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ACEA 1021: Flip or Flop?

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ABSTRACT

Inasmuch as glutamate is the main excitatory neurotransmitter in the central nervous system, strategies aimed at counteracting glutamate excitotoxicity, which is at least partially involved in many acute neurologic, chronic neurodegenerative and psychiatric diseases, are challenging. Blockade of the NMDA receptor was identified as one way of achieving selective antagonism and overcoming glutamate neurotoxicity, yet not without liabilities. Glycine site antagonism of the NMDA receptor in 1987 offered a significant advance in blocking this receptor because such drugs were shown to lack most of the side effects, such as memory impairment, ataxia, lack of motor coordination and psychotomimetic effects, which accompanied competitive and non-competitive NMDA receptor antagonists. To date, much has been done to improve the structure-activity relationship (SAR) of compounds resulting in the synthesis of ACEA 1021. It is unclear, however, whether further chemical substitutions will lead to an improved compound. Many studies have been performed with ACEA 1021 and although there are much *in vitro* and *in vivo* data to support its neuroprotective effects and improved safety profile, there is very little published information regarding its clinical pharmacology. In order to properly evaluate the true potential for ACEA 1021 in acute and chronic CNS disorders additional longer term safety and efficacy data in humans are needed.

INTRODUCTION

Abnormal glutamate neurotransmission via the ionotropic, N-methyl-D-aspartate (NMDA) receptor is at least partially involved in a wide variety of acute neurologic, chronic neurodegenerative and psychiatric disorders, including Alzheimer's disease, anxiety,

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depression, drug dependency, epilepsy, Huntington's disease, pain, Parkinson's disease, schizophrenia, stroke and traumatic brain injury, and even alcoholism (26,32,64).

Structurally, the NMDA receptor is a cationic channel that is permeable to Ca^{2+}, Na^{+}, and K^{+}. In addition to the NMDA recognition site there is a binding site recognized by dissociative anesthetics and MK-801 that is located within the channel. This divalent cation-binding site binds Mg^{2+} and its modulatory sites are sensitive to Zn^{2+}, polyamines and glycine (63).

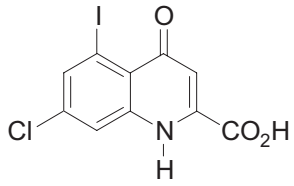
Functionally, the NMDA receptor can be inhibited at four different sites: a) the primary transmitter site (competitive inhibition); b) the polyamine site (NR2B selective); c) the phencyclidine site within the ion channel; and d) the strychnine-insensitive, glycine site. The glycine site on the NMDA receptor was discovered by Johnson and Ascher (27). It represents a unique characteristic of the NMDA receptor in which another amino acid, glycine, acts as a co-agonist with glutamate to drive the glutamatergic response. By the early 1990s a tremendous interest in glycine site antagonism of the NMDA receptor as a therapeutic target led to the discovery of many compounds at major pharmaceutical companies, including, Glaxo (GV150526), Hoechst Marion Roussel/Aventis Pharma (MDL 105,519), Merck (L-687414, L-701324), Aventis Pharma (RPR104632, RPR118723), Symphony (ACPC), and Zeneca (ZD9379). The research programs at these companies focused on the same mechanism of action (5,10,30). ACEA 1021 (CoCensys/Ciba) was one of the experimental compounds that were studied extensively in a variety of animal models (30).

CHEMISTRY

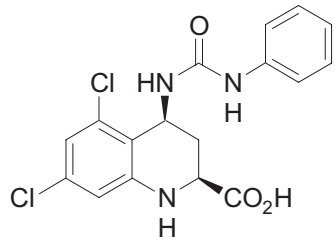
Several classes of glycine antagonists, shown in Fig. 1, have been developed (30). A significant problem with the first generation of these antagonists is their lack of systemic activity. Although these compounds displayed significant *in vitro* and *in vivo* activity following intraventricular injection, by systemic administration most of them had poor blood-brain barrier penetration (33,35). The dihydro-2,3-quinoxalinediones (QXs) overcame this deficiency (46). In the initial structure-activity relationship (SAR) studies QXs were substituted at C-6 and C-7 with electron withdrawing groups (29) leading to ACEA 1021 (6a, $R_6 = R_7 = Cl$) (Fig. 2) (72,73). Extension of these studies encompassed electron donating groups and led to 6b ($R_6 = R_7 = CH_3$) (37,38). This compound has been shown to be more potent *in vivo* as an anticonvulsant in the maximal electroshock (MES) test in mice, but was only about 1/5 as potent at the glycine site (63). The combination of electron donating and electron withdrawing groups produced QXs with an excellent combination of *in vitro* and *in vivo* potencies with 6c ($C_6 = CH_3$, $C_7 = Cl$) having the best combination of the QXs studied (12,25).

