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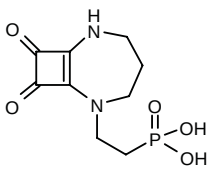
EAA-090

Neuroprotectant
Competitive NMDA Antagonist

WAY-126090

2-[8,9-Dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl]ethylphosphonic acid



$C_9H_{13}N_2O_5P$
Mol wt: 260.1847
CAS: 144912-63-0
EN: 189797

Abstract

EAA-090 is a novel squaric acid amide derivative that has been identified as a potential treatment for the ischemic brain damage resulting from stroke. EAA-090 is a competitive inhibitor at the NMDA-selective subtype of the glutamate receptor. The compound demonstrates potent inhibitory activity in both *in vitro* and *in vivo* models of NMDA-induced excitotoxicity and provides neuroprotective efficacy in several animal models of stroke. EAA-090 is unique among competitive NMDA antagonists in displaying a clear separation between predicted efficacious dose and doses that induce PCP-like psychotomimetic side effects in both animals and humans. This unique profile makes EAA-090 an exciting candidate for assessing the neuroprotective potential of the competitive NMDA mechanism.

Synthesis

EAA-090 is synthesized in four steps. Combining (3-aminopropyl)carbamic acid benzyl ester (CBZ-protected propylene diamine) (I) with 2-oxoethylphosphonic acid diethyl ester under reductive amination conditions in the presence of sodium cyanoborohydride gives the CBZ-protected phosphonoethyl-substituted propylene diamine (II). The condensation of (II) with 3,4-diethoxy-3-cyclobutene-1,2-dione at room temperature in ethanol leads to the squaric acid analog (III). Removal of the CBZ protecting group under catalytic transfer hydrogenation conditions followed by spontaneous cyclization provides the penultimate diethylphosphonate ester (IV), which is hydrolyzed in the presence of bromotrimethylsilane in refluxing 1,2-dichloroethane to yield the final product in 15% overall yield (1, 2). Scheme 1.

An alternate preparative process has also been patented (3).

Description

Yellow solid, m.p. 260-78 °C (decomp).

Introduction

Stroke is the third leading cause of medically related death in North America, Western Europe and Japan. In the United States alone, approximately 600,000 strokes (first ever and recurrent) occur each year and the estimated costs for stroke-related care and lost productivity may approach nearly USD 49 billion. The widely accepted excitotoxic hypothesis of ischemic neuronal cell death in stroke holds that the hypoxia and hypoglycemia caused by occlusion of the brain blood vessels (due to clot, hemorrhage or vasospasm) induce energy failure. The resulting loss of ionic homeostasis and depolarization-induced neurotransmitter release lead to an overactivation of the excitatory glutamatergic neurotransmitter system which, in turn, leads to excessive intracellular calcium ion concentrations, mainly via an influx of calcium through the *N*-methyl-D-aspartate (NMDA)-specific subtype of the glutamate receptor ion channel complex. The elevated calcium ion concentrations induce a complex cascade of biochemical events which eventually leads to neuronal cell death (4-6).

Intense research over the past 20 years has identified numerous agents that interrupt the excitotoxic cascade at various stages. While many of these potential drugs have

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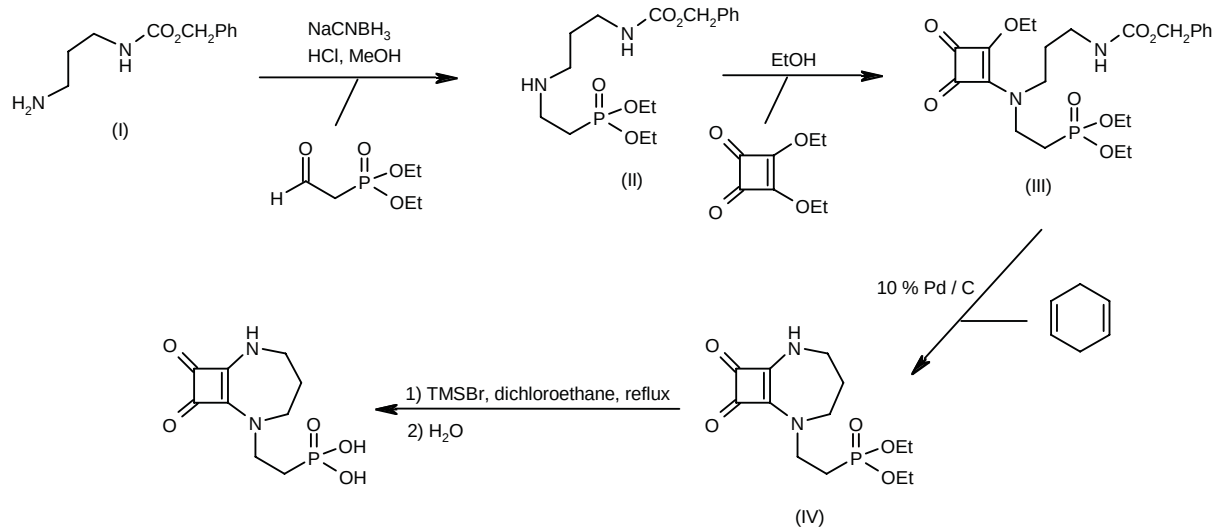
Scheme 1: Synthesis of EEA-090



