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CGS 27830, A POTENT NONPEPTIDE ENDOTHELIN RECEPTOR ANTAGONIST

Ben Mugrage*, John Moliterni, Leslie Robinson, Randy L. Webb, Suraj S. Shetty,
Kenneth E. Lipson, Michael H. Chin, Robert Neale and Catherine Cioffi
Ciba-Geigy Corp., Pharmaceuticals Div., 556 Morris Ave., Summit, NJ 07901

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Abstract: Treatment of the dihydropyridine carboxylic acid **1** with EDCI produced a diastereomeric mixture of chemically stable anhydrides. The meso-isomer, **CGS 27830**, was isolated by crystallization and found to be a potent (16 nM) endothelin receptor antagonist for the ET_A receptor subtype.

We wish to report the synthesis and biological activity of a potent nonpeptide endothelin antagonist derived from the dihydropyridine class of calcium ion modulators.[1] The symmetric anhydride (**CGS 27830**) was found to have rather poor potency in inhibiting the binding of [^{3}H]PN200-110 to L-type calcium channels (IC_{50} = 592 ± 94 nM) in rat cortex membranes.[2] However, **CGS 27830** was found to potently inhibit the binding of [^{125}I]ET-1 to the ET_A receptor subtype (IC_{50} = 15.9 ± 1.3 nM) in porcine thoracic aorta membranes.[3,12] Using rat cerebellum membranes, **CGS 27830** was also able to compete for binding to the ET_B receptor subtype, but with considerably less affinity (IC_{50} = 295 ± 19 nM).

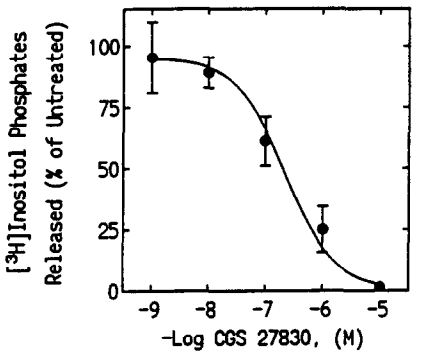


Figure 1. Ability of CGS 27830 to inhibit [3H]inositol phosphate accumulation in A7r5 cells. Intact cells were preincubated with CGS 27830 for 30 min and then challenged with 5 nM ET-1 for an additional 30 min. Values are shown as the mean±SEM from 3 independent experiments.

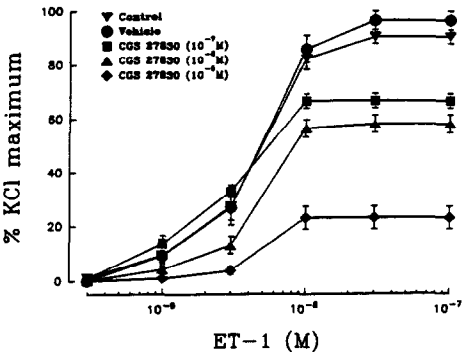


Figure 2. Effect of CGS 27830 on contractions evoked by ET-1 in isolated rabbit aortic strips. The tissues were equilibrated with CGS 27830 for 30 min before exposure to cumulative concentrations of ET-1. All changes in force are expressed as percentage of maximal response to KCl and expressed as the mean ± SEM.

Further biological profiling showed that **CGS 27830** inhibited ET-1-induced phosphoinositide (PI) turnover (EC_{50} = 143 ± 58 nM, n = 3) in A7r5 smooth muscle cells (fig. 1), while having no effect on basal PI turnover.[4] Since the ET_A receptor predominates in this cell line, these data demonstrate the potency of **CGS 27830** as an ET_A antagonist. A 30 min pretreatment with **CGS 27830** (0.1 - 10 μM) produced a dose dependent, insurmountable inhibition of the ET-1 induced contractile response in isolated rabbit aorta. The maximal response to ET-1 was attenuated by 33, 42 and 77% at 0.1, 1 and 10 μM, respectively (fig. 2). Similar pretreatment with **CGS 27830** (1 μM) did not alter the concentration curves for phenylephrine or KCl suggesting that the antagonist action of **CGS 27830** is specific for ET-1. A 5 min pretreatment with **CGS 27830** (10 mg/kg, i.v.) abolished the pressor response and attenuated the depressor response to ET-1 (0.25 nmol/kg i.v.) in the conscious rat (fig. 3). Unfortunately, an assessment of the duration of action of **CGS 27830** revealed a relatively short half-life ($T_{1/2}$ < 60 min) as shown in fig. 4.

