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Effect of BKCa Channel Opener LDD175 on Erectile Function in an In Vivo Diabetic Rat Model

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ABSTRACT

Introduction: The development of novel therapeutic options is imperative in patients with erectile dysfunction, especially those non-responsive to phosphodiesterase type 5 inhibitors. LDD175, a potent BKCa channel opener, has a relaxation effect on the in vitro cavernosal smooth muscle strip.

Aim: To investigate the effect of LDD175 on erectile function using in vivo animal disease model.

Methods: Male Sprague-Dawley rats were assigned to a normal control group and seven diabetic groups: diabetic control, sildenafil (1 and 5 mg/kg), LDD175 (5 and 10 mg/kg), LDD175 5 mg/kg plus sildenafil 1 mg/kg, and LDD175 10 mg/kg plus tetraethylammonium.

Main Outcome Measures: Intracavernosal pressure (ICP), ratio of ICP to mean arterial pressure (MAP), and the area under curve of ICP/MAP of eight groups were compared using in vivo pelvic nerve stimulation.

Results: The ICP, ICP/MAP ratio, and area under curve of the ICP/MAP ratio of the normal control rats increased with an increase in electrical field stimulation voltage. All parameters in the diabetic control group were significantly lower than those in the normal control rats, with an electrical field stimulation ranging from 1 to 5 V ($P < .05$). LDD175 improved the erectile response in diabetic rats in a dose-dependent manner. The combination of sildenafil (1 mg/kg) and LDD175 (5 mg/kg) showed a significant additive effect ($P < .05$) on the improvement of erectile function compared with sildenafil (1 mg/kg) alone. The enhancement of erectile function by LDD175 was completely blocked by tetraethylammonium.

Conclusion: The results showed that the BKCa channel opener LDD175 improved erectile function in an in vivo diabetic rat model. Furthermore, combination therapy of LDD175 and sildenafil had an additive effect on the improvement of erectile function in diabetic rats. LDD175 could be a new candidate for the treatment of erectile dysfunction.

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Key Words: Erectile Dysfunction; BK Channel; LDD175; Phosphodiesterase Type 5

INTRODUCTION

Erectile dysfunction (ED) is a prevalent condition in men. Current approaches to ED treatment have been based on pharmacotherapy, especially on the use of phosphodiesterase type 5 inhibitors (PDE5Is), since the historical introduction of sildenafil.[1] However, PDE5Is used for medical therapy of ED are not effective in a considerable number of men with ED, especially in those with risk factors such as diabetes, old age, and prostatectomy.[2,3] These men whose ED is unresponsive to PDE5Is can undergo second- and third-line therapy; however, these modalities are relatively invasive, are not easy to access, and can even cause a feeling of hatred between a man and his partner.[4] Thus, the development of novel therapeutic options is imperative for patients whose ED is non-responsive to PDE5Is.

During the past decade, more insight has been gained into the molecular and pathophysiologic pathways involved in erections. This has allowed the development of new strategies for the treatment of ED, such as gene therapy, low-intensity shockwave therapy, and non-PDE5I pharmacotherapies.[5–7] Our institution also has investigated potential candidates for the treatment of

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patients with ED, from compounds derived from natural products to gene therapy.[8–10] As we previously reported, LDD175 (4-chloro-7-trifluoromethyl-10*H*-benzo[4,5]furo[3,2-*b*]indole-1-carboxylic acid), a novel BKCa channel opener, caused a concentration-dependent relaxation of erectile tissue in rabbit cavernosal smooth muscle strips in vitro.[11] This relaxation was endothelium independent and occurred primarily by opening the BKCa channel. Improved erectile function was confirmed in an in vivo animal model using cavernous nerve electrical stimulation tests.[11] These responses were comparable to those seen with PDE5Is. The aim of the present study was to verify the effect and toxicity and safety profile of LDD175 using an in vivo animal disease model.

METHODS

This study was conducted in accordance with the guideline of the institutional animal care and use committee of the Samsung Biomedical Research Institute (Seoul, Korea).

Preparation of Animal Model

Forty-eight male Sprague-Dawley rats were used in this study. The rats were assigned to one of eight groups: (i) a normal control group (n = 5); (ii) a diabetic control group (n = 8); (iii) a diabetic group treated with sildenafil (1 mg/kg, n = 6; 5 mg/kg, n = 7); (iv) a diabetic group treated with LDD175 (5 mg/kg, n = 7; 10 mg/kg, n = 8); (v) a diabetic group treated with LDD175 (5 mg/kg) plus sildenafil (1 mg/kg; n = 4); and (vi) a diabetic group treated with LDD175 (10 mg/kg) plus tetraethylammonium (a non-selective potassium channel blocker; 30 mg/kg; n = 6).

Induction of Diabetes in Rats

Diabetes mellitus was induced for a 4-month period in experimental animals (8 weeks old at the time of injection) using a single intraperitoneal injection of streptozotocin (65 mg/kg) dissolved in citrate buffer (60 mL of citric acid 100 mmol/L and 40 mL of disodium phosphate 200 mmol/L, pH 4.6), as previously described.[12] An age-matched control group received an injection of the vehicle alone. Rats were included in the study when they were confirmed to be diabetic for 1 week (blood glucose levels > 250 mg/dL).

