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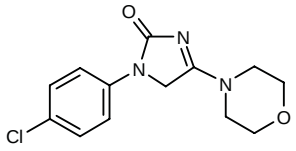
CCC: 0377-8282/98

AWD-131-138

Anxiolytic
Anticonvulsant

ADD-233089

1-(4-Chlorophenyl)-4-(4-morpholinyl)-2,5-dihydro-1*H*-imidazol-2-one



$C_{13}H_{14}ClN_3O_2$ Mol wt: 279.73

CAS: 188116-07-6

EN: 250021

Synthesis

The synthesis of AWD 131-138 is obtained as illustrated in Scheme 1:

The alkylation of 4-chloroaniline (I) with chloroacetic acid methyl ester in ethanol gives *N*-(4-chlorophenyl)-glycine methyl ester (II), which is added to cyanate by means of hydrochloric acid in glacial acetic acid to yield 3-(4-chlorophenyl)hydantoic acid methyl ester (III). The cyclization in hydrochloric acid yields 1-(4-chlorophenyl)-hydantoin (IV), which is finally condensed with morpholine.

Description

White crystalline powder, m.p. 264 C.

Introduction

AWD 131-138 was selected for development from a synthesis program because of its potency in tests predictive for anxiolytic effects and a broad spectrum anticonvulsant activity. In tests measuring CNS-related unwanted effects, AWD 131-138 is clearly less effective. AWD 131-138 is structurally different from other clinically effective anxiolytics and antiepileptics. This report will summarize the *in vivo* experimental and *in vitro* studies with AWD 131-138.

Pharmacological Actions

Anxiolytic activity

The anxiolytic potential of AWD 131-138 was assessed in tests considered to be predictive for anxiolytic effects and compared with those of diazepam. In the Vogel conflict test (1), drinking response by water-deprived rats is simultaneously rewarded with water and punished by footshock. The response rate was increased by oral doses of 3-50 mg/kg of AWD 131-138 in a dose-dependent manner (Table I).

In a further test, the elevated plus maze, the natural aversion of rats for exposed places (open arms) is diminished by anxiolytic compounds. Following oral administration of 10 and 30 mg/kg AWD 131-138, the number of entries into and time spent in the open arms were increased. In an alternative approach, the light-dark box, rats prefer dark enclosed spaces, and this preference is reflected in the portion of time spent in each part of the box. Anxiolytic compounds increase the time spent in the open lighted area. After oral treatment of rats with 3, 10 and 30 mg/kg AWD 131-138, the total time spent in the lighted part was increased by 154, 176 and 84%, respectively.

Anticonvulsant activity and minimal motor impairment

AWD 131-138 exerted broad anticonvulsant activities in rats and mice which could be clearly separated from behavioral toxicity (Table II). Clonic convulsions induced in rats and mice by pentylenetetrazol (80 mg/kg s.c. in rats, 85 mg/kg in mice, a dose eliciting seizures in 97% of the animals) were prevented following oral administration of AWD 131-138, with an ED_{50} of 27.4 mg/kg in rats and following i.p. administration, an ED_{50} of 17.2 mg/kg in mice. Tonic convulsions induced in the traditional MES test were prevented with an oral ED_{50} of 205 mg/kg in rats and an i.p. ED_{50} of 94.3 mg/kg in mice. Motor impairment was assessed after drug administration by forced motor