shown promise in preclinical animal stroke models of permanent and temporary occlusion of the middle cerebral artery (MCA), this promise has not translated to the clinical setting. In fact, with the recent failure of several neuroprotective drug trials, the thrombolytic agent recombinant tissue plasminogen activator (r-tPA) remains the only currently approved drug for the treatment of acute ischemic stroke and its use may be limited to a relatively small number of patients due to safety considerations. Thus, a true medical need for a safe, efficacious and widely applicable neuroprotective agent still exists (5).

Blockade of the NMDA receptor has received a great deal of attention as a potential mechanism for neuroprotection. Compounds acting at four sites on the NMDA receptor ion channel complex, namely the competitive glutamate binding site, the glycine co-agonist binding site, the MK-801 noncompetitive binding site within the ion channel, and the polyamine modulatory binding site have been examined both preclinically and in the clinic. The first NMDA antagonists to enter clinical trials were ones that acted at the competitive glutamate and noncompetitive (MK-801) binding sites. Clinical trials on almost all of these compounds have been discontinued, primarily due to the occurrence of unpleasant and sometimes violent psychotomimetic side effects which are similar to those induced by the illicit recreational drug phencyclidine (PCP). In particular, the two competitive NMDA antagonists D-CPPene (SDZ-EAA-494) and selfotel (CGS-19755) were discontinued in the clinic because they elicited adverse experiences (agitation, hallucinations, confusion, paranoia and delirium) at blood concentrations which were well below the levels predicted to be required for neuroprotective efficacy. Because of these early failures, more recent stroke clinical trials involve other glutamate-based mechanisms, such as modulatory sites on the NMDA receptor complex (*e.g.*, glycine and polyamine), other glutamate receptor subtypes (*e.g.*, AMPA) or low-affinity ligands for the MK-801 binding site. However, the lack of therapeutic index seen with competitive NMDA antagonists such as D-CPPene and selfotel, which can be demonstrated in a preclinical setting, has made it impossible to accurately assess the neuroprotective potential of competitive NMDA antagonists since predicted efficacious blood levels have never been achieved. Thus, a competitive NMDA antagonist that possesses a clear separation between efficacious neuroprotective dose and the dose that induces psychotomimetic effects would not only be a truly useful tool with which the actual clinical efficacy of NMDA antagonism could finally be assessed, but also a potentially efficacious neuroprotective agent (5, 7-13).

Pharmacological Actions

EAA-090 is an intravenously administered competitive NMDA-selective glutamate receptor antagonist developed as a neuroprotective agent for the treatment of ischemic stroke. The compound is structurally distinct from almost all other competitive NMDA antagonists in that it possesses a bioisosteric squaric acid amide grouping in place of the amino acid moiety, eliminating any zwitterionic character.