In Vivo Erectile Function Test Using Pelvic Nerve Stimulation

In vivo pelvic nerve stimulation was performed to investigate erectile function 10 minutes after the intravenous injection of vehicle, sildenafil, LDD175, or sildenafil plus LDD175. The detailed methodology of measuring the erectile response to pelvic nerve stimulation has been described previously.[10,12] The electrical field stimulation (EFS) parameters were 1, 2.5, and 5 V, 5 ms, 2 Hz, and a duration of 60 seconds using an electric stimulator (model PG 721A; Austin Electronic Specialties, Inc, Palo Alto, CA, USA). Submaximal EFS was introduced with 1 and 2.5 V as previously described.[11] We considered that it would be difficult for LDD175 or sildenafil to induce an additional effect of relaxation vs control under maximal stimulation. Submaximal EFS was performed 10 minutes after a series of control (buffer solution alone), LDD175, and sildenafil injections. At the end of submaximal stimulation, the full response was measured using an EFS of 5 V to compare it with submaximal stimulation and to ensure that the cavernous nerve was intact. LDD175 (5 and 10 mg/kg) and sildenafil (1 and 5 mg/kg) were administered intravenously in the tail vein at intervals of 30 minutes for stabilization in each rat. Systemic arterial blood pressure (BP) was monitored by carotid artery cannulation with polyethylene-50 tubing. The ratio of maximal intracavernosal pressure (ICP) to mean arterial pressure (MAP) obtained at the peak of the erectile response was determined to control for variations in arterial BP. In addition, the area under the curve (AUC) of the ICP/MAP ratio was calculated.

Toxicity and Safety Evaluations of LDD175

Non-diabetic rats (normal control, n = 6; LDD175, n = 9) were prepared to check the toxicity and safety of LDD175. Toxicity of LDD175 was evaluated 1 week after daily oral administration of LDD175 (10 mg/kg) and compared with that of buffer solution. Mortality, change in body weight, and BP were measured. The complete blood cell count and chemistry, including renal function, hepatic function, and cholesterol, were compared between the two in vivo models. Gross and microscopic evaluations of the main organs, including the liver, lung, kidney, spleen, testis, and penis, were performed.

Drugs and Solutions

LDD175 was kindly provided by AnyGen Co, Ltd (Gwangju, Korea). All other chemical agents were purchased from Sigma Chemical Co (St Louis, MO, USA), except sildenafil, which was supplied by Hanmi Pharmaceutical (Seoul, Korea). Sildenafil was dissolved in normal saline at 30 mg/kg. For the in vivo functional studies, LDD175 was dissolved in 10% Tween 20 as described in previous studies.[11] All other drugs were dissolved in normal saline.

Data Analysis

Erectile responses were measured with pelvic nerve EFS and were compared among the normal control, diabetic control, sildenafil, LDD175, and sildenafil plus LDD175 groups. This comparison also was performed by serially increasing electrical stimulation voltage. After investigation of the effect of LDD175 on erectile function in pelvic nerve stimulation, tetraethylammonium was added to confirm the mechanism of action of LDD175 in the erectile response as a BKCa channel opener.

Statistical Analysis

Levels of maximal ICP, ICP/MAP, and AUC of ICP/MAP were described as mean ± standard error. All data were compared using a two-tailed Student t-test. All statistical analyses were conducted using IBM SPSS 23.0 (IBM, Armonk, NY, USA), and a P value less than .05 was considered statistically significant.

RESULTS

The mean weights of the normal and diabetic rats were 597.1 ± 22.0 and 336.5 ± 6.1 g at 16 weeks, respectively ($P < .05$). The mean blood glucose levels of the normal and diabetic rats were 108.2 ± 2.4 and 526.6 ± 9.6 mg/dL at 16 weeks, respectively ($P < .05$). The ICP, ICP/MAP, and AUC of ICP/MAP of the normal control rats increased with an increase in EFS voltage. All tracings of ICP measurements for each group are shown in Figure 1. All parameters in the diabetic control group were significantly lower than those of the normal control group, with an EFS ranging from 1 to 5 V ($P < .05$). LDD175 (10 mg/kg) increased the erectile response to pelvic nerve stimulation in the diabetic groups ($P < .05$; Figure 2). This effect of LDD715 on erectile function in the diabetic groups was dose dependent. At 2.5 V of EFS, the LDD175 group showed a full response, and ICP and ICP/MAP significantly increased compared with values in the diabetic control group ($P < .05$). Sildenafil (5 mg/kg) also triggered a higher erectile response than in the diabetic control group for ICP, ICP/MAP, and AUC ($P < .05$).

The effect of sildenafil (1 mg/kg) on erectile function was not significant ($P > .05$), and LDD175 (5 mg/kg) also increased only ICP and the ICP/BP ratio at 2.5 V. The combination of sildenafil (1 mg/kg) and LDD175 (5 mg/kg) showed a significant additive effect on erectile function (Figure 3). Compared with sildenafil (1 mg/kg) alone, the combination of LDD175 (5 mg/kg) and sildenafil (1 mg/kg) showed a significant improvement in ICP/MAP at 5 V of EFS (77.6 ± 1.8% vs 55.4 ± 5.2%, $P < .05$) and in maximal ICP at 2.5 and 5 V ($P < .05$; Figure 3).

