

### 3. ENDOTHELIN ANTAGONISTS: DISCOVERY OF EMD 122946, A HIGHLY POTENT AND ORALLY ACTIVE ET<sub>A</sub> SELECTIVE ANTAGONIST<sup>1</sup>

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**Abstract:** The discovery, *in vitro* and *in vivo* studies of the highly potent ET<sub>A</sub> antagonist EMD 122946 are presented. This compound displayed high binding affinity and functional antagonism [ IC<sub>50</sub> = 3.2 × 10<sup>-11</sup> M, pA<sub>2</sub> = 9.5 (ET<sub>A</sub>) ] and inhibited the ET-1 induced pressor response in pithed rats with an ED<sub>50</sub> of 0.3 mg/kg. In conscious spontaneously hypertensive rats and in DOCA-salt hypertensive rats the compound lowered mean blood pressure with an ED<sub>50</sub> of 0.06 mg/kg. EMD 122946 exhibited high bioavailability in rats and monkeys.

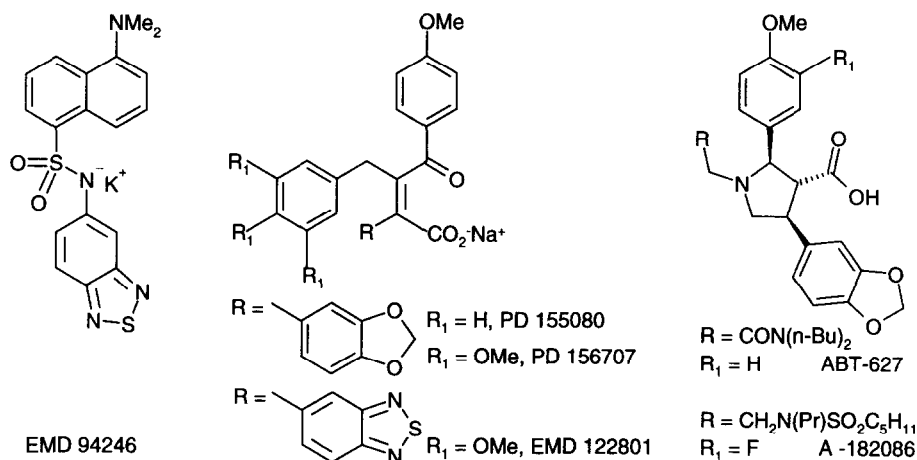
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**Keywords:** benzothiadiazole, butenolide, endothelin, ET<sub>A</sub> antagonist, antihypertensive activity

#### Introduction:

Our first efforts in the search for endothelin antagonists resulted in the discovery of the clinical candidate EMD 94246, <sup>2</sup> an ET<sub>A</sub> selective compound (Figure I). In recent articles in this journal,<sup>1,3</sup> our group described the identification of several classes of endothelin receptor antagonists by means of a Kohonen neural network<sup>4</sup> approach. In particular, we reported on the synthesis and *in vitro* results of the potent benzothiadiazole EMD 122801, derived from the reference compound of Parke-Davis PD 156707 (Figure I).<sup>1</sup> In the present paper we describe the full structure-activity relationships (SAR) of EMD 122801 which led to the picomolar binding ET<sub>A</sub> selective antagonist EMD 122946. We also describe the *in vivo* pharmacology and pharmacokinetics of this compound.

Figure I



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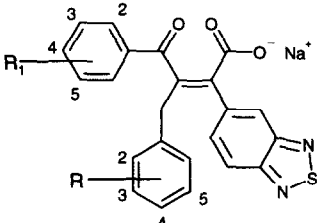
**Chemistry:**

Benzothiadiazoles **1** - **22**, EMD's 122801 and 122946 in Table 1 were prepared as previously described.<sup>1,5</sup>

**Biological results and discussion:**

*In vitro* studies: The compounds were screened for their ability to inhibit specific [<sup>125</sup>I]-ET-1 binding to rat aorta membranes (ET<sub>A</sub>) and porcine kidney (inner medulla) membranes (ET<sub>B</sub>).<sup>6</sup> The functional assay was performed by obtaining ET-1 concentration-response curves [pA<sub>2</sub>(ET<sub>A</sub>)] in isolated rat aortic rings without endothelium and sarafotoxin 6c concentration-response curves [pA<sub>2</sub>(ET<sub>B</sub>)] in isolated rabbit jugularis vein in the