PHARMACOLOGY

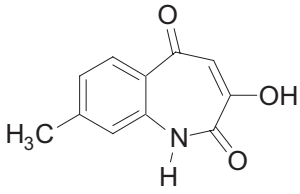
ACEA 1021 (5-nitro-6,7-dichloro-1,4-dihydro-2,3-quinoxalinedione; licostinel) is a selective antagonist at the strychnine-insensitive glycine site associated with the NMDA receptor complex (Table 1). It is a potent, competitive antagonist of glycine at NMDA re-



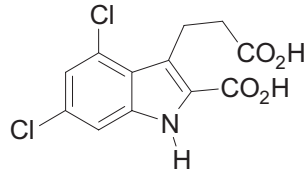
7-chloro-5-iodokynurenic acid



trans-2-carboxy-5,7-dichlorotetrahydro-quinoline-4-phenylurea



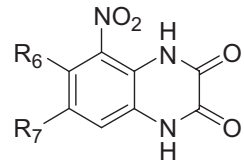
2,3-dihydro-2.5-dioxo-3-hydroxy-8-methyl-1*H*-benzazepine



2-carboxy-4,6-dichloroindole-3-propionic acid

Fig. 1. NMDA glycine receptor antagonists.

ceptors in *Xenopus* oocytes expressing rat brain mRNA (K_b = 6–8 nM), in rat cortical neurons (K_b = 5–7 nM), and in *Xenopus* oocytes expressing cloned NMDA receptors (K_b = 2–13 nM) (29,72,73). With respect to subtypes of non-NMDA receptors, ACEA 1021 has similar activity at cortical AMPA and dorsal root ganglion kainate receptors (K_b = 2.5 and 0.9 μM, respectively) (69).



$R_6 = R_7 = Cl$ ACEA-1021
$R_6 = R_7 = Me$ ACEA-1328
$R_6 = Me, R_7 = Cl$ ACEA-1416

Fig. 2. 1,4-Dihydroquinoxaline-2,3-diones.

TABLE 1. Inhibition of NMDA and non-NMDA receptors by ACEA 1021

Receptor	Agonist	IC_{50} of ACEA 1021, μM	Reference
Rat brain NMDA receptor, expressed in oocytes	Glycine	0.17	72
Rat brain NMDA cortical membrane receptor	5,7-Dichlorokynurenic acid	0.0059	75
NMDA receptor in rat cortical neurons	Glycine	0.042	72
Rat brain non-NMDA receptor, expressed in oocytes	AMPA	1.7	72
Non-NMDA receptors in rat cortical neurons	AMPA	3.9	72
Rat brain non-NMDA receptor, expressed in oocytes	Kainate	0.76	72
Non-NMDA receptor in rat cortical neurons	Kainate	1.7	72

In vivo ACEA 1021 reduced the rate of propagation of cortical spreading depression (39), an effect consistent with blockade of NMDA receptors. ACEA 1021 also decreased audiogenic myoclonus in resuscitated rats following cardiac arrest (40), and the minimum alveolar concentration for halothane (42), effects which suggest a reduction of excitatory amino acid neurotransmission. Moreover, ACEA 1021 decreased *in vivo* [^{125}I]MK-801 binding in the penumbral zone beneath experimentally induced acute subdural hematoma, indicating that it reduces pathological activation of NMDA receptors in ischemic brain tissue (17). Hence, *in vivo* studies are consistent with the *in vitro* demonstration of the blockade of NMDA receptors by ACEA 1021.

NEUROPROTECTION

The excessive increase of glutamate in the synaptic cleft is one of many deleterious mechanisms leading to neuronal damage following ischemia (50). The subsequent excessive stimulation of NMDA type receptors initiates a cascade of events leading to neuronal death mediated by calcium (7). Several studies have indicated that many compounds acting at excitatory amino acid receptors have beneficial effects in conditions of cerebral ischemia. MK-801, a noncompetitive antagonist at the phencyclidine site of the NMDA receptor complex, decreases stroke lesions in animal models of cerebral ischemia (21,54, 55). Competitive NMDA receptor antagonists such as CGS 19755 and CPP reduce ischemic damage in gerbils (9,20,55).