In vitro studies confirmed EAA-090 as a potent, selective competitive NMDA antagonist (14). It competitively inhibited the binding of [^{3}H]-CPP to NMDA receptors in rat brain homogenates (IC_{50} = 28 nM) and prolonged the equilibration time required for [^{3}H]-TCP to bind within the

Table I: Summary of in vitro *and* in vivo *activity for EAA-090.*

In vitro data	
Affinity for NMDA receptor	K_i = 28 nM
Inhibition of NMDA-stimulated TCP binding	IC_{50} = 7.7 μM
Inhibition of NMDA-induced currents (hippocampal neurons)	IC_{50} = 0.48 μM
Inhibition of glutamate-induced neurotoxicity (hippocampal neurons)	IC_{50} = 1.5 μM
In vivo data	
Inhibition of NMDA-induced lethality	ED_{50} = 2.1 mg/kg i.p.
Inhibition of MES-induced seizures	ED_{50} = 4.8 mg/kg i.p.

NMDA ion channel (IC_{50} = 7.7 μM). EAA-090 did not possess any appreciable affinity for kainate, AMPA or strychnine-insensitive glycine receptors at concentrations up to 100 μM. Likewise, EAA-090 did not show any appreciable affinity for 36 other CNS receptors, ion channels, uptake sites and second messenger recognition sites at concentrations up to 10 μM. In electrophysiological experiments, EAA-090 blocked currents induced by iontophoretically applied NMDA (IC_{50} = 0.48 μM). In cultured rat hippocampal neurons, EAA-090 blocked glutamate-induced neurotoxicity (IC50 = 1.6 μM) (14) (Table I).

In vivo, EAA-090 blocked the lethality induced by administering an ED_{90} dose of NMDA (ED_{50} = 2.1 mg/kg i.p.). It also demonstrated anticonvulsant efficacy in the maximal electroshock model when administered systemically (ED_{50} = 4.8 mg/kg i.p.) (14) (Table I).

EAA-090 provided neuroprotection in various experimental models of ischemic stroke. In a gerbil global ischemia model employing a 5-min occlusion and a 4-day recovery period, a 100 mg/kg i.p. dose of EAA-090 (given 4 times, 2 h apart beginning immediately prior to occlusion) resulted in a statistically significant reduction in the hippocampal neuronal injury score when animals' body temperatures were allowed to free-regulate. Neuroprotection was maintained in a similar experiment when hypothermia was excluded by maintaining normothermic body temperature for 2 h postocclusion (14).

EAA-090 was examined in multiple models of rat focal ischemia. In rats subjected to permanent tandem occlusion of the MCA (using an electrocoagulation technique) and ipsilateral common carotid artery, a 10 mg/kg dose of EAA-090 administered as a single i.v. bolus provided a maximal 57% reduction in infarct volume (evaluated 24 h postocclusion). This result was similar to that obtained with the known competitive NMDA antagonist selfotel (50% reduction in infarct volume at 10 mg/kg i.v.). The range of efficacious doses seen with EAA-090 was quite wide, spanning from 3-30 mg/kg i.v. Hypothermia did not contribute significantly to the neuroprotection of EAA-090, as fluctuations in body temperature during the 4-h postsurgery interval were within 1 °C. Neuroprotective efficacy in the permanent focal ischemia model was lost when administration of EAA-090 was delayed until 1 h postocclusion. In spontaneously hypertensive rats subjected to permanent MCA occlusion for 24 h by an intraluminal suture approach, treatment with EAA-090 (10 mg/kg i.v. 5 min postocclusion) resulted in a 33% reduction in infarct, while a similar dose of selfotel was inactive (1, 15, 16) (Table II).

EAA-090 also provided neuroprotective efficacy in transient focal ischemia models using the intraluminal suture approach. In Wistar rats subjected to 90 or 120 min of transient MCA occlusion, a 10 mg/kg i.v. dose of EAA-090 provided a 40-50% reduction in infarct volume. In spontaneously hypertensive rats, an injury dose-response curve was generated by subjecting animals to transient MCA occlusion of varying duration from 5-180 min. EAA-090 (10 mg/kg i.v.) treatment significantly prolonged the duration of MCA occlusion resulting in half-maximal infarct. Selfotel (10 mg/kg i.v.) was not active in either of these two models (16, 17) (Table II).

The histological neuroprotection seen from treatment with EAA-090 in the transient and permanent focal ischemia models correlated with improved performance in motor function assessments, including vertical screen, vertical beam and the Bederson motor testing battery. Additionally, the histological neuroprotection and improvement in motor function resulting from treatment with EAA-090 in a transient focal ischemia model correlated with a reduction in serum levels of neuron-specific enolase. These results were similar to those obtained with preocclusion administration of MK-801. Increased serum and CSF levels of neuron-specific enolase have been observed in animal models of brain ischemia and epilepsy as well as in human stroke patients, and have been identified as a potential diagnostic and prognostic tool for assessing the injury to the CNS in stroke victims (16-20).