The improvement of erectile function by LDD175 was completely inhibited by the addition of tetraethylammonium. This result showed that the therapeutic effects of LDD175 were activated through BKCa channels in the diabetic rat model ($P < .05$; Figure 4).

In the toxicity evaluation of LDD175, body weight, heart rate, and BP did not differ between the LDD175 (10 mg/kg) and normal saline groups 1 week after oral administration (Table 1). There was no acute effect of LDD175 on systemic BP just after administration of the drug. In particular, there were no differences in organ weights of the brain, heart, lung, kidney, liver, testis, penis, and spleen. In addition, the complete blood cell counts and serum chemistry levels of the rats treated with LDD175 did not differ from those of the normal group. Pathologic evaluation of the rats treated with LDD175 showed no focal necrotic changes, inflammation, hemorrhage, abnormal proliferation of normal or atypical cells, and damaged cells in any organ compared with normal rats. Moreover, no mortality cases, abnormal conditions, or sickness behaviors were observed in the rats treated with LDD175.

DISCUSSION

In a previous report, LDD175 relaxed pre-contracted rabbit cavernosal smooth muscle strips in a dose-dependent manner. This relaxation of cavernosal smooth muscle strips was significantly inhibited by iberiotoxin, a BKCa channel blocker. In addition, the effect of LDD175 was not dependent on denudation of the endothelium. These responses were comparable to those seen with udenafil. After this in vitro research, the present study confirmed an excellent effect of LDD175 as a BKCa channel opener on the improvement of erectile response in an in vivo animal disease model. Furthermore, the safety profile of LDD175 in the animals was shown in this study.

LDD175 was introduced as a benzofuroindole analog and it is the most potent and effective activator of BKCa channels among these analogs (Figure 5).[13] Its relaxation effect on smooth muscles by BKCa channel activation has been verified in the urinary bladder, uterine, ileum, and prostate.[14–16] The BK channel is ubiquitously expressed in the body.[17–19] BKCa channels have been reported to play an important role in maintaining normal vasomotor tone by regulating potassium efflux. Hyperpolarization of the BKCa channel plays an important role in vascular reactivity by providing negative feedback with excessive constriction.[19] Unlike other potassium channels, such as the adenosine triphosphate–sensitive potassium channel opener, BKCa channels are known to be less expressed in the cardiovascular system and have fewer unstable hemodynamic side effects.[20] Thus, many pharmaceutical companies have interest in the research of BKCa channel openers and the role of BKCa channels in diseases. With the use of BKCa channel openers, evidence of the role of the channel and its possible therapeutic potential have been established for several indications. However, despite the intense academic and industrial focus, no drugs targeting BKCa channels are on the market, and to our knowledge, only one drug, andolast, is currently in clinical development for mild to moderate asthma.[19,21] In the field of ED, LDD175 has shown an efficacy and acceptable safety profile in in vitro and in vivo studies. In this regard, we expect that the successful preclinical work and previous basic research of LDD175 might translate to the clinic.

Drug-related adverse reactions are an important concern and one of the main factors of failure in new drug development. Many in vitro studies have failed to progress to in vivo evaluation, preclinical, and clinical studies owing to an adverse