Another approach to reduce ischemic brain damage is to target the glycine recognition site of the NMDA receptor complex. Studies have reported that several glycine site antagonists of the NMDA receptor provide neuroprotection in models of focal cerebral ischemia. ACEA 1021 is a systemically active antagonist at the NMDA receptor glycine site with minimal phencyclidine-like side effects (4). It has been reported to be neuroprotective in many different models of focal cerebral ischemia (Table 2). Administered intraperitoneally at 55 min before middle cerebral artery (MCA) occlusion, at the termination of ischemia and at 180 min after the onset of reperfusion ACEA 1021 (10 and 30 mg/kg) protected against cortical ischemic brain damage following 90 min of focal cerebral ischemia (66). This protection was associated with a reduced incidence of hemiparesis. ACEA 1021 administered as an i.v., bolus at 15 min after the onset of ischemia followed by an infusion for 6 h significantly, reduced the infarct volume in two models of transient MCA, one of which was combined with permanent ligation of the ipsilateral common carotid artery (57). Magnetic resonance imaging confirmed these effects in the transient model, in that infarct volume observed using apparent diffusion coefficient (ADC) maps, was significantly smaller and the increase in perfusion signal intensity after reperfusion was more pronounced in the ACEA 1021-treated group (57). In rats with permanent occlusion of the MCA (modified Tamura model), ACEA 1021, injected as an i.v. bolus followed by a 6 h infusion, induced a dose-related decrease in infarct volume with a 2 h therapeutic window-of-opportunity. The reduction in infarct volume was associated with an improvement in neurological outcome (57). Sarhan et al. (60) confirmed that ACEA 1021 is neuroprotective and can reduce the infarcted area induced by permanent focal cerebral ischemia. They showed that the reduction was achieved in two models of permanent MCA occlusion in two species of rats, and that protection was possible even when ACEA 1021 was administered as late as 2 h after the onset of the insult. In mice with permanent MCA

TABLE 2. Neuroprotective effects of ACEA 1021 in animal models of cerebral ischemia

Model	Animals	Observation period h	Dose, mg/kg prime + mg/kg/h infusion	Route	Time of administration in relation to ischemic insult	% Reduction in infarct volume	Reference
90-min MCAO	Wistar rats	96	10 or 30	i.p.	–55, +90, and +180 min	50 and 75	67
2 h MCAO and 24 h reperfusion	Sprague–Dawley rats	24	10 + 7	i.v.	+15 min, + 6 h of infusion	39	57
2 h MCAO + permanent carotid artery occlusion, 24 h reperfusion	Wistar rats	24	10 + 7	i.v.	+15 min, + 6 h of infusion	32	
Permanent MCAO (modified)	Fisher 344 rats	24	10 + 7	i.v.	+15 min, + 6 h of infusion	74	
		24	5 and 3	i.v.	+15 min, + 6 h of infusion, + 2 h, + 6 h of infusion	52	
		24	10 + 7	i.v.		78	
Permanent MCAO	CD-1 mice		5	i.v.	+ 5 min	43	
			30	s.c.	1 and 4 h		
Permanent MCAO (modified)	Fisher 344 rats	24	10 + 7	i.v.	+15 min, + 6 h of infusion	62	60
Permanent MCAO (intraluminal filament)	Sprague–Dawley rats	24	10 + 7	i.v.	+2 h, + 6 h of infusion	42	

MCAO, Middle cerebral artery occlusion.

occlusion ACEA 1021 decreased infarct volume, if administered as an i.v. bolus 5 min after occlusion followed by two s.c. injections, at 1 and 4 h after MCA occlusion (57).

ACEA 1021 significantly reduced the volume of ischemic brain damage caused by acute subdural hematoma in rats with a pretreatment and also high-dose posttreatment paradigm (65). Moreover, ACEA 1021 reduced [^{125}I]MK-801 binding *in vivo* by 28% in the penumbral zone beneath experimentally induced acute subdural hematoma in rats (17). These authors suggest that, since ACEA 1021 reduces the penumbral NMDA receptor activation after the onset of subdural hematoma, it is likely to reduce the associated cell swelling.