As described above, competitive NMDA antagonism has been associated with a number of psychotomimetic side effects, including those associated with the administration of PCP. A lack of separation between these undesired side effects and efficacious dose has hampered the development of several competitive NMDA antagonists, including D-CPPene and selfotel (9).

The preclinical side effect profile of EAA-090 suggests that the compound possesses a separation between efficacy and undesired CNS side effects. EAA-090 induced deficits in traction reflex in mice (ED_{50} = 16 mg/kg i.p.). However, it performed favorably in a drug discrimination paradigm in rats, which examined the ability of

Table II: Results for EAA-090 and comparators in animal models of stroke.

Occusion method	Compound	Dose	% Reduction in infarct volume
		Permanent focal ischemia	
Electrocoagulation (Fisher 344)	EAA-090	3 mg/kg*	29
		10 mg/kg*	57
		30 mg/kg	28
	Selfotel	3 mg/kg*	Inactive
		10 mg/kg*	50
		20 mg/kg*	Inactive
Intraluminal suture (SHR)	EAA-090	10 mg/kg*	33
	Selfotel	10 mg/kg*	Inactive
		Transient focal ischemia	
Intraluminal suture (Wistar) 90-120 min	EAA-090	3 or 10 mg/kg*	40-50
	Selfotel	10 mg/kg*	Inactive
Intraluminal suture (Wistar) 90 min	EAA-090	3 mg/kg*	50
	MK-801	0.5 mg/kg**	55

*Administered as a single i.v. bolus 5 min postocclusion of MCA. **Administered as a single i.v. bolus 15 min prior to occlusion of MCA.

compounds to generalize to the stimulus properties of PCP. When EAA-090 was administered to rats trained to recognize the PCP cue, partial generalization occurred only in some animals at higher doses (ED_{50} > 54 mg/kg i.p.; ED_{50} = 35 mg/kg i.v.). In contrast, selfotel completely generalized to the PCP cue at doses which overlapped the efficacious dose in the rat focal ischemia model (ED_{50} = 7 mg/kg i.p.; ED_{50} = 11 mg/kg i.v.). Thus, EAA-090 displayed a separation between efficacious dose and the dose that induced PCP-like psychotomimetic effects which is not seen with selfotel. Olney *et al.* correlated the PCP-like psychotomimetic effects resulting from administration of NMDA antagonists with an acute vacuole reaction in regions of the cingulate and retrosplenial cortices. EAA-090 demonstrated a separation between efficacious dose in the rat focal ischemia model and the dose that induced this vacuolization reaction. Doses of 10-40 mg/kg i.v. caused no vacuole formation. At 50 mg/kg i.v., EAA-090 induced a low frequency of vacuole formation in 50% of the rats. In comparison, a 20 mg/kg i.v. dose of selfotel induced a high rate of vacuole formation in 100% of the animals tested. Thus, preclinical studies indicate that EAA-090 possesses a separation between efficacious dose and psychotomimetic dose that is unique among competitive NMDA antagonists (14, 21, 22).

Pharmacokinetics

Single and multiple intravenous bolus doses of EAA-090 were administered to rats and monkeys at doses spanning the pharmacological and toxicological ranges. Humans received single 15-min i.v. infusions at doses ranging from 30-960 mg. Plasma concentrations of EAA-090 were determined by HPLC with UV detection and pharmacokinetic parameters were determined using a noncompartmental approach. Clearance, volume of distribution at steady-state and half-life ($t_{1/2}$) in humans were predicted by allometric scaling of the preclinical data and were compared to actual clinical data (23) (Table III).

Table III: Summary of pharmacokinetic parameters for EAA-090.