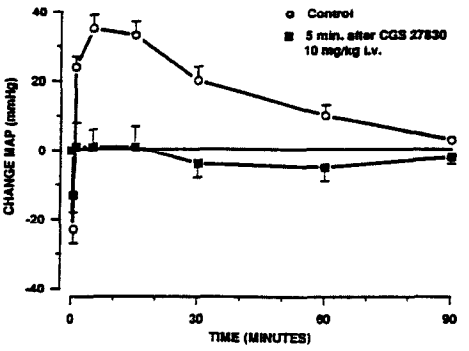


Figure 3. Effect of CGS 27830 on ET-1-induced changes in mean arterial blood pressure in the conscious Wistar Kyoto rat. CGS 27830 (10 mg/kg, i.v.) or vehicle were administered 5 min prior to ET-1 (0.25 nmol/kg, i.v.)

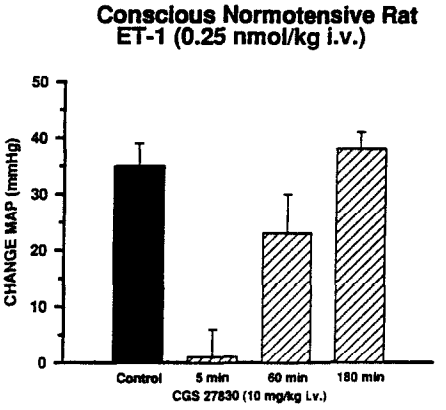
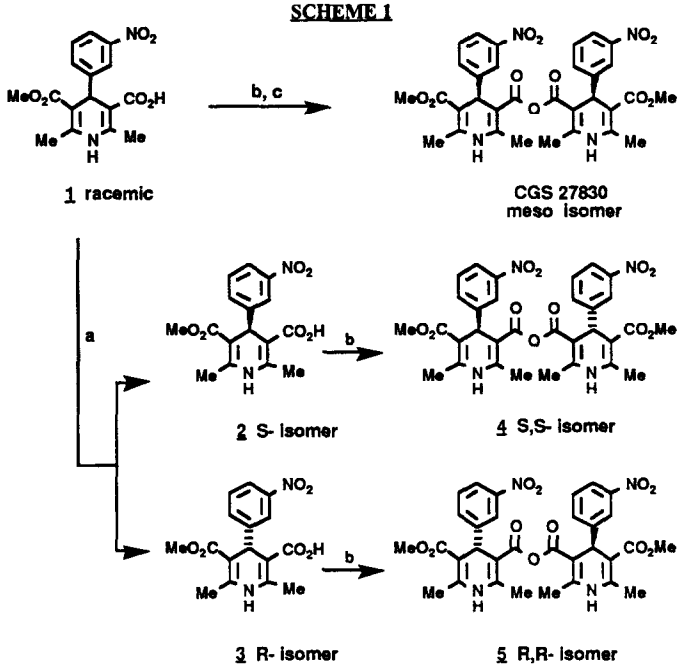


Figure 4. Effect of CGS 27830 on ET-1-induced increases in blood pressure. CGS 27830 was given either 5, 60 or 180 min prior to an ET-1 challenge (hatched bars). Changes in blood pressure evoked by ET-1 in the absence of the antagonist are also shown (solid bar)

The other possible diastereomeric anhydrides were also examined for ability to antagonize ET-1 binding. The R,R-isomer (**5**) was found to only moderately inhibit binding (IC_{50}; ET_A = 422 ± 56 nM, ET_B = 2.7 ± .5 μM), while the S,S-isomer (**4**) was found to be inactive at both endothelin receptor subtypes.

SCHEME 1



a) Resolution (Ref. 10), b) EDCI•HCl / DMAP, c)Recrystallize from THF / Et_2O

A diastereomeric mixture of anhydrides was produced, as shown in Scheme 1, by treatment of the known dihydropyridine carboxylic acid[5] with a dehydrating agent. The most efficient method was the utilization of 1-ethyl-3-(3-dimethylamino-propyl)carbodiimde hydrochloride (EDCI•HCl) with a catalytic amount of dimethylaminopyridine (DMAP) in CH_2Cl_2, at room temperature for 1h.[6] Other dehydrating conditions such as oxalyl chloride / pyridine or diethylazodicarboxlate (DEAD) with triphenylphospine also gave the mixture of diastereomeric anhydrides, although in much lower yields.[7] The anhydride proved remarkably stable and could be washed with water or 1N K_2CO_3 solution without appreciable hydrolysis. Another reflection of the surprising stability of the anhydride was that flash chromatography did not result in decomposition. **CGS 27830** was separated from the mixture of stereo-isomers, as a light yellow solid, by careful crystallization from THF / Et_2O. A second crystallization from THF / Et_2O produced crystals suitable for x-ray structure determination (fig. 5).[8]