**Table 1** Biological data of benzothiadiazole and reference compounds



Cpd.	R	R <sub>1</sub>	Binding ET <sub>A</sub> <sup>a</sup>	Binding ET <sub>B</sub> <sup>a</sup>	Function ET <sub>A</sub> <sup>b</sup>	Function ET <sub>B</sub> <sup>b</sup>
<b>1</b>	H	4-OCH <sub>3</sub>	26.0	1,200	7.6	-
<b>2</b>	2-OCH <sub>3</sub>	4-OCH <sub>3</sub>	3.4	820		
<b>3</b>	3-OCH <sub>3</sub>	4-OCH <sub>3</sub>	3.4	1,000		
<b>4</b>	4-OCH <sub>3</sub>	4-OCH <sub>3</sub>	28.0	1,800		
<b>5</b>	4-SCH <sub>3</sub>	4-OCH <sub>3</sub>	17.0	3,200		
<b>6</b>	4-O- <i>t</i> -C <sub>4</sub> H <sub>9</sub>	4-OCH <sub>3</sub>	580.0	1,600		
<b>7</b>	3,4-OCH <sub>2</sub> O-	4-OCH <sub>3</sub>	66.0	1,500		
<b>8</b>	3,4-(CH <sub>2</sub> ) <sub>2</sub> O-	4-OCH <sub>3</sub>	3.4	1,400		
<b>9</b>	3-F, 4-OCH <sub>3</sub>	4-OCH <sub>3</sub>	14.0	2,900		
<b>10</b>	3,4,5-O- <i>i</i> -C <sub>3</sub> H <sub>7</sub>	4-OCH <sub>3</sub>	57.0	220		
EMD 122801	3,4,5-OCH <sub>3</sub>	4-OCH <sub>3</sub>	0.3	340	8.5	6.1
<b>11</b>	3,4,5-OCH <sub>3</sub>	3-OCH <sub>3</sub>	4.6	1,800		
<b>12</b>	3,4,5-OCH <sub>3</sub>	2-OCH <sub>3</sub>	16.0	4,800		
<b>13</b>	3,4,5-OCH <sub>3</sub>	4-O- <i>i</i> -C <sub>3</sub> H <sub>7</sub>	64.0	550		
<b>14</b>	3,4,5-OCH <sub>3</sub>	4-OCHF <sub>2</sub>	160.0	1,900		
<b>15</b>	3,4,5-OCH <sub>3</sub>	3,4,5-OCH <sub>3</sub>	3.7	340		
<b>16</b>	3,4,5-OCH <sub>3</sub>	3,4-OCH <sub>2</sub> O-	6.5	400		
<b>17</b>	3,4,5-OCH <sub>3</sub>	3,4-(CH <sub>2</sub> ) <sub>2</sub> O-	28.0	460		
<b>18</b>	3,4,5-OCH <sub>3</sub>	3-Me, 4-OCH <sub>3</sub>	1.4	260		
<b>19</b>	3,4,5-OCH <sub>3</sub>	3-Cl, 4-OCH <sub>3</sub>	1.0	180		
EMD 122946	3,4,5-OCH <sub>3</sub>	3-F, 4-OCH <sub>3</sub>	0.032	160	9.5	6.0
<b>20</b>	3,4,5-OCH <sub>3</sub>	3-F, 4-O- <i>i</i> -C <sub>3</sub> H <sub>7</sub>	65.0	290		
<b>21</b>	3,4,5-OCH <sub>3</sub>	2-F, 4-OCH <sub>3</sub>	3.0	-		
<b>22</b>	3,4,5-OCH <sub>3</sub>	4-F, 2-OCH <sub>3</sub>	12.0	-		
PD 155080 <sup>c</sup>	--	--	7.4	4,550		
PD 156707	--	--	1.3	340	7.6	<5.5
A-127722 <sup>d</sup>	--	--	1.5	110	8.6	6.1

<sup>a</sup> = IC<sub>50</sub>(nM); <sup>b</sup> = pA<sub>2</sub>; <sup>c</sup> literature values<sup>8</sup>; <sup>d</sup> = racemic mixture of ABT-627

absence or presence of the antagonist.<sup>7</sup> Sarafotoxin 6c mediates vasoconstriction *via* the ET<sub>B</sub> receptor. The receptor binding affinities of compounds **1** - **22**, EMD 122801 and EMD 122946 and of selected reference compounds as well as functional antagonism of characteristic derivatives are summarized in Table 1.

