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3. ENDOTHELIN ANTAGONISTS: DISCOVERY OF EMD 122946, A HIGHLY POTENT AND ORALLY ACTIVE ET_A SELECTIVE ANTAGONIST[1]

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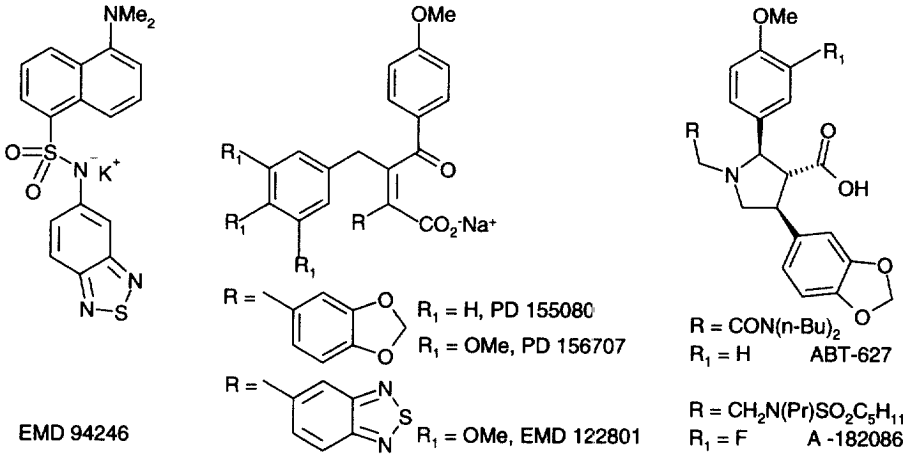
Abstract: The discovery, *in vitro* and *in vivo* studies of the highly potent ET_A antagonist EMD 122946 are presented. This compound displayed high binding affinity and functional antagonism [IC_{50} = 3.2 x 10^{-11} M, pA_2 = 9.5 (ET_A)] and inhibited the ET-1 induced pressor response in pithed rats with an ED_{50} 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_{50} of 0.06 mg/kg. EMD 122946 exhibited high bioavailability in rats and monkeys.

Keywords: benzothiadiazole, butenolide, endothelin, ET_A antagonist, antihypertensive activity

Introduction:

Our first efforts in the search for endothelin antagonists resulted in the discovery of the clinical candidate EMD 94246, [2] an ET_A selective compound (Figure I). In recent articles in this journal,[1,3] our group described the identification of several classes of endothelin receptor antagonists by means of a Kohonen neural network[4] 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).[1] In the present paper we describe the full structure-activity relationships (SAR) of EMD 122801 which led to the picomolar binding ET_A selective antagonist EMD 122946. We also describe the *in vivo* pharmacology and pharmacokinetics of this compound.

Figure I



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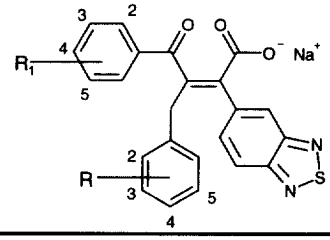
Chemistry:

Benzothiadiazoles **1** - **22**, EMD's 122801 and 122946 in Table 1 were prepared as previously described.[1, 5]

Biological results and discussion:

In vitro studies: The compounds were screened for their ability to inhibit specific [^{125}I]-ET-1 binding to rat aorta membranes (ET_A) and porcine kidney (inner medulla) membranes (ET_B).[6] The functional assay was performed by obtaining ET-1 concentration-response curves [$pA_2(ET_A)$] in isolated rat aortic rings without endothelium and sarafotoxin 6c concentration-response curves [$pA_2(ET_B)$] in isolated rabbit jugularis vein in the