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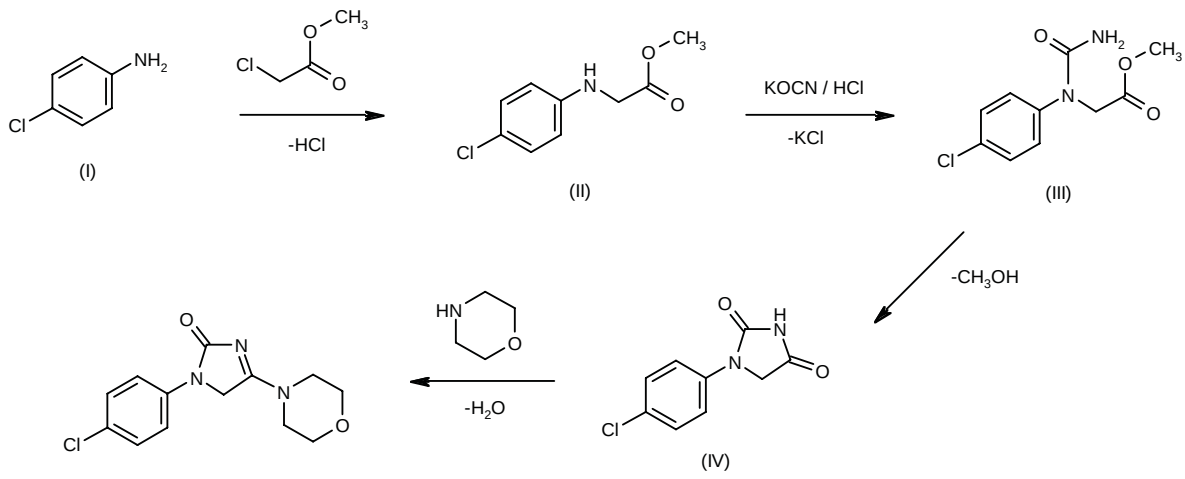
Scheme 1: Synthesis of AWD-131-138



activity in the rotarod test. In rats and mice, the locomotor coordination was not impaired by doses which were anticonvulsive effective. Following oral administration, there was no ataxia up to high doses in rats (ED_{50} = > 500 mg/kg), and in mice after i.p. administration (ED_{50} = 176 mg/kg). AWD 131-138 was also capable of increasing the threshold for the different seizure types elicited by i.v. administration of PTZ in mice. While an oral dose of 30 mg/kg was needed to increase the threshold for the first myoclonic twitch, an oral dose of 15 mg/kg was effective in increasing the threshold for induction of the first generalized clonus.

AWD 131-138 was proven to be very active in the amygdala kindling model of focal epilepsy, which is considered to be the most predictive model for complex partial seizures in humans (2). The compound showed dose-dependent anticonvulsant effects in amygdala-kindled rats following oral administration. The effective dose for increasing the afterdischarge threshold was 10 mg/kg, and the seizure severity was significantly suppressed by 30 mg/kg.

To determine the potential of AWD 131-138 to lose efficacy due to development of tolerance and to determine the potential to induce withdrawal symptoms, the MES threshold test was utilized as a sensitive and graded measure of anticonvulsant and, after withdrawal of the drug, possible preconvulsant rebound-associated effects. During twice-daily oral administration of 100 mg/kg for 10 days, the anticonvulsant effect remained stable during the entire treatment period. No rebound hyperexcitability was seen after substance withdrawal.

Sedative effects

Central sedative effects of a compound can be determined by the ethanol interaction test. There was no significant ethanol interaction after oral doses of 48 mg/kg and 200 mg/kg (s.c. PTZ ED_{50} and 4 x s.c. PTZ ED_{50}) of AWD 131-138 in mice. In comparison, a strong increase in duration over the control situation by administration of equivalents of diazepam (0.3 mg/kg and 1.33 mg/kg) was shown. The lack of sedative potential was further substantiated by the results in a passive avoidance test in rats. The normal performance was not impaired by AWD 131-138 up to 30 mg/kg p.o. In comparison, the perfor-

Table I: Anxiolytic effects of AWD 131-138 and diazepam in the Vogel conflict test in rats. The number of punished responses and the effect in % is given.

