method was mainly based on simply combinatorial assembly of diversely commercial available units and assembly of pharmacophore of the same chemical functionality while Schreiber's method was primary based on organic method of synthesis. Thereby, we have firstly discovered two types of lead structures for selective agonists of ETA and found out two drug candidates, DMHPPP and PPVP, by further optimization of type-1 lead structures. And we have also first demonstrated that ETA could be desired target for protection of endothelium and for prophylaxis and treatment of cardiovascular endothelial dysfunction such as atherosclerosis and thrombosis in animal models[10] (as shown in *Supplementary materials*). Experimental results indicated that the proposed procedure was efficient.[11] The two drug candidates, DMHPPP and PPVP, are in further studies. Optimization of type-2 lead structure will be reported elsewhere later.

Supplementary materials for partial physico-chemical data of synthesized compounds and pharmacological data of in vivo assays on DMHPPP and PPVP are available.

Acknowledgements

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11. All compounds in Tables 1,3, and 4 were assayed at the concentration of 10^{-5} mol L^{-1}. The number of animals tested was above four. The screening methods were general methods as shown in Refs. 3,7,10.