Table I Biological data of benzothiadiazole and reference compounds



Cpd.	R	R_1	Binding ET_A[a]	Binding ET_B[a]	Function ET_A[b]	Function ET_B[b]
1	H	4-OCH_3	26.0	1,200	7.6	-
2	2-OCH_3	4-OCH_3	3.4	820		
3	3-OCH_3	4-OCH_3	3.4	1,000		
4	4-OCH_3	4-OCH_3	28.0	1,800		
5	4-SCH_3	4-OCH_3	17.0	3,200		
6	4-O-*t*-C_4H_9	4-OCH_3	580.0	1,600		
7	3,4-OCH_2O-	4-OCH_3	66.0	1,500		
8	3,4-$(CH_2)_2O$-	4-OCH_3	3.4	1,400		
9	3-F, 4-OCH_3	4-OCH_3	14.0	2,900		
10	3,4,5-O-*i*-C_3H_7	4-OCH_3	57.0	220		
EMD 122801	3,4,5-OCH_3	4-OCH_3	0.3	340	8.5	6.1
11	3,4,5-OCH_3	3-OCH_3	4.6	1,800		
12	3,4,5-OCH_3	2-OCH_3	16.0	4,800		
13	3,4,5-OCH_3	4-O-*i*-C_3H_7	64.0	550		
14	3,4,5-OCH_3	4-$OCHF_2$	160.0	1,900		
15	3,4,5-OCH_3	3,4,5-OCH_3	3.7	340		
16	3,4,5-OCH_3	3,4-OCH_2O-	6.5	400		
17	3,4,5-OCH_3	3,4-$(CH_2)_2O$-	28.0	460		
18	3,4,5-OCH_3	3-Me, 4-OCH_3	1.4	260		
19	3,4,5-OCH_3	3-Cl, 4-OCH_3	1.0	180		
EMD 122946	3,4,5-OCH_3	3-F, 4-OCH_3	0.032	160	9.5	6.0
20	3,4,5-OCH_3	3-F, 4-O-*i*-C_3H_7	65.0	290		
21	3,4,5-OCH_3	2-F, 4-OCH_3	3.0	-		
22	3,4,5-OCH_3	4-F, 2-OCH_3	12.0	-		
PD 155080[c]	--	--	7.4	4,550		
PD 156707	--	--	1.3	340	7.6	<5.5
A-127722[d]	--	--	1.5	110	8.6	6.1

[a] = IC_{50}(nM); [b] = pA_2; [c] literature values[8]; [d] = racemic mixture of ABT-627

absence or presence of the antagonist.[7] Sarafotoxin 6c mediates vasoconstriction *via* the ET_B 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_A binding affinty and is a functional antagonist of the ET_A receptor with a pA_2 value of 8.5.

Initial attempts to improve the ET_A 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[8] 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_1. 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_1 position keeping the 3,4,5-trimethoxy substituent as R. Replacement of the *para* methoxy in R_1 by various electron-donating groups led to a loss in ET_A 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_1 were nearly optimized for ET_A binding. Any significant additional enhancement regarding ET_A 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_B affinity while ET_A affinity remained unaffected [*cf* Figure I and Table 1: *ent* Abt-627 (*rac* A-127722) and *ent* A-182086],[9] we combined this *ortho* substitution at R_1 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_A activity. The 3-fluoro analogue EMD 122946 displayed a 10-fold improvement in ET_A binding affinity relative to the unsubstituted derivative EMD 122801. This compound also exhibited high functional activity for the ET_A receptor with a pA_2 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_A 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_A receptor with ET_B / ET_A ratios greater than 150 for the most of them.

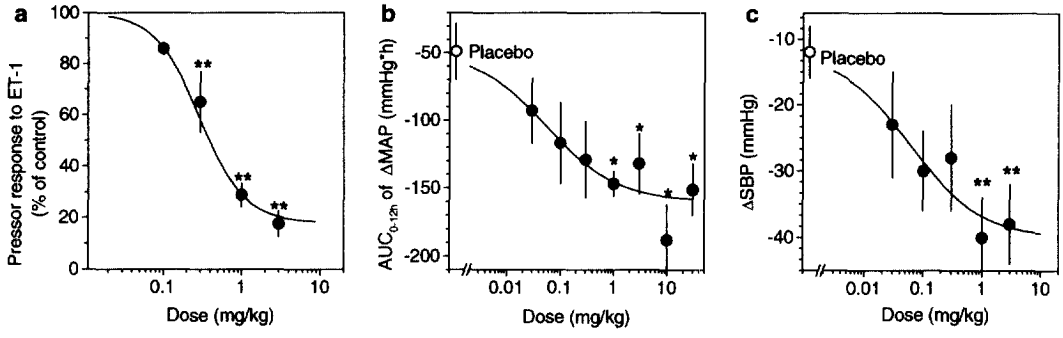
As a potent representative of this class of compounds, EMD 122946 was selected for *in vivo* evaluation as an antihypertensive drug.