Oral Dose (mg/kg)	Control	AWD 131-138	% Effect over Control	Control	Diazepam	% Effect over Control
0.3	NE	NE	NE	62 ± 7.1	71 ± 6.9	14
1	51 ± 7.7	61 ± 8.7	20		91 ± 3.2**	48
3		83 ± 7.2**	63		104 ± 12**	68
10		80 ± 9.2*	57	28 ± 5.8	92 ± 9.2**	229
30	40 ± 7.2	126 ± 16**	215		NE	
50		135 ± 16**	238		NE	

*$p < 0.05$, **$p < 0.01$ substance groups in comparison with controls. NE = not evaluated.

Table II: Anticonvulsant activity and minimal toxicity of AWD 131-138 in mice and rats, ED_{50} (mg/kg).

Species	Route	s.c.PTZ	MES	Rotarod
Mouse	i.p.	17.2* (13.6-21.4)	94.3* (69.8-125)	176* (133-236)
Rat	p.o.	27.4 (12.7-59.0)	205 (116-367)	>500

*Experiments were performed within the NIH sponsored Anticonvulsant Screening Project at the University of Utah, Salt Lake City, Utah, USA.

mance of rats in this test was disturbed by diazepam at doses needed to induce anxiolytic and anticonvulsant effects.

Mode of Action

The effects of AWD 131-138 on ligand-gated ion channels were examined using standard patch-clamp techniques and single mouse cortical neurons (13-16 DIV) (3). Whole-cell currents (V_m = –75 mV) were evoked by 1-sec applications of 10 μM glutamate plus 1 μM glycine or 1 μM GABA. While AWD 131-138 (10 μM) did not affect the glutamate-evoked currents, the amplitude of GABA-evoked whole cell currents (176 ± 20% of predrug control) was markedly enhanced.

The receptor binding of AWD 131-138 was tested using different receptors in preparations of male Wistar rat and mouse brains. We found marginal affinity to the benzodiazepine binding site on the $GABA_A$ receptor complex by using [^{3}H]-flunitrazepam as radioactive ligand. AWD 131-138 inhibited the binding to this receptor in rat and mouse brain membrane preparations with an IC_{50} of 5804 nM and of 5460 nM, respectively. The corresponding K_i values were 4350 and 5140 nM. AWD 131-138 therefore has a weak affinity to the benzodiazepine binding site of the $GABA_A$ receptor complex.

AWD 131-138 has also been tested for its interaction with more than 40 other receptors, ion channels or second messenger systems in assays which were chosen based on the presumed location of action (central nervous system) or with respect to side effects. The compound did not inhibit binding or activity at any of these targets.

It is questionable whether the weak affinity of AWD 131-138 to the benzodiazepine binding site on the $GABA_A$ receptor complex could be the only reason for the strong anxiolytic and anticonvulsant activities. The mode of action of AWD 131-138 remains to be elucidated.

Pharmacokinetics and Metabolism

The results of exploratory pharmacokinetic studies in rats indicate that single oral doses lead to high plasma concentrations and therefore to a significant systemic exposure of the treated animals to AWD 131-138. These results also suggest a lack of an extensive first-pass effect after oral administration. The high plasma levels and early time points of maximum plasma concentrations argue for a rapid absorption. Additionally, the interindividual variability of AWD 131-138 in rats is low.

The metabolic stability of AWD 131-138 was studied in rats and human liver microsomes at a substrate concentration of 70 μM, corresponding to the range of maximal concentrations of AWD 131-138 in rat plasma after oral administration of 30 mg/kg. Metabolite profiles from these in vitro incubates obtained by HPLC showed a pronounced stability of the drug candidate against phase I metabolic attack in these in vitro models of both species. Eleven percent of the parent drug was converted into metabolites in rat liver microsomes and 5% of the parent drug was converted into metabolites in human liver microsomes. The first LC/MS characterization of metabolites gave evidence for the involvement of oxidative metabolic reactions.

Acknowledgements

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Manufacturer

Arzneimittelwerk Dresden GmbH (DE), a subsidiary of ASTA Medica AG (DE).

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