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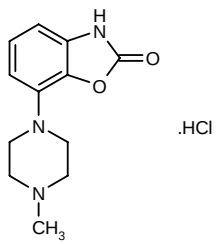
SLV308

Antiparkinsonian
Antidepressant
Anxiolytic
Dopamine D_2 Partial Agonist
5-HT_{1A} Agonist

7-(4-Methyl-1-piperazinyl)benzoxazol-2(3*H*)-one monohydrochloride



$C_{12}H_{15}N_3O_2$.HCl Mol wt: 269.7304

CAS: 269718-83-4
CAS: 269718-84-5 (as free base)

EN: 290288

Synthesis

The synthesis of SLV308 was obtained as follows: The first step is the reduction of just one nitro group, which was accomplished by treating 2,6-dinitrophenol (I) with sodium sulfide in the presence of sodium hydrogencarbonate dissolved in a water/MeOH mixture to yield 2-amino-6-nitrophenol (II) in 62%. To construct the heterocyclic ring, (II) was reacted with carbonyldiimidazole in dry tetrahydrofuran to give the nitro benzoxazolinone (III) almost quantitatively. The reduction of the nitro group of (III) was performed in acetone/Raney-Ni, resulting in the corresponding aniline (IV) (72%). Transforming the aniline (IV) into the piperazine was done by heating (IV) and bis(2-chloroethyl)amine (V) in chlorobenzene at reflux temperature; after 70 hours the desired piperazine (VI) was obtained after chromatographic purification in 60% yield. The last step, introduction of the methyl group, was achieved by a reductive amination; formaldehyde, sodium triacetoxyborohydride and triethylamine dissolved in dichloroethane were added to (VI). After work-up, chromatographic purification and subsequent treatment with ethanolic HCl, SLV308 was isolated in 81% yield (1). Scheme 1.

Description

White solid, m.p. 302-3 °C.

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder, primarily affecting dopaminergic neurons arising from the substantia nigra which project into the caudate nucleus and putamen. This system is critical in controlling motor patterns, and neurodegeneration in this pathway results in primary symptoms such as postural rigidity, bradykinesia and resting tremor. Moreover, many Parkinsonian patients also suffer from secondary symptoms like depression, sleep disturbances and dementia which can become more disabling than the primary ones.

Current therapy for Parkinson's disease focuses on alleviating the motor symptoms. Use of L-dopa as the precursor for dopamine synthesis has been shown to be effective in improving the motor functioning of patients. By using L-dopa, the rate-limiting step for the synthesis of dopamine, tyrosine hydroxylase, is by-passed, especially in combination with peripherally acting decarboxylase inhibitors. However, the effects of L-dopa treatment decline over the years and patients may experience characteristic situations in their performance, known as "on-off" effect. During the off-state, patients suffer weakness, akinesia and "freezing". In addition, patients may suffer

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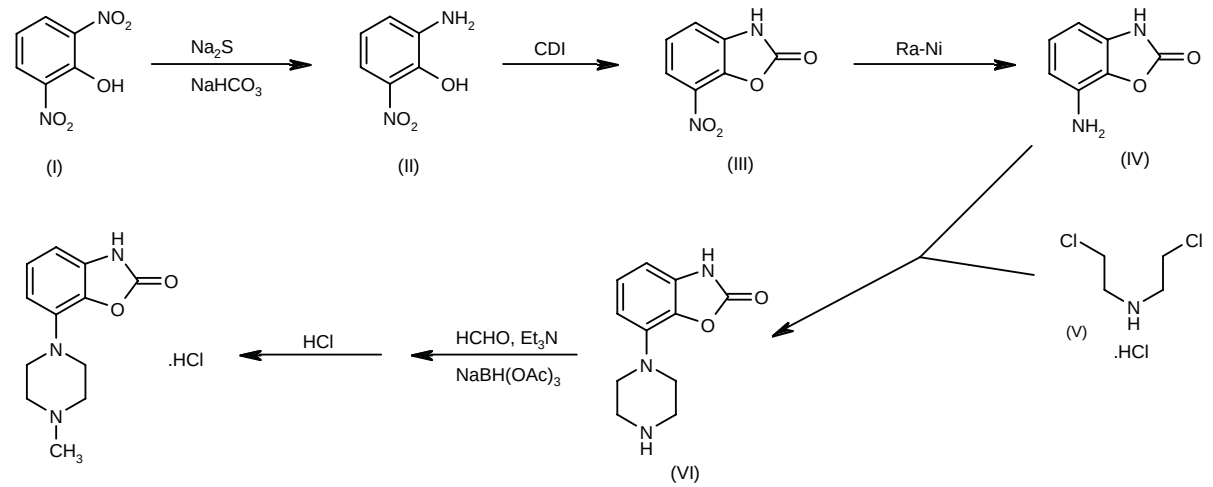
Scheme 1: Synthesis of SLV308



from “end of dose” deterioration in which the benefit of each dose becomes progressively shorter. For these reasons, the opinion to delay L-dopa treatment as long as possible has become more widely accepted.