Species	Cl (l/h/kg)	V_{ss} (l/kg)	$t_{1/2}$ (h)
Rat	0.66	0.47	1-12 (dose-dependent)
Monkey	0.28	0.35	1.4-9 (dose-dependent)
Human	0.15	0.43	5.7 (dose-independent)

In rats receiving a single dose of EAA-090, clearance was dose-independent between 1.1 and 40 mg/kg and decreased slightly at the highest dose (280 mg/kg). Volume of distribution remained constant over the entire dose range. The $t_{1/2}$ increased with dose and ranged from 1-12 h. Multiple dosing had no effects on the kinetics of EAA-090 in rats. In the monkey, clearance and volume of distribution were dose-independent between 1 and 70 mg/kg. The $t_{1/2}$ values increased with dose and ranged from 1.4-9 h. Slight decreases in clearance and volume of distribution were noted in monkeys after repeated administration of the highest dose (70 mg/kg). No gender differences were observed. In humans, clearance, volume of distribution and $t_{1/2}$ were constant over the dose range. Allometric equations determined from the preclinical data predicted values in humans which were 87% (clearance), 68% (volume of distribution) and 65% ($t_{1/2}$) of those actually measured following administration of a single dose to healthy volunteers.

Clinical Studies

A dose-escalation safety and tolerability study of EAA-090 was conducted in 40 normal, healthy male volunteers. Ten groups of 4 subjects were randomized (3:1), with 30 subjects receiving a single dose of EAA-090 and 10 subjects receiving placebo. Drug was administered as a slow intravenous bolus over 15 min at escalating doses of 30-960 mg. Blood pressure, heart rate, ECG, symptoms and neurological signs were closely monitored for 48 h following administration (24).

No significant changes in mean arterial blood pressure (MAP) were observed at doses of EAA-090 up to 480 mg. Significant rises in MAP were observed in 1 volunteer at the 600 mg dose (27 mmHg), all 3 subjects at the 720 mg dose (13-22 mmHg), 1 at the 840 mg dose (23 mmHg) and 2 at the 960 mg dose (18 and 23 mmHg). Blood pressure rises peaked at 30 min for all doses other than the 960 mg dose, which peaked at 2 h. All elevated blood pressures returned to normal within 4 h, and no serious adverse events and no laboratory safety abnormalities were observed.

No significant CNS effects were observed in subjects receiving doses of EAA-090 up to and including 120 mg. CNS symptoms were reported more frequently at higher doses, with minimal symptoms reported in the dose range of 120-600 mg and significant CNS effects being observed in the range of 720-960 mg. Symptoms included dizziness, poor concentration, sedation, dysarthria, paraesthesia and altered taste. Dizziness was most commonly reported (33%) followed by paraesthesia and poor concentration (both 17%). Dizziness and poor concentration were severe enough to be considered as adverse effects at the 840 and 960 mg doses, respectively. These effects peaked between 15 min and 2 h from the start of the infusion and persisted for up to 8 h in the 960 mg group. All subjects in the 960 mg group reported CNS events. One subject described a mild auditory and visual hallucination between 4.5 h and 9 h after the infusion. Numerous CNS symptoms, including depersonalization, were also reported by 1 subject who received placebo.

Phase I data indicate that single doses of up to 720 mg were well tolerated in healthy male volunteers. Given the fact that the projected optimal neuroprotective plasma concentration (16.3 μg/ml, based on the rat focal ischemia studies) is achieved at the 240 mg dose, the data suggest that a separation between efficacy and undesirable cardiovascular and psychotomimetic side effects exists for EAA-090. An analysis of the ratio between therapeutic effects and various side effects reveals a separation of potencies of approximately 3.5. The favorable therapeutic index seen with EAA-090 is unique among competitive NMDA antagonists, where efficacious and psychotomimetic doses have historically overlapped.

Conclusions

In conclusion, EAA-090 represents a potent, selective competitive NMDA antagonist that is structurally distinct from other competitive NMDA antagonists in lacking an amino acid moiety. EAA-090 is active *in vivo* when given systemically, and demonstrates neuroprotective efficacy in numerous animal models of cerebral ischemia. The reduction in infarct volume in these models correlates with improvement in several motor function assessments. Most importantly, when assessed in both animals and humans, EAA-090 appears to be well tolerated and to possess a clear and significant separation between predicted efficacious dose and doses that induce the PCP-like psychotomimetic side effects which doomed earlier NMDA antagonists to failure in clinical trials. This unique profile makes EAA-090 an exciting candidate for truly assessing the neuroprotective potential of the competitive NMDA antagonist mechanism.

Source

Wyeth (US).

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