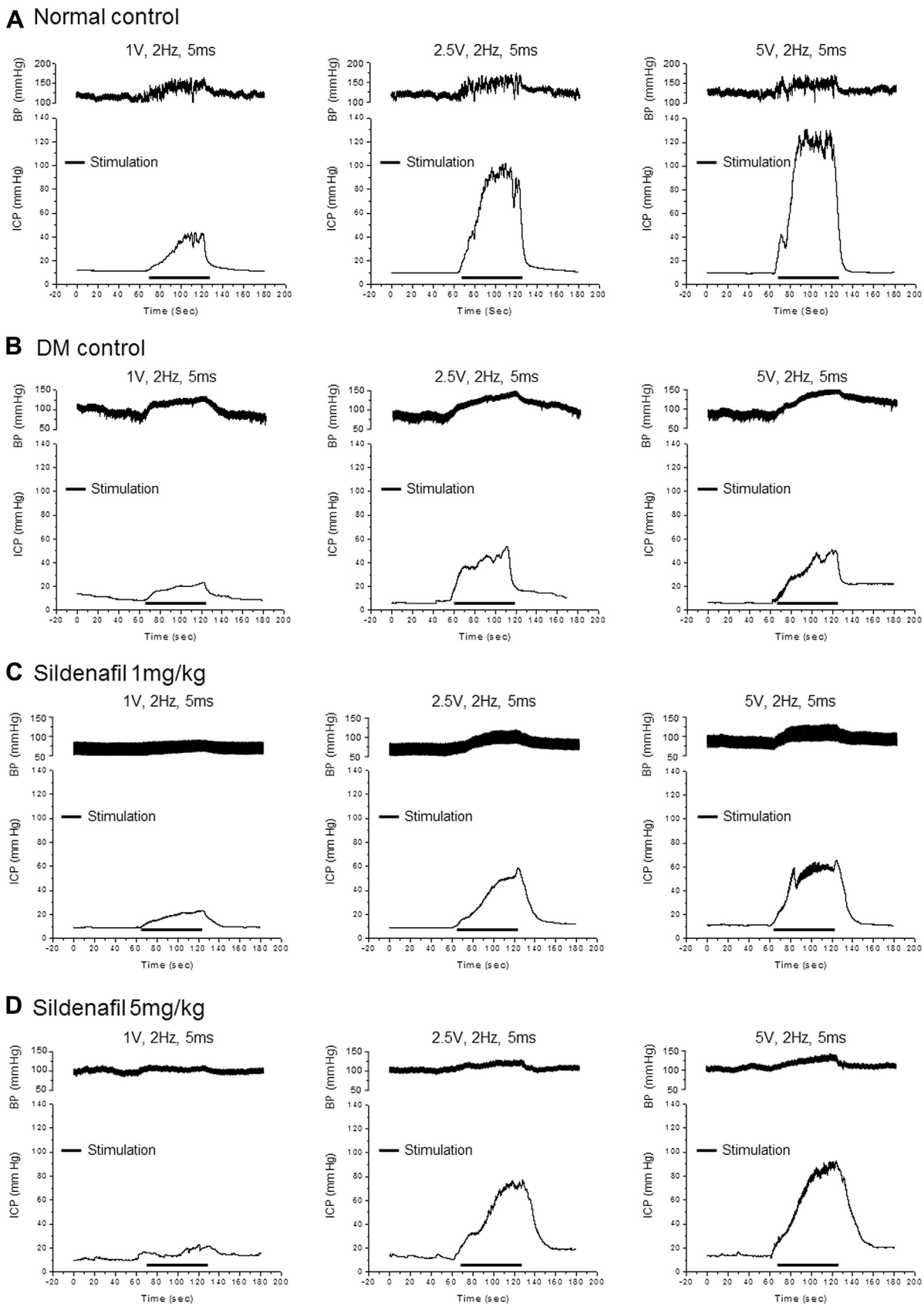


Figure 1. Tracings of intracavernosal pressure (ICP) measurements for the (A) normal control group, (B) diabetic control group, (C) diabetic group treated with sildenafil 1 mg/kg, (D) diabetic group treated with sildenafil 5 mg/kg, (E) diabetic group treated with LDD175 5 mg/kg, (F) diabetic group treated with LDD175 10 mg/kg, (G) diabetic group treated with LDD175 5 mg/kg plus sildenafil 1 mg/kg, and (H) diabetic group treated with TEA plus LDD175 10 mg/kg. BP = blood pressure; DM = diabetes mellitus; TEA = tetraethylammonium.