Trying to substitute L-dopa treatment by dopamine agonists has led to the identification of the ergot compounds bromocriptine and pergolide as effective in PD. More recently, nonergot compounds such as ropinirole and pramipexole have been introduced and found equally effective in treating the symptoms (4,5) but with less side effects. These compounds all share the property of being full dopamine D_2 receptor agonists, when assayed for cAMP accumulation or GTPγS binding.

In the search for new therapeutic approaches for PD, it was considered that by using partial D_2 agonists, efficacious pharmacotherapy could be significantly prolonged while the incidence of side effects such as nausea, vomiting and hallucinations would be avoided or substantially decreased. In parkinsonian patients, with supersensitive postsynaptic dopamine receptors, full receptor agonists may rapidly desensitize the receptors. However, partial receptor agonists, such as SLV308, are less likely to induce this undesirable effect. In addition, partial receptor agonists are likely to induce a more consistent degree of postsynaptic receptor activation. Under conditions where synaptic levels of endogenous dopamine are low, SLV308 would act to supplement postsynaptic dopamine receptor stimulation. However, under conditions where high levels of endogenous dopamine are present, a partial agonist will antagonize the maximal effect of the endogenous agonist, thereby preventing overstimulation of the receptors. These pharmacological actions of SLV308 give the opportunity to create a situation in which the tone at postsynaptic dopamine receptors can be finely tuned in a sustainable manner.

When screened *in vitro*, compounds were selected that yielded partial agonism; subsequently, they were tested for their antiparkinsonian and potential antidepressant activity *in vivo.* Using this profile, SLV308 was selected as a compound showing extremely potent partial dopamine D_2 receptor agonism in combination with weaker full 5-HT_{1A} agonism, ultimately providing an antiparkinsonian as well as antidepressant and anxiolytic profile.

Pharmacological Actions

In order to assess dopamine D_2 receptor affinity, receptor binding was done using competition assays at rat striatal membrane preparations using [^{3}H]-spiperone as the radioligand (2). SLV308 was found to compete with radiolabeled spiperone with a pK_i of 7.5. A more complete receptor binding profile is shown in Table I.

To assess agonist efficacy and potency of compounds at dopamine D_2 receptors, we used cloned human D_2 receptors, stably transfected into CHO cells. Accumulation of radioactive cAMP from radiolabeled ATP (3) was induced by incubation with forskolin and in the presence of the phosphodiesterase inhibitor IBMX. D_2 receptor agonists can concentration-dependently attenuate cAMP accumulation. SLV308 was found to be a partial agonist with a pEC_{50} value of 7.5 and an efficacy of 0.55 (Fig. 1). Moreover, SLV308 was able to concentration-dependently antagonize the agonist actions of quinpirole with a pA_2 of 8.4, to about 50% with respect to control values. Therefore, it is expected that SLV308 will interactively control DA neurotransmission, *i.e.*, at low ambient DA concentrations, SLV308 will mimic DA effects by itself, whereas at high ambient DA concentrations, SLV308 is likely to exert antagonist properties, suppressing the maximal effect induced by endogenous DA. SLV308 also

Table I: Receptor binding profile of SLV308 (pK_i) for various receptors.

Receptor	pK_i value	Radioligand	Material
D_1	7.5	[^{3}H]-Dopamine	Rat striatum
D_2	7.5	[^{3}H]-Spiperone	Rat striatum
D_4	7.6	[^{3}H]-Spiperone	Human D4.2-CHO
5-HT_{1A}	7.5	[^{3}H]-8-OH-DAPT	Rat frontal cortex
5-HT_7	7.1	[^{3}H]-5-CT	Rat 5-HT_7-CHO
α_{1a}	7.1	[^{3}H]-Prazosine	Rat liver
α_{1b}	7.1	[^{3}H]-Prazosine	Rat liver
Others	< 6.0	Miscellaneous	

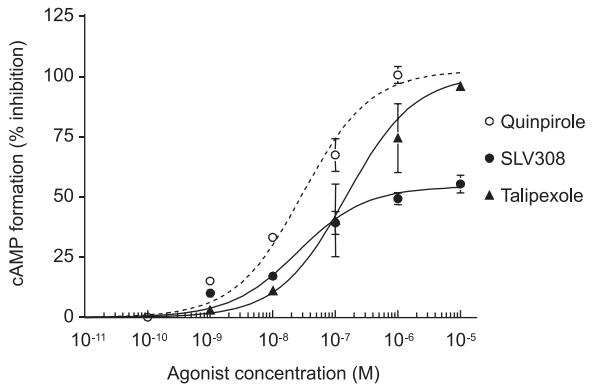


Fig. 1. Interaction of SLV308, quinpirole and talipexole with CHO cells, stably transfected by human dopamine D_2 receptors, as measured by cAMP accumulation. Agonist activity can be measured by the concentration-dependent attenuation of cAMP accumulation. D_2 receptor activation inhibits the accumulation of cAMP stimulated by forskolin (10^{-7} M). Quinpirole and talipexole completely attenuated cAMP formation, whereas SLV308 acts as a partial agonist.

weakly antagonized the effects of the full dopamine D_1 receptor agonist SKF-38393 with a pA_2 of 6.1.