In vivo studies: The effect of EMD 122946 on ET-1 induced changes in blood pressure was investigated in anesthetized pithed rats (Figure IIa).[7a] 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 administatration 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_{50} for oral administration of this compound was 0.3 mg/kg. Like the ET_A antagonists PD

156707[10] and A-127722[11] 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_A selective antagonists to interfere with the vasoconstrictor effects of ET-1 at ET_B receptors.[12] EMD 122946 had no effect on the depressor response to ET-1, a fact which supports the selectivity of this substance for the ET_A receptor *in vivo*.

In conscious spontaneously hypertensive rats the effect of EMD 122946 on blood pressure after single oral administration was studied by telemetry.[13] 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_{50} 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 ($AUC_{0\text{-}12h}$ 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 ± 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.[14] Lowering of blood pressure has been shown with the ET_A receptor selective antagonists BQ-123 in DOCA-salt treated spontaneously hypertensive rats [15] and BMS-182874 in uninephrectomized DOCA-salt rats [16] suggesting that endothelin may play a role in blood pressure elevation in

these models. In conscious hypertensive DOCA-salt rats [17] 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 IIc). The ED_{50} for oral administration of EMD 122946 in DOCA-salt hypertensive rats was 0.06 mg/kg and corresponds to the ED_{50} 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	0.03	0.1	0.3	1	3	10	30
	n = 13	n = 6	n = 6	n = 6	n = 13	n = 7	n = 6	n = 7
Body weight (g)	430 ± 10	433 ± 16	430 ± 17	432 ± 18	428 ± 11	427 ± 14	425 ± 15	429 ± 12
Prior to drug administration								
MAP (mmHg)	163 ± 5	170 ± 6	170 ± 5	170 ± 5	164 ± 4	157 ± 3	159 ± 4	156 ± 3
HR (min^{-1})	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
After drug administration								
AUC0-12h of								
ΔHR (min^{-1}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[a,c]		po[b,c]		iv[a,d]	po[b,d]
	male	female	male	female	female	female
C_{max} [ng/ml]			1010	3160		3830
tmax [h]			0.5	0.5		0.5
AUC [ng/ml x h]	2500	15400	1630	21300	3300	4260
$t_{1/2}$ [h][e]	1.6	1.7			1.0	
CL [L/h/kg]	0.4	0.1			0.3	
V_{ss} [L/kg]	0.5	0.2			0.1	
bioavailability [%]			~ 30	~ 70		~ 65

[a] dose: 1 mg/kg; [b] dose: 2 mg/kg; [c] n = 3; [d] n = 1; [e] 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_A-selective antagonist. Thus, this compound should prove useful for the evaluation of any potential benefit of ET_A-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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5. All new compounds were characterized by IR, NMR and mass spectra. For EMD 122946: mp > 200°C (dec.); IR (KBr) 1592 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ ppm: 7.97 (d, J = 9.1 Hz, 1H), 7.87 (tr, J = 1.0 Hz, 1H), 7.56 (dd, J = 1.6 Hz, J = 9.0 Hz, 1H), 7.51 (dd, J = 2.2 Hz, J = 8.5 Hz, 1H), 7.39 (dd, J = 2.2 Hz, J = 12.1 Hz, 1H), 7.03 (tr, J = 8.5 Hz, 1H), 5.75 (s, 2H), 3.88 (s, 3H), 3,45 (s, 3H), 3.42 (s, 6H), 3.02 (s, 2H); MS (FAB), m/z = 583 (M^+ + Na). Anal. calcd. for $C_{27}H_{22}FN_2NaO_7$ (560.54): C, 57.86; H, 3.96; N, 5.00; S, 5.72; found: C, 57.6; H, 4.0; N, 5.0; S, 5.9.
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