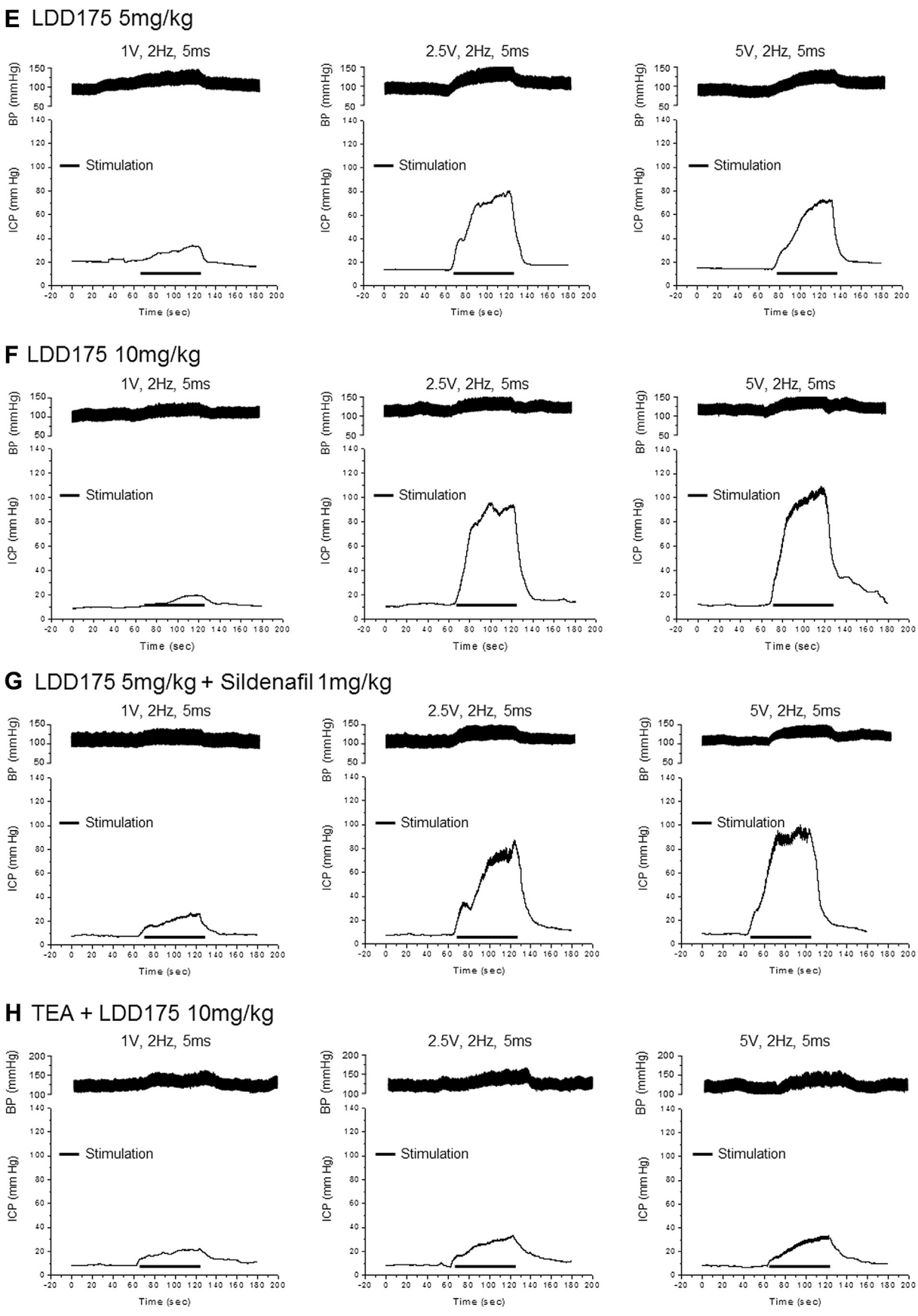
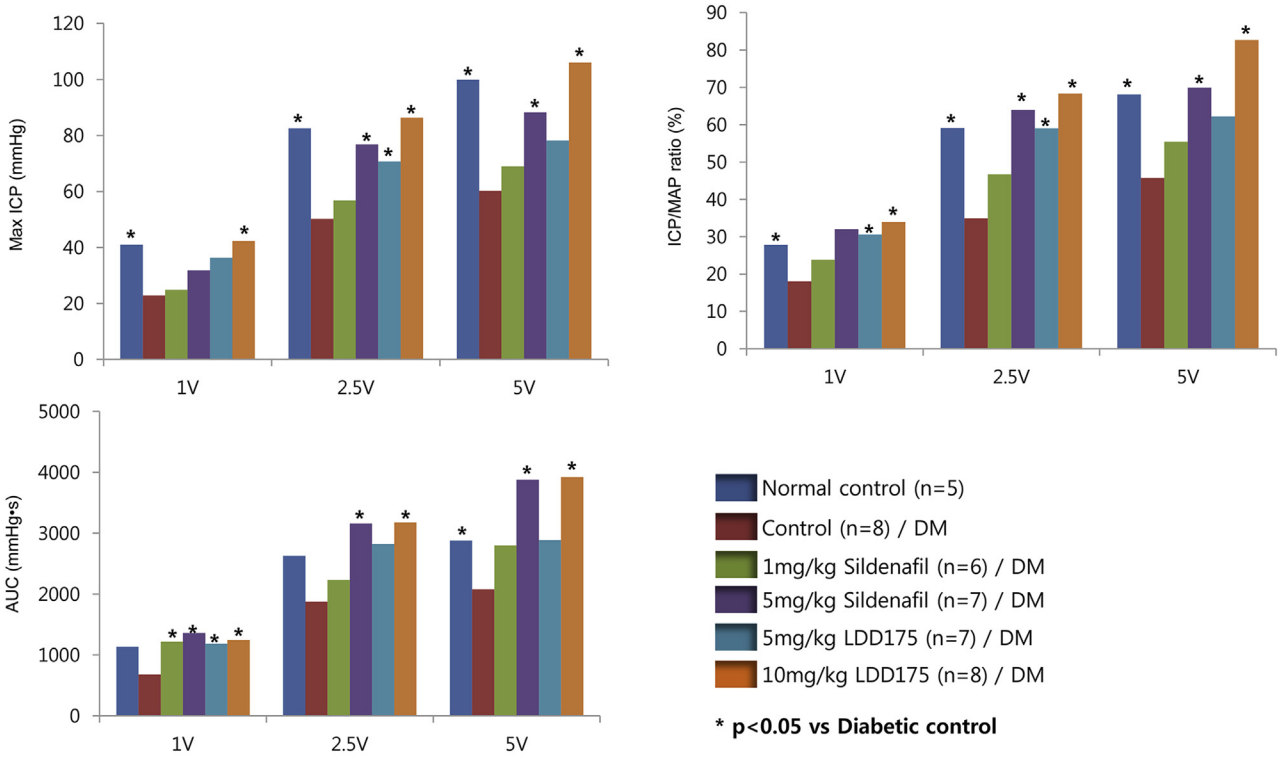