Furthermore, SLV308 was found to be a weak but full 5-HT_{1A} receptor agonist with a pEC_{50} of 6.3. *In vivo*, SLV308 was found to possess α_1-adrenoreceptor agonistic activity.

The activity of SLV308 was further investigated by assessing its activity at presynaptic dopamine D_2 receptors. Striatal slices were prelabeled with [^{3}H]-dopamine and subsequently used in superfusion experiments. Release was stimulated by increased K^+ concentrations in the absence or presence of SLV308. SLV308 did not yield agonist activity in this preparation, but was found to completely and potently antagonize the agonist quinpirole with a pA_2 of 8.5. Apparently, SLV308 does not have enough intrinsic activity to exert agonist activity at presynaptic D_2 receptors.

Using microdialysis in rat nucleus accumbens, SLV308 lowered extracellular DA content with a lowest effective dose (LED) of 0.3 mg/kg p.o. and an ED_{75} of 0.4 mg/kg p.o., whereas on extracellular 5-HT no significant effects were observed at these doses. SLV308 was tested for its activity on locomotor behavior in open field tests. SLV308 lowered spontaneous activity with ED_{50} values of 0.02 mg/kg i.p. and 0.03 mg/kg p.o. However, in rats that were lesioned unilaterally by 6-OH-dopamine and checked for turning behavior using apomorphine, SLV308 produced contralateral turning behavior with an LED of 0.03 mg/kg p.o. In MPTP-treated common marmosets SLV308 at doses of 0.1 mg i.p. and above produced marked and long-lasting antiparkinsonian effects. These results indicate that SLV308 acts as a potent agonist in animal models of Parkinson's disease.

The putative antidepressant properties of SLV308 were assessed in the forced swim test and the differential reinforcement of low rates of responding (DRL-72). In the forced swim test SLV308 exerted potent antidepressant activity as measured by decreasing the immobility time by at least 8 sec. The observed ED_{50} was 0.2 mg/kg i.p. in Wistar rats and was 0.03 mg/kg i.p. in Hooded Lister rats. The antidepressant properties of SLV308 are probably mediated by D_2 receptors and 5-HT_{1A} receptors, as both 5-HT_{1A} receptor agonists such as 8-OH-DPAT and flesinoxan and D_2 receptor agonists like quinpirole and talipexole are active in the forced swim test.

In the DRL-72 paradigm, SLV308 showed an antidepressant profile with an LED of 0.87 mg/kg i.p. These effects are shared with both dopamine agonists and antagonists, as well as with 5-HT_{1A} receptor agonists and α_2 receptor agonists. Together, these data suggest antidepressant activity of SLV308.

SLV308 was also tested in behavioral paradigms, predictive for anxiolytic efficacy. Thus, in recording stress-induced ultrasonic pup vocalizations, SLV308 dose-dependently attenuated the number of calls with an LED_{50} of 0.1 mg/kg i.p. However, in the adult stress-induced ultrasonic vocalization paradigm, SLV308 attenuated the number of shock-induced ultrasonic calls with an ED_{50} of 0.006 mg/kg p.o. Moreover, when used for duration of action, SLV308 was still found to be effective 4 h after administration, with an ED_{50} of 0.09 mg/kg p.o.

In conclusion, the pharmacological profile of the non-ergot partial D_2 agonist SLV308 suggests that both motor and mood disturbances associated with Parkinson's disease can be treated.

Pharmacokinetics and Metabolism

The absorption of SLV308 in rats and cynomolgus monkeys was rapid and complete. The bioavailability was 60-80%. The $t_{1/2}$ was 2-8 h in rats and 5-12 h in monkeys. Several metabolic routes exist. To date, no pharmacologically active metabolites were found. *In vitro* metabolism by human microsomes was mediated primarily by CYP 1A2. No inhibitory effects on CYP 1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1 and 3A4 were observed. Excretion was mainly in urine. At high doses such as 6.5 mg/kg deviations from linear kinetics were observed. In the low and middle dose range kinetics were linear and not

sex-specific. SLV308 is considered to be an intermediate clearance drug.