Many studies have demonstrated that the extent of ischemic damage is influenced by brain temperature during the ischemic period, and have described the beneficial effects of hypothermia on infarct size (6,44). The beneficial effects of ACEA 1021 on infarct volume have been associated with a fall in body temperature (57,67), suggesting that the induced hypothermia may be responsible for the decrease in infarct volume. ACEA 1021 has been, however, reported to induce a significant neuroprotection even when brain temperature is controlled within ±0.1°C during ischemia and the first 6 h of reperfusion (62).

Although ACEA 1021 reduces infarct volumes following focal cerebral ischemia, it appears to be inactive in models of global cerebral ischemia. Hicks et al. (23) reported that ACEA 1021 failed to provide any neuroprotection against ischemia-induced cell death in the hippocampus, as a result of bilateral common carotid artery occlusion in the gerbil. Similarly, Warner et al. (67) found no beneficial effects of ACEA 1021 on delayed neuronal cell death in the two-vessel model of global forebrain ischemia in rats.

PAIN

The excitatory amino acid neurotransmitters have been demonstrated to play a prominent role in spinal nociceptive transmission (18,58). The NMDA receptor is located postsynaptically to an interneuron mediating a polysynaptic excitation, that is responsible for the spinal nociceptive processing that results in a central facilitatory state induced by ongoing C fiber input (74). The strychnine-insensitive glycine site is thought to facilitate this excitatory transmission.

The use of selective antagonists has demonstrated the different roles of excitatory amino acid transmitters in the processing of nociception. Blockade of NMDA receptors by inhibition of the glycine site elicits antinociception against prolonged (chemical) noxious stimulation without any effects on motor coordination (43). In mice, pretreatment with ACEA 1021, injected intraperitoneally (1–60 mg/kg) or intrathecally (1–40 μg/mouse), attenuated both the early and late tonic phases resulting from a formalin injection into the plantar region of a hind paw (36). In addition, an antinociceptive effect was demonstrated in the tail flick test following intrathecal administration of ACEA 1021. Furthermore, the microinjection of NMDA into the spinal lumbar intrathecal space produced caudally directed biting and scratching behaviors, which were blocked by pretreatment with ACEA 1021. The potency of ACEA 1021 was about the same against the biting and scratching behaviors, and in attenuating the nociceptive responses in the tail flick and formalin tests, suggesting that ACEA 1021 is exerting its effects through NMDA receptors (36). Näsström et al. (47) examined the antinociceptive effect of different classes of

NMDA receptor antagonists in the tail flick, hot plate and formalin tests. They found that NMDA receptor antagonists that are devoid of non-NMDA receptor antagonist activity display antinociceptive effects only in the formalin test, whereas blockade of non-NMDA receptors appears to be critical for analgesic effects in the hot plate and tail flick tests. ACEA 1021 shows at least a 250-fold selectivity ratio for NMDA receptor glycine sites (72). Nevertheless, its antinociceptive effect appears to be due to the blockade of spinal non-NMDA receptors in the tail flick test and a combination of NMDA and non-NMDA receptors in the formalin test (36). Nishiyama (49), however, was unable to demonstrate any antinociceptive activity of ACEA 1021 in the rat formalin paw test following intrathecal administration of the drug of doses up to 24 μg/300 g rat. He suggested that this dose was insufficient to act on non-NMDA receptors, blocking only the glycine site on the NMDA receptor complex. Higher doses could not be utilized, however, since they induced motor disturbances (49). Brennan and Zahn (11) examined the effects of intrathecal ACEA 1021 in a rat model of postoperative pain. Spontaneous nociceptive behaviors induced by NMDA, AMPA, kainate, or plantar incision were decreased or abolished by ACEA 1021, suggesting that inhibition of pain behaviors by ACEA 1021 is produced by blockade of spinal non-NMDA receptors.

Systemically active glycine site antagonists attenuate both dependence on morphine and the development of tolerance to the antinociceptive effects of opioids after repeated administration. ACEA 1328 (20 mg/kg, i.p.) completely blocked tolerance to morphine-induced antinociception in the tail flick test, without affecting the basal nociceptive response or potentiating morphine-induced antinociceptive effects. ACEA 1021 exhibited a time- and dose-dependent antinociceptive effect (ED_{50} = 8.5 μg/mouse, intrathecal) (38,39).