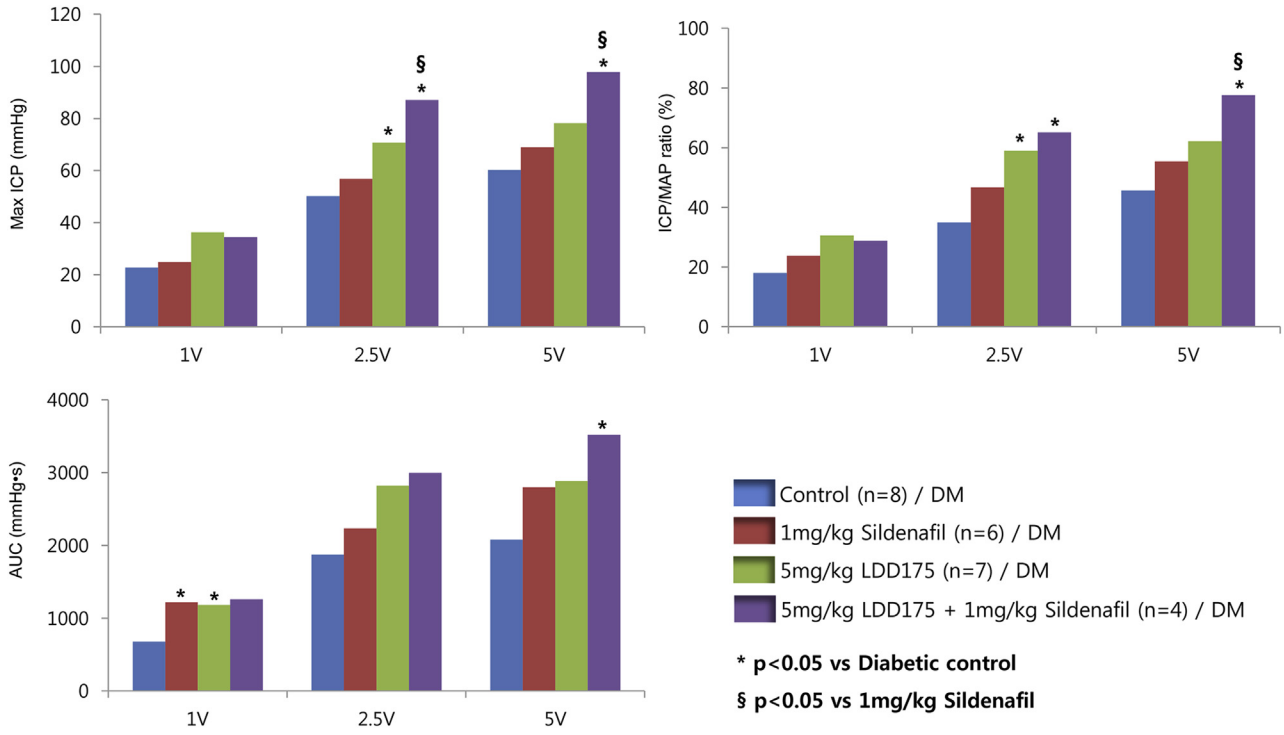
Figure 1. (*continued*).



		1V	2.5V	5V
MaxICP	Normal control (N=5)	41.0 ± 6.8	81.5 ± 15.5	99.9 ± 18.4
	Control (N=8) / DM	22.8 ± 3.7	50.2 ± 5.9	60.2 ± 5.1
	1mg/kg Sildenafil (N=6) / DM	24.8 ± 1.3	56.8 ± 8.5	68.9 ± 5.8
	5mg/kg Sildenafil (N=7) / DM	31.8 ± 5.1	76.8 ± 7.7	88.2 ± 9.5
	5mg/kg LDD175 (N=7) / DM	36.3 ± 6.8	70.7 ± 7.5	78.2 ± 10.4
	10mg/kg LDD175 (N=8) / DM	42.3 ± 7.6	86.4 ± 4.9	106.0 ± 3.5
ICP/BP	Normal control	27.8 ± 3.6	59.1 ± 12.1	68.1 ± 10.3
	Control / DM	18.0 ± 3.3	34.9 ± 4.2	45.7 ± 4.5
	1mg/kg Sildenafil / DM	23.8 ± 2.6	46.7 ± 7.3	55.4 ± 5.2
	5mg/kg Sildenafil / DM	32.0 ± 6.1	64 ± 5.5	69.9 ± 5.4
	5mg/kg LDD175 / DM	30.6 ± 5.0	59 ± 7.2	62.2 ± 9.4
	10mg/kg LDD175 / DM	33.9 ± 5.3	68.3 ± 5.4	82.7 ± 4.6
AUC	Normal control	1133.7 ± 181.0	2628.5 ± 486.9	2878.1 ± 284.0
	Control / DM	676.1 ± 138.8	1871.3 ± 378.2	2078.9 ± 217.0
	1mg/kg Sildenafil / DM	1217.7 ± 177.7	2232.9 ± 412.4	2797.9 ± 280.1
	5mg/kg Sildenafil / DM	1360.6 ± 366.3	3159.2 ± 284.7	3876.0 ± 432.7
	5mg/kg LDD175 / DM	1182.1 ± 187.5	2820.9 ± 501.3	2886.4 ± 645.8
	10mg/kg LDD175 / DM	1244.1 ± 187.2	3178.0 ± 309.8	3921.0 ± 421.8

Figure 2. The erectile response of normal rats increased with an increase in electrical field stimulation voltage. All parameters in the diabetic control group were significantly lower than those in the normal control group with an electrical field stimulation ranging from 1 to 5 V ($P < .05$). LDD175 increased the erectile response to pelvic nerve stimulation in diabetic rats. This effect of LDD715 on erectile function in diabetic rats was dose dependent. AUC = area under the curve; BP = blood pressure; DM = diabetes mellitus; ICP = intracavernosal pressure; MAP = mean arterial pressure; MaxICP = maximum intracavernosal pressure. Figure 2 is available in color at www.jsm.jsexmed.org.

safety profile. In the present study, hemodynamically unstable features were not observed by carotid artery monitoring during in vivo pelvic nerve stimulation after an intravenous injection of LDD175. Oral administration of LDD175 did not affect body weight, heart rate, BP, and several serum parameters during a short period of the experiment. To translate this work to the clinic, additional safety profiling studies are mandatory.