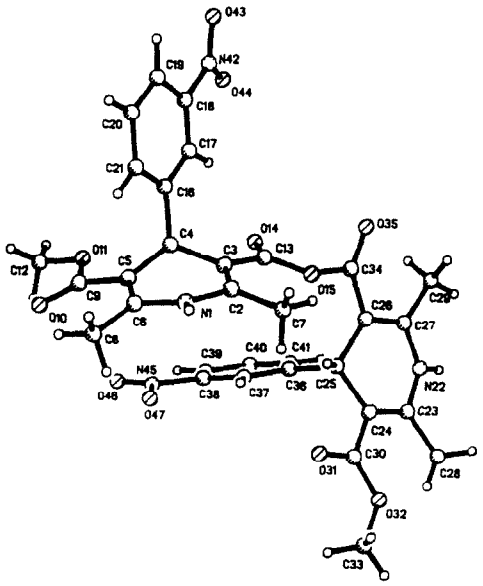


Figure 5. The x-ray structure of CGS 27830

The R,R and S,S-stereoisomers[9] (**4, 5**) were produced from the corresponding optically active dihydropyridine carboxylic acids[10] (**2, 3**) in a similar fashion. The des-nitro, *m*-chloro and *m*-methoxy anhydride analogs were also tested, as diastereomeric mixtures, and found to have receptor binding activities similar to the *m*-nitro compound.

We have shown CGS 27830 to be a potent and selective endothelin receptor antagonist. Results from receptor binding studies indicate that **CGS 27830** displays more than an 18-fold selectivity for the ET_A receptor subtype. However, results from in vitro and in vivo studies suggest some functional ET_B antagonist activity as well. Given the insurmountable nature of the receptor antagonism and the potential for anhydrides to act as acylating agents, covalent modification of the receptor by **CGS 27830** is a distinct possibility. Experiments to determine the mode of action of **CGS 27830** are ongoing. Nevertheless, the potent and readily synthesized receptor antagonist **CGS 27830** represents a valuable phamacologic tool for the investigation of the role of ET-1 in models of vascular disease states.[11]

Acknowledgment: Dr. Frank H. Clarke is gratefully thanked for the x-ray structure determination studies. Drs. Herman Rodriguez and Gary Ksander are thanked for valuable discussions.

References and Notes

1. F. Bossert, H. Meyer and E. Wehinger; *Angew. Chem. Int. Ed. Eng.*, **20**, 762, (1981).
2. The binding of [^{3}H]PN200-110 to rat cortex membranes was performed by the method of Lee *et al.*: Lee, H.R., Roeske, W.R., and Yamamura, H.I., *Life Sci*, **35**, 721 (1984).
3. The binding of [^{125}I]ET-1 to porcine thoracic aorta membranes (ET_A), or rat cerebellum membranes (ET_B) was performed as previously described for rat lung membranes: Cioffi, C., Neale Jr., R.F., Jackson, R.H., and Sills, M.A., *J. Pharmacol. Exp. Ther.*, **262**, 611 (1992).
4. The release of [^{3}H]inositol phosphates from [^{3}H]inositol-labeled A7r5 cells was measured as previously described: Cioffi, C. and Garay, J., *J. Cardiovas. Pharmacol.*, in Press (1993).
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6. Preparation of **CGS 27830**: 1,4-Dihydro-5-methoxycarbonyl-2,6-dimethyl-4-(3-nitrophenyl)-3-pyridine-carboxylic acid (**1**) (21.4 g, 0.065 mol) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (23.0 g, 0.120 mol) were suspended in dry CH_2Cl_2 (300 ml) and treated with a catalytic amount (25 mg) of 4-dimethylaminopyridine. The reaction was stirred, at room temperature, under an N_2 atmosphere, until a clear yellow solution was obtained (~2h). Tlc (SiO_2 / Et_2O) indicated the reaction was complete. The solution was transferred to a separatory funnel and washed sequentially with water (200 ml), 1N K_2CO_3 (100 ml) and brine (100 ml). The organic layer was dried ($MgSO_4$) and concentrated to a yellow foam. The crude product was purified by passing through a short plug of flash silica gel eluted with 1:1 Et_2O / EtOAc followed by EtOAc to produce, after evaporation, a 2:1 mixture of diastereomers favoring the meso-isomer. The isomeric mixture was then dissolved in THF (600 ml) and Et_2O added until the solution became slightly cloudy. The solution was kept at 4°C overnight to give **CGS 27830** (6.0 g) as a light yellow solid (mp 223-4° dec) which was isolated by decantation. ^{1}H-NMR (DMSO-d_6) 9.38,s,2H; 8.03, d, 2H, J = 8.3; 7.70, s, 2H; 7.46-7.57, m, 4H; 4.58, s, 2H; 3.50, s, 6H; 2.26, s, 6H; 2.19, s, 6H., ^{1}H-NMR ($CDCl_3$) 8.00, m, 2H; 7.85, m, 2H; 7.33-7.50, m, 4H; 5.95, s, 2H; 4.72, s, 2H; 3.60, 6H; 2.30, s, 6H; 2.26, s, 6H. IR (CH_2Cl_2) 3350, 1751, 1701, 1652, 1609, 1530, 1472, 1435, 1351, 1225, 1185, 1112, 1061, 950 cm^{-1}. Calc. for $C_{32}H_{30}N_4O_{11}$: 59.44 %C, 4.68 %H, 8.67 %N; Found: 59.25 %C, 4.50 %H, 8.52%N.