SIDE EFFECTS AND SAFETY PHARMACOLOGY

Animal studies suggested that glycine site antagonists may not produce many of the side effects observed with competitive NMDA receptor antagonists. Balster et al. (4) reported that ACEA 1021 at doses up to 25 mg/kg i.p. in rats and 10 mg/kg i.v. in *Rhesus* monkeys, neither substituted nor attenuated the phencyclidine (a non-competitive NMDA antagonist) cue. In rats it failed to act as a discriminative stimulus alone. It had no effect on either working or reference memory errors in rats, but increased the time to complete the task, which probably reflects alterations in motor coordination (31).

ACEA 1021 has also been characterized for its activities in a variety of anticonvulsant and side effect tests performed in rats and mice. It had anticonvulsant activity in chemical, electrical and genetic seizure models (5), being more potent against audiogenic seizures and quinolinic acid-induced seizures in mice, but was less active against electrically induced seizures in rats. Many compounds that block the NMDA complex induce disturbances of motor coordination (14). ACEA 1021 was more potent in producing motor disruption, as measured in the rotarod test, than at inhibiting quinolinic acid induced seizures, with a "safety index" of 0.3 (5); demonstrating that ACEA 1021 induces ataxia at doses lower than those required to prevent seizures. In fact, in comparison with L-701,324 (another glycine-site antagonist from Merck), it possessed a worse safety index relative to MDL 103,371 (HMR), D-CPPene (Sandoz), CGS 19755 (Ciba Geigy), CNS 1102 (Cambridge Neuroscience), and MK-801 (Merck) (Baron et al., personal communication).

Olney et al. (51,52) observed that high doses of competitive or non-competitive NMDA receptor antagonists produce neuronal vacuolization in the cingulate/retrosplenial cortex in rodents. Some of the neurons containing vacuoles (emerging from mitochondria) eventually die through necrosis and possibly apoptosis (19). No pathological changes were observed with ACEA 1021 in doses of up to 50 mg/kg i.v. (22).

Psychomimetic effects are often apparent in humans treated with high doses of either competitive or non-competitive NMDA receptor antagonists (45). ACEA 1021 induced a moderate, not clearly dose-dependent increase in stereotyped sniffing in rats (31), a behavioral effect often indicative of psychomimetic potential.

In general, NMDA receptor antagonists inhibit sensitization (locomotor response) to repetitive administration of cocaine (13,29). ACEA 1021 attenuated cocaine toxicity and the development and expression of behavioral sensitization in rats (8,41,59). These findings suggest a potential role for this NMDA glycine site antagonist in the treatment of drug-cue reactivity in drug abusers.

During studies conducted to examine the neuroprotective effect of ACEA 1021, cardiovascular parameters such as heart rate, blood pressure, gases and glucose were measured. They were not significantly affected in the ACEA 1021-treated group as compared to the vehicle-treated control animals. Although there were transient (1–3 min) decreases (11%) in mean arterial blood pressure (MABP) following initial bolus administration of ACEA 1021, blood pressure tended to remain near pretreatment levels following continuous infusion of the drug lasting several hours. Thus, the initial decrease in MABP is likely to have been a bolus effect and not due to any sustained cardiovascular effect of ACEA 1021 (57,60). Thus ACEA 1021, whilst it appears to show efficacy in permanent and transient focal, but not global ischemia, does not possess the adverse toxicologic profile seen with other NMDA receptor antagonists (2).

CLINICAL EXPERIENCE WITH ACEA 1021

Studies in normal, healthy volunteers with ACEA 1021 showed that 15 min infusions of ≤2.0 mg/kg resulted in plasma levels of 30.8 ± 5.3 μg/mL which is well within the protective plasma concentration necessary for neuroprotection in rodents. The drug was well tolerated without neurologic, psychiatric, cardiovascular or laboratory abnormalities (68). Albers et al. (1) tested ACEA 1021 in stroke patients and determined that by short infusions at doese up to 3.0 mg/kg the drug was safe and tolerable in acute stroke patients. At higher doses, there were transient mild to moderate adverse neurologic and gastrointestinal complaints including transient agitation, dizziness, somnolence, memory impairment, nausea and vomiting. No psychotomimetic effects were observed. It was noted that patients were not treated in the hyperacute period in this trial, and infusion periods were short (15–30 min). Peak, neuroprotective serum levels were achieved rapidly and maintained for 1–2 h only. Thus, longer infusion periods of ACEA 1021 in stroke patients in the hyperacute phase of stroke onset would need to be examined in a larger phase III clinical trial.