At the outset of our SAR studies we examined the effects of the Parke-Davis benzothiadiazole analogues **1** and EMD 122801. The 3,4,5-trimethoxy derivative EMD 122801 displayed clearly superior activity compared to **1**, PD 155080 and PD156707. This compound showed subnanomolar ET<sub>A</sub> binding affinity and is a functional antagonist of the ET<sub>A</sub> receptor with a pA<sub>2</sub> value of 8.5.

Initial attempts to improve the ET<sub>A</sub> receptor binding affinity of EMD 122801 failed. On the basis of the results of the Parke-Davis scientists who employed the Topliss decision tree approach<sup>8</sup> only the effects of electron-donating substituents at each aromatic ring were investigated.

For optimization of the R site (compounds **2** - **8** and **10**), the *para* methoxy substituent was kept constant as R<sub>1</sub>. However, the binding affinities achieved in this study were significantly lower than with the 3,4,5-(trimethoxy)phenyl-substituted derivative EMD 122801.

We also attempted to optimize activity at the R<sub>1</sub> position keeping the 3,4,5-trimethoxy substituent as R. Replacement of the *para* methoxy in R<sub>1</sub> by various electron-donating groups led to a loss in ET<sub>A</sub> binding affinity (compounds **11** - **18**) compared to compound EMD 122801.

In the course of that study, it became clear that the substituents R and R<sub>1</sub> were nearly optimized for ET<sub>A</sub> binding. Any significant additional enhancement regarding ET<sub>A</sub> affinity had to be derived from other approaches. When Abbott scientists discovered that introduction of a fluorine atom *ortho* to the aromatic methoxy group significantly improved ET<sub>B</sub> affinity while ET<sub>A</sub> affinity remained unaffected [cf Figure I and Table 1: *ent* Abt-627 (*rac* A-127722) and *ent* A-182086 ],<sup>9</sup> we combined this *ortho* substitution at R<sub>1</sub> with the 3,4,5-trimethoxy substituent at the R site. To our surprise, introduction of a 3-fluoro substituent next to the 4-methoxy in EMD 122801 in contrast to the Abbott results only improved the ET<sub>A</sub> activity. The 3-fluoro analogue EMD 122946 displayed a 10-fold improvement in ET<sub>A</sub> binding affinity relative to the unsubstituted derivative EMD 122801. This compound also exhibited high functional activity for the ET<sub>A</sub> receptor with a pA<sub>2</sub> value of 9.5. Replacement of the 3-fluorine atom with a methyl group or a chlorine atom led to a loss of binding affinity (compounds **18** and **19**). Replacement of the 4-methoxy group in EMD 122946 by a bulkier isopropoxy group also worsened the binding affinity (compound **20**). To explore the substitution pattern of the methoxy-fluoro groups two analogues **21** and **22** were synthesized. However, both compounds showed diminished affinities for the ET<sub>A</sub> receptor. The introduction of a fluoro substituent next to a methoxy at the R site delivered compound **9**, which is much less active than EMD 122946.

All the compounds described herein were selective for the ET<sub>A</sub> receptor with ET<sub>B</sub> / ET<sub>A</sub> ratios greater than 150 for the most of them.

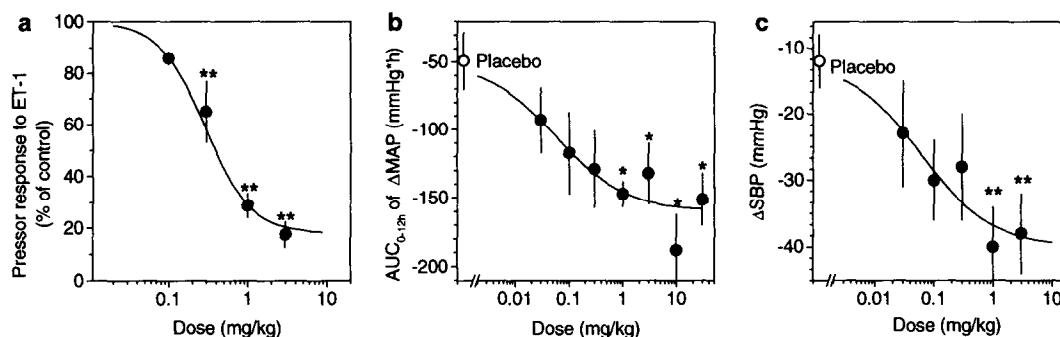
As a potent representative of this class of compounds, EMD 122946 was selected for *in vivo* evaluation as an antihypertensive drug.