7. When the dihydropyridine carboxylic acid **1** and EDCI•HCl are stirred in the presence of 1-hydroxybenzotriazole, a stable HOBT adduct is formed that can be isolated and flash-chromatographed.
8. The refined coordinates, bond distances, bond angles and torsion angles with esd's for the crystal structure of **CGS 27830** have been submitted for deposition at the Cambridge Crystallographic Data Centre.
9. Analytical data for **4**: ^{1}H-NMR ($CDCl_3$) 8.05, m, 2H; 7.98, m, 2H; 7.60, d, 2H, J = 8.1 Hz; 7.33, t, 2H, J = 8.1 Hz; 6.05, s, 2H; 5.12, s, 2H; 3.70, s, 6H; 2.37, s, 6H; 2.23, s, 6H. Calc. for $C_{32}H_{30}N_4O_{11}$: 59.44 %C, 4.68 %H, 8.67 %N; Found: 59.13 %C, 4.50 %H, 8.33%N. $[\alpha]_D$ (c 0.54, $CHCl_3$) = -269°.
Analytical data for **5** ^{1}H-NMR ($CDCl_3$) 8.02, m, 2H; 7.95, m, 2H; 7.59, d, 2H, J = 8.1 Hz; 7.31, t, 2H, J = 8.1 Hz; 6.19, s, 2H; 5.10, s, 2H; 3.70, s, 6H; 2.34, s, 6H; 2.20, s, 6H. Calc. for $C_{32}H_{30}N_4O_{11}$: 59.44 %C, 4.68 %H, 8.67 %N; Found: 59.63 %C, 4.53 %H, 8.46%N. $[\alpha]_D$ (c 0.81, $CHCl_3$) = +329°.
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12. Using the cyclic pentapeptide endothelin antagonist, BQ-123, as a reference in our assays the following results were obtained: ET_A receptor binding, IC_{50} = 22 nM; PI-turnover IC_{50} = 87 nM; Isolated rabbit aorta BQ-123 (1 μM) inhibited 28% of the contraction caused by ET-1(0.1 μM); In Vivo, in conscious rats, a 5 min. pretreatment with BQ-123 (10 mg/kg, i.v.) inhibited 57% of the pressor response caused by ET-1 (0.25 nmol/kg, i.v.).
13. Nonpeptide endothelin receptor antagonists has been reported previously see: a) S-i. Mihara and M. Fujimoto, *Eur. J. Pharacol., Mol. Pharmacol Sect.*, **246**, 33 (1993). b) M. Fujimoto, S-i. Mihara, M. Ueda, M. Nakamura and K-s Sakurai, *FEBS Lett.*, **305**, 41 (1992). c) R. Cousins *et al.*, World Pat. 9308799 (1993). d) K. Burri *et al.*, Eur. Pat. 510,526A (1992). e) O. Nobutaka *et al.*, Eur. Pat. 405,421 (1991).