		1V	2.5V	5V
Max ICP (mmHg)	Control (N=8) / DM	22.8 ± 3.7	50.2 ± 5.9	60.2 ± 5.1
	1mg/kg Sildenafil (N=6) / DM	24.8 ± 1.3	56.8 ± 8.5	68.9 ± 5.8
	5mg/kg LDD175 (N=7) / DM	36.3 ± 6.8	70.7 ± 7.5	78.2 ± 10.4
	5mg/kg LDD175 + 1mg/kg Sildenafil (N=4) / DM	34.4 ± 10.2	87.1 ± 6.2	97.8 ± 3.9
ICP/BP (%)	Control (N=8) / DM	18.0 ± 3.3	34.9 ± 4.2	45.7 ± 4.5
	1mg/kg Sildenafil (N=6) / DM	23.8 ± 2.6	46.7 ± 7.3	55.4 ± 5.2
	5mg/kg LDD175 (N=7) / DM	30.6 ± 5.0	59.0 ± 7.2	62.2 ± 9.4
	5mg/kg LDD175 + 1mg/kg Sildenafil (N=4) / DM	28.8 ± 8.8	65.1 ± 4.4	77.6 ± 1.8
AUC (mmHg*s)	Control (N=8) / DM	676.1 ± 138.8	1871.3 ± 378.2	2078.9 ± 217.0
	1mg/kg Sildenafil (N=6) / DM	1217.7 ± 177.7	2232.9 ± 412.4	2797.9 ± 280.1
	5mg/kg LDD175 (N=7) / DM	1182.1 ± 187.5	2820.9 ± 501.3	2886.4 ± 645.8
	5mg/kg LDD175 + 1mg/kg Sildenafil (N=4) / DM	1257.6 ± 384.2	2996.9 ± 346.0	3521.5 ± 211.6

Figure 3. The effect of sildenafil (1 mg/kg) on erectile function was not significant ($P > .05$), and LDD175 (5 mg/kg) increased only ICP and the ICP/BP ratio at 2.5 V. The combination of sildenafil (1 mg/kg) and LDD175 (5 mg/kg) showed a significant effect on erectile function. Compared with sildenafil (1 mg/kg) alone, LDD175 (5 mg/kg) plus sildenafil (1 mg/kg) showed significant improvements in ICP/MAP at 5 V of electrical field stimulation and in maximal ICP at 2.5 and 5 V. AUC = area under the curve; BP = blood pressure; DM = diabetes mellitus; ICP = intracavernosal pressure; MAP = mean arterial pressure; Max ICP = maximum intracavernosal pressure. Figure 3 is available in color at www.jsm.jsexmed.org.

The effect of LDD175 on improving erectile responses was enhanced by sildenafil in the present study. This additive effect also was shown with another PDE5I, udenafil, in a previous in vitro study.[11] The exact mechanism of action of this effect of LDD175 in combination with PDE5Is is unknown. However, previous studies have suggested some rationale for this effect. The vasodilation effects of nitric oxide are mediated in part by the activation of BKCa channels.[22,23] Kun et al[22] reported that the relaxant responses elicited by sildenafil involve the activation of BKCa channels in intracavernous artery and human corpus cavernosum strips. However, they also suggested that there are species differences with regard to the involvement of BKCa channels in sildenafil relaxation in erectile tissue. A more recent study also reported that BKCa channel activation produces an endothelium-mediated potentiation of the relaxation of human penile arteries mediated by nitric oxide and cyclic guanosine monophosphate, which results in an improved vasodilatory capacity of PDE5Is in these human vessels. This