*In vivo* studies: The effect of EMD 122946 on ET-1 induced changes in blood pressure was investigated in anesthetized pithed rats (Figure IIa).<sup>7a</sup> The maximum change in mean arterial blood pressure after ET-1 administration was measured. The ET-1 (0.3 nmol/kg *iv*) induced increase in mean arterial pressure after oral administration of placebo was 37 ± 3 mmHg (n = 6). The ET-1 induced increase in mean arterial pressure following oral administration of different doses of EMD 122946 was after 0.1 mg/kg 32 ± 1 mmHg (n = 6, P = NS vs. placebo), after 0.3 mg/kg 24 ± 4 mmHg (n = 6, P < 0.01), after 1 mg/kg 11 ± 2 mmHg (n = 6, P < 0.01), and after 3 mg/kg 7 ± 2 mmHg (n = 6, P < 0.01). Consequently, EMD 122946 inhibited the ET-1 induced pressor response in a dose dependent manner. The maximum inhibition of ET-1 induced pressor response was 82%. The ED<sub>50</sub> for oral administration of this compound was 0.3 mg/kg. Like the ET<sub>A</sub> antagonists PD

156707<sup>10</sup> and A-127722<sup>11</sup> EMD 122946 was unable to completely block the pressor response to ET-1 in rats. Part of the reason for this may have been the inability of ET<sub>A</sub> selective antagonists to interfere with the vasoconstrictor effects of ET-1 at ET<sub>B</sub> receptors.<sup>12</sup> EMD 122946 had no effect on the depressor response to ET-1, a fact which supports the selectivity of this substance for the ET<sub>A</sub> receptor *in vivo*.

In conscious spontaneously hypertensive rats the effect of EMD 122946 on blood pressure after single oral administration was studied by telemetry.<sup>13</sup> The maximum effects of EMD 122946 on blood pressure were -9 mmHg with 0.1 mg/kg, -19 mmHg with 1 mg/kg, and -24 mmHg with 10 mg/kg compared to placebo treated animals. The overall effect on blood pressure, heart rate, and motor activity was calculated over 12 hours as AUC (area under the curve) of the change in mean arterial pressure, heart rate, and motor activity compared to predrug values (Table II). This substance lowered mean arterial pressure in spontaneously hypertensive rats in a dose-dependent manner (Figure IIb). The antihypertensive effect developed gradually and persisted over 24 hours at a dose of 1 mg/kg and the ED<sub>50</sub> for oral administration in spontaneously hypertensive rats was 0.06 mg/kg. EMD 122946 had no effect on heart rate and motor activity. Body weight, baseline mean arterial pressure, heart rate, and motor activity before administration of EMD 122946 did not differ significantly between groups.

**Figure II** Effect of EMD 122946 on blood pressure in rats



a. Inhibition of the increase in mean arterial pressure (MAP) induced by ET-1 (0.3 nmol/kg i.v.) in pithed rats 1.5 hours after oral administration of the drug or placebo. For fitting of the curve, 100% was considered the starting point.  $n = 6$  animals. b. Dose-response curve of the effect on mean arterial pressure (MAP) in spontaneously hypertensive rats. The MAP was measured by telemetry. Drug and placebo were administered at 12:00. The change of MAP to a 3 hours predrug period (9:00 to 12:00) was calculated and expressed as the area under the drug effect-time curve (AUC<sub>0-12h</sub> of ΔMAP) over 12 hours (12:00 to 24:00). For fitting of the curve, the value of the placebo group was considered the starting point.  $n = 6-13$  animals. c. Dose-response curve of the effect on systolic blood pressure (SBP) in DOCA-salt hypertensive rats. Blood pressure was measured by plethysmography 4 hours after oral administration. The effect was calculated as change in SBP compared to the predrug value.  $n = 5-14$  animals. Statistical comparisons between control rats (5% arabic gum) and those which received EMD 122946 were performed by analysis of variance (ANOVA; Dunnett's *t* test): \*  $P < 0.05$ , \*\*  $P < 0.01$ . For fitting of the curves in b. and c., the value of the placebo group was considered the starting point. Data points represent mean values  $\pm$  SEM.