Toxicology

To date, acute and chronic toxicology studies have been conducted in rats, mice, dogs and cynomolgus monkeys with the focus on rats and monkeys, being the species that mimic human metabolism of SLV308.

At low doses (about 0.2 mg/kg in rats and 0.07 mg/kg in monkeys; systemic exposure of about 30 ng·h/ml in rats to 100 ng·h/ml in monkeys) mild, CNS-mediated clinical signs were observed. At higher doses the same clinical signs were more pronounced.

Also observed (in rats but not monkeys) at the lower dose levels were a mild disturbance of water and electrolyte balance (indicated by increased water consumption and urine production) and slight changes in clinical chemistry parameters. Vomiting was a prominent effect in dogs and occurred occasionally in monkeys. In addition, at high doses (6.5 mg/kg) in rats body weight, liver weight and thymus weight were slightly reduced. At high doses (0.6 mg/kg) in monkeys, tremors were occasionally observed, water consumption (in females only) was increased and liver weight was increased or decreased, probably representing effects on glycogen storage. In rats, SLV308 caused transient increase in corticosterone levels (but not in prolactin or growth hormone levels); some of the effects on water/electrolyte balance and blood chemistry are consistent with increased glucocorticoid levels or other effects on the hypothalamic-pituitary-adrenal axis. Even at high exposures (about 750 ng·h/ml) there were no adverse, treatment-related effects in the 3-month study in monkeys upon body weight, food consumption, ophthalmology, electrocardioraphy, blood pressure, pulse rate, hematology, clinical chemistry, urinalysis, 24-h urine volume or macroscopic or microscopic pathology. At high doses (6.5 mg/kg) in the 6-month rat study there were also no adverse macroscopic or histopathologic abnormalities observed in any tissue. In this latter study in rats, all observed adverse responses and clinical signs were reversed and returned to normal after a 1-month recovery period.

The therapeutic index for SLV308 is considered large: in rats, the most sensitive species, a dose of about 1 mg/kg, corresponding to an exposure of 190-310 ng·h/ml, appeared to be the lowest dose that caused mild, reversible adverse effects while doses as low as 0.01 mg/kg were pharmacologically active.

SLV308 was tested in genotoxicity assays and there was no evidence to indicate a genotoxic risk to humans.

Clinical Studies

The safety and tolerability of SLV308 were assessed in a total of 51 healthy male subjects. Twenty seven subjects received one or more single doses ranging from 0.01-0.5 mg. In a multiple-dose study, 18 subjects received doses of up to 0.7 mg b.i.d. This dose was achieved after gradual titration over a period of 14 days, starting with a dose of 0.1 mg b.i.d. In a second multiple-dose study, 6 subjects received doses up to 1 mg t.i.d.

In the single-dose study, clear drug-related adverse events were observed with doses of 0.2 mg and higher. The tolerability after single administration of 0.5 mg was poor. The following adverse events were reported in decreasing order of frequency: nausea, general malaise, dizziness, asthenia, syncope, headache, vomiting, abdominal pain, somnolence and flu syndrome. Dose-related orthostatic hypotension was observed, especially in systolic blood pressure, which was accompanied by dizziness, nausea and general malaise.

The multiple-dose studies show that SLV308 is well tolerated up to doses of 1 mg t.i.d. Adverse events were reported with doses of 0.7 mg t.i.d. and higher. Compared to the single-dose study, the tolerability of SLV308 in this study was better. It can be concluded that tolerance to adverse events developed after slow upward titration.

The effects of SLV308 on hormones and body temperature in these studies suggest that, in the investigated dose range, the compound acts as an agonist at 5-HT_{1A} and D_2 receptors. In the single-dose study a dose-related increase in cortisol and hGH levels was observed with doses of 0.2 mg and higher. Decreases in prolactin levels were seen with doses of 0.01 mg and higher.

A slight decrease in body temperature was observed after administration of 0.4 mg (under fasting conditions) and 0.5 mg. Cognitive performance tests showed a slight trend for a dose related effect on the execution of the tests. In the multiple-dose studies decreases of prolactin levels were observed after all investigated doses, but evels were normalized within 12 h postdosing.

SLV308 was rapidly absorbed. Peak concentrations were reached within 0.5-4 h postdose. The mean half-life was around 1-3 h. The AUC and C_{max} data suggest linear kinetics in the dose range up to 1.0 mg t.i.d.

The excretion of unchanged SLV308 in urine is low (around 1%). Four subjects were genotyped as poor metabolizers for CYP2D6. These subjects had plasma levels in the same range as the extensive metabolizers.

Both the preclinical and clinical data suggest that SLV308 will be of value in treating parkinsonian patients. This is presently under examination.

Manufacturer

Solvay Pharmaceuticals, Inc. (NL).

References

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