According to the Investigational Drug database (IDdb) Ciba-Geigy (now Novartis) discontinued in 1997 their participation in the development of this compound, in part, due to the finding of ACEA 1021 crystals in the urine of some subjects in a phase I safety clinical

trial, indicating a potential dose-limiting effect. In 1998, whilst searching for a new development partner for ACEA 1021, CoCensys (now Purdue and formerly Acea Pharmaceuticals) suspended all clinical trials. In 2003, Purdue confirmed that the project had been discontinued.

CONCLUSION

By the end of the twentieth century it was clear that glycine-site antagonists have robust neuroprotective effects in many animal models. Based upon *in vitro* and *in vivo* studies, ACEA 1021 was demonstrated to be a potent selective antagonist at the glycine site of the NMDA receptor complex. ACEA 1021 crosses the blood-brain barrier and blocks the pathophysiologic consequences of NMDA receptor overstimulation. It was neuroprotective with a favorable therapeutic window in models of transient and permanent cerebral ischemia, epilepsy and pain. ACEA 1021 is not without liabilities, since it is light-sensitive and has poor aqueous solubility. It is conceivable, however, that further SAR studies may lead to a better compound.

The side effect profiles of glycine-site antagonists were improved over prior NMDA antagonists. They lack cingulate cortex vacuolization and pre-pulse inhibition impairment indicative of psychotomimetic effects. They are not, however, devoid of sedation, mild ataxia and myorelaxation (17,59). In *in vivo* animal experiments ACEA 1021, at therapeutic doses, did not exhibit these liabilities.

Literature searches conducted by the authors revealed only 2 studies examining the clinical pharmacology of ACEA 1021 in human subjects. Without clinical trials examining the long-term tolerance of the drug at therapeutic doses it is unclear whether such drugs can be used in the treatment of acute neurologic or chronic neurodegenerative conditions. Since many modifications of ACEA 1021 molecule led to potent and selective glycine-site blockers, it is not clear that additional SAR studies will result in a more efficacious compound (16). Nevertheless, it is likely that further SAR studies can eliminate or improve the light-sensitivity and solubility liabilities of ACEA 1021. Also, the elimination of the transient mild-to-moderate neurologic and gastrointestinal side effects of ACEA 1021, observed at the highest doses in humans, would improve the clinical profile of the drug. Such improvements will permit a more comprehensive evaluation of glycine-site antagonism as a drug target, and lead to a new drug for multiple acute and chronic CNS indications.

Addendum.

The chemical names of compoinds referred to by code numbers as follows:

GV150526: 4,6-Dichloro-3-[(1E)-3-oxo-3-(phenylamino)-1-propenyl)-1*H*-indole-2-carboxylic acid, monosodium salt;

MDL 105,519: 3-[(1E)-2-Carboxy-2-phenylethenyl)-4,6-dichloro-1*H*-indole-2-carboxylic acid;

L-687417: (3R,4R)-3-amino-1-hydroxy-4-methyl-2-pyrrolidinone;

L-701324: 7-Chloro-4-hydroxy-3-(3-phenoxyphenyl)-2(1*H*)-quinolinone;

(+)-RPR104623: (+)-2-[(3-Bromophenyl)methyl]-6,8-dichloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-3-carboxylic acid, 1,1-dioxide;

RPR118723: 6-Chloro-2,3,4,9-tetrahydro-9-methyl-2,3-dioxo-1*H*-indeno[1,2-b]pyrazine-9-acetic acid;

ACPC: 1-Amino-cyclopropanecarboxylic acid;

ZD9379: 7-Chloro-2,3,4,9-tetrahydro-9-methyl-2,3-dioxo-1*H*-indeno[1,2-b]quinoline-1,4,10(5*H*)-trione, monosodium salt;

MDL 103371: 3-[(1E)-2-(3-Aminophenyl)-2-carboxyethenyl]-4,6-dichloro-1*H*-indole-2-carboxylic acid;

D-CPPene: (2R)-4-[(2E)-3-Phosphono-2-propenyl]-2-piperazinecarboxylic acid;

CGS 19755: (2R,3S)-4-(Phosphonomethyl)-2-piperidinecarboxylic acid;

CNS 1102: N-(3-Ethylphenyl)-N-methyl-N′-1-naphthalenyl-guanidine monohydrochloride.

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