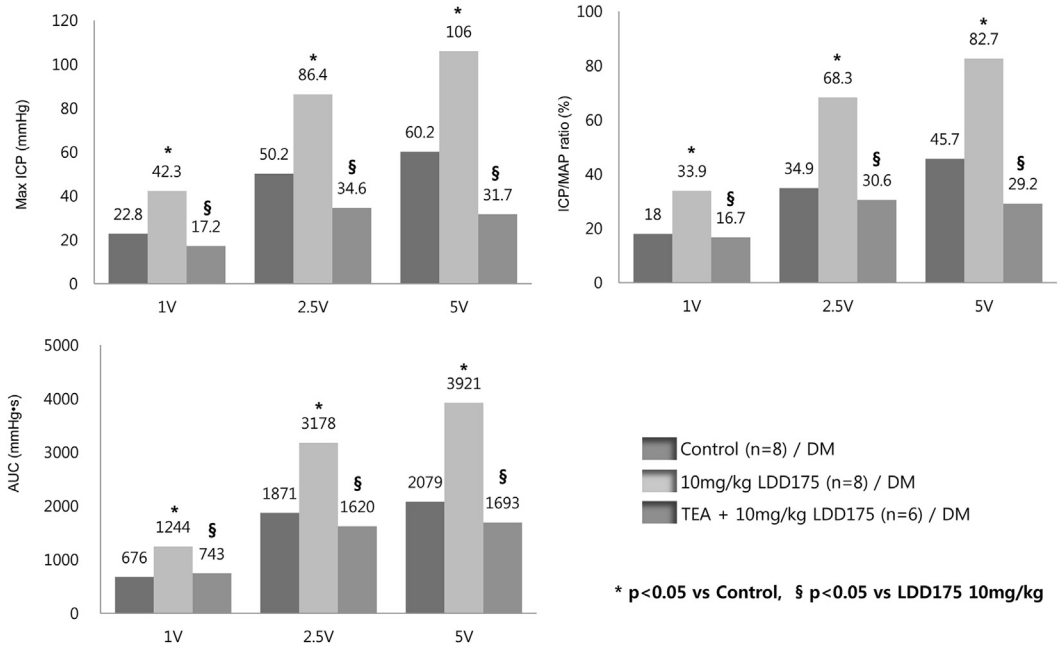


Figure 4. LDD175 (10 mg/kg) was completely inhibited by the addition of TEA. The therapeutic effects of LDD175 were activated through BKCa channels in the diabetic rat model. AUC = area under the curve; BP = blood pressure; DM = diabetes mellitus; ICP = intracavernosal pressure; MAP = mean arterial pressure; Max ICP = maximum intracavernosal pressure; TEA = tetraethylammonium.

potentiating effect increases the efficacy of PDE5 inhibition to enhance erectile responses in vivo.[24] The researchers concluded that these results provide a rationale for considering the therapeutic potential of BKCa channel openers to improve the efficacy of PDE5Is in the management of diabetic ED. In the real-world treatment of ED, the additive effect of PDE5Is and LDD175 could provide a promising opportunity for salvage treatment in men whose ED does not respond to PDE5Is, although further investigation is warranted.

Table 1. Safety and toxicity evaluation of LDD175 (10 mg/kg) in rats 1 week after oral administration*

	Normal control group (n = 6)	LDD175 group (n = 9)
Mortality, n	0	0
Body weight (g)	393 ± 18	385 ± 9
Systolic BP (mm Hg)	124 ± 5	130 ± 4
Heart rate (beats/min)	464 ± 6	460 ± 16
WBC count ($\times 10^3/\mu L$)	13.3 ± 1.0	15.1 ± 1.2
Hemoglobin (g/dL)	15.8 ± 0.2	16.0 ± 0.2
Platelets ($\times 10^3/\mu L$)	808 ± 55	837 ± 60
Glucose (mg/dL)	167.7 ± 8.8	144.3 ± 13.6
Creatinine (mg/dL)	0.20 ± 0.03	0.10 ± 0.02
Total bilirubin (mg/dL)	0.20 ± 0.02	0.20 ± 0.01
ALT (IU/L)	36.0 ± 2.7	31.8 ± 1.6
Total protein (g/dL)	5.5 ± 0.5	6.0 ± 0.1

ALT = alanine transaminase; BP = blood pressure; WBC = white blood cell.
*In all categories, there was no difference between the two groups.

This study has several limitations. First, an investigation of tissue selectivity of LDD175, such as corpus cavernosum vs vascular smooth muscles, should follow. Second, LDD175 is commercially unavailable on the market owing to a patent issue. Third, the work using LDD175 in ED should be repeated. Fourth, this study lacked the evaluation of a long-term safety profile for preclinical and clinical studies.

In conclusion, the present study confirmed that the BKCa channel opener LDD175 improved erectile function in an in vivo diabetic rat model using cavernosal nerve electrical stimulation tests after a previous in vitro experimental study. Combination therapy of LDD175 and sildenafil provided an additive effect on the improvement of erectile function in diabetic rats. In addition, there were no abnormal safety or toxicity effects in rats treated with LDD175. Based on these

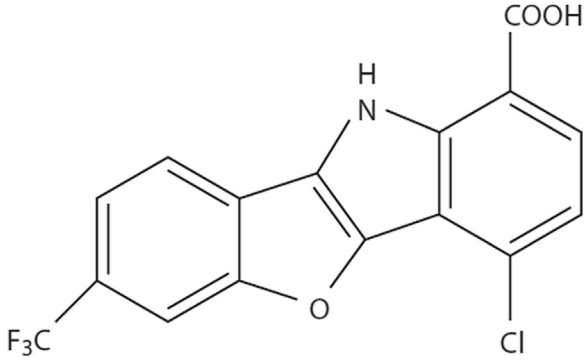


Figure 5. Structure of LDD175 (4-chloro-7-trifluoromethyl-10*H*-benzo[4,5]furo[3,2-*b*]indole-1-carboxylic acid).

results, LDD175 could be a new candidate for the pharmacotherapy of ED.

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