Endothelin has been implicated in the pathogenesis of DOCA-salt hypertension because of the increased ET-1 content in the vasculature of DOCA-salt hypertensive rats and the decreased vascular responsiveness to ET-1 in blood vessels of hypertensive rats.<sup>14</sup> Lowering of blood pressure has been shown with the ET<sub>A</sub> receptor selective antagonists BQ-123 in DOCA-salt treated spontaneously hypertensive rats<sup>15</sup> and BMS-182874 in uninephrectomized DOCA-salt rats<sup>16</sup> suggesting that endothelin may play a role in blood pressure elevation in

these models. In conscious hypertensive DOCA-salt rats<sup>17</sup> the effect of EMD 122946 on blood pressure was studied by plethysmography 4 hours after single oral administration. This substance decreased blood pressure in conscious DOCA-salt hypertensive rats in a dose-dependent manner (Figure 1Ic). The ED<sub>50</sub> for oral administration of EMD 122946 in DOCA-salt hypertensive rats was 0.06 mg/kg and corresponds to the ED<sub>50</sub> in spontaneously hypertensive rats.

**Table II** Effect of EMD 122946 on heart rate and motor activity of spontaneously hypertensive rats after single oral administration

	Control	Dose of EMD 122946 (mg/kg)						
	5% arab. gum n = 13	0.03 n = 6	0.1 n = 6	0.3 n = 6	1 n = 13	3 n = 7	10 n = 6	30 n = 7
Body weight (g)	430 ± 10	433 ± 16	430 ± 17	432 ± 18	428 ± 11	427 ± 14	425 ± 15	429 ± 12
<u>Prior to drug administration</u>								
MAP (mmHg)	163 ± 5	170 ± 6	170 ± 5	170 ± 5	164 ± 4	157 ± 3	159 ± 4	156 ± 3
HR (min <sup>-1</sup> )	329 ± 4	340 ± 6	341 ± 5	344 ± 6	335 ± 3	335 ± 6	332 ± 3	326 ± 5
MA (units)	34 ± 3	37 ± 2	33 ± 5	37 ± 4	39 ± 2	38 ± 3	38 ± 3	37 ± 3
<u>After drug administration</u>								
AUC <sub>0-12h</sub> of								
ΔHR (min <sup>-1</sup> xh)	-333 ± 36	-384 ± 43	-341 ± 25	-413 ± 75	-344 ± 23	-356 ± 25	-349 ± 33	-260 ± 47
ΔMA(unitsxh)	-60 ± 38	-97 ± 24	-72 ± 52	-93 ± 53	-131 ± 33	-102 ± 43	-131 ± 46	-99 ± 22

Statistical comparisons between control rats (5% arabic gum) and those which received EMD 122946 were performed by analysis of variance (ANOVA; Dunnett's t test). Values shown are the mean ± SEM; n represents the number of animals examined in each group. AUC: area under the curve; HR: heart rate; MA: motor activity

Orientating studies of the pharmacokinetics of EMD 122946 were investigated in Wistar rats and cynomolgus monkeys after treatment with single intravenous and oral doses. A compilation of the pharmacokinetic parameters is presented in Table III.

**Table III**

	Wistar rats				Cynomolgus monkey	
	iv <sup>a,c</sup>		po <sup>b,c</sup>		iv <sup>a,d</sup>	po <sup>b,d</sup>
	male	female	male	female	female	female
C <sub>max</sub> [ng/ml]			1010	3160		3830
t <sub>max</sub> [h]			0.5	0.5		0.5
AUC [ng/ml x h]	2500	15400	1630	21300	3300	4260
t <sub>1/2</sub> [h] <sup>e</sup>	1.6	1.7			1.0	
CL [L/h/kg]	0.4	0.1			0.3	
V <sub>ss</sub> [L/kg]	0.5	0.2			0.1	
bioavailability [%]			~ 30	~ 70		~ 65

<sup>a</sup> dose: 1 mg/kg; <sup>b</sup> dose: 2 mg/kg; <sup>c</sup> n = 3; <sup>d</sup> n = 1; <sup>e</sup> interval: 1-8 h post administration

EMD 122946 is characterized by rapid absorption from the gastrointestinal tract, plasma elimination half-lives of 1.0 to 1.7 h and absolute bioavailability of 30 to 70 %.

In summary, EMD 122946 represents a novel, highly potent, orally active, non-peptide ET<sub>A</sub>-selective antagonist. Thus, this compound should prove useful for the evaluation of any potential benefit of ET<sub>A</sub>-receptor

selective antagonism in diseases associated with myocardial infarction, congestive heart failure, acute and chronic renal failure, (pulmonary) hypertension, atherosclerosis, percutaneous transluminal coronary angioplasty, and subarachnoid hemorrhage.

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