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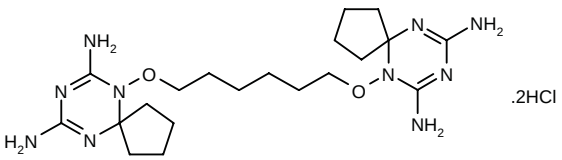
Trybizine Hydrochloride

Antitrypanosomal

SIPI-1029
T-46

10,10'-[1,6-Hexanediylbis(oxy)]bis(6,8,10-triazaspiro[4.5]deca-6,8-diene-7,9-diamine) dihydrochloride



$C_{20}H_{36}N_{10}O_2.2HCl$ Mol wt: 521.4942

CAS: 172280-69-2

EN: 269255

Synthesis

The reaction of benzoylhydroxamic acid (I) with 1,6-dibromohexane gives hexamethylene-1,6-bis(benzoylhydroxamate) (II) which is converted by hydrolysis into *O*,*O*'-diamino-1,6-hexanediol dihydrochloride (III). The reaction of (III) with dicyandiamide affords *O*,*O*'-bis(diguanidyl)-1,6-hexanediol dihydrochloride (IV). Finally, trybizine hydrochloride is obtained by cyclization of (IV) with cyclopentanone in acidic conditions (1). Scheme 1.

Description

Crystal, m.p. 244-6 °C (EtOH/H_2O) (1).

Introduction

Trypanosomiasis is found both in human and in animals. African trypanosomiasis (African sleeping sickness) caused by *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* in humans affects millions of people in 36 countries in sub-Saharan Africa, where it is a public health problem with a major impact on social and economic development. In addition, the loss due to African trypanosomiasis in livestock is estimated to be US$ 5000 million a year. Other species of trypanosomes such as *T. evansi*, *T. equiperdum* and *T. congolense* cause similar disease in domestic livestock and are major obstacles to agricultural development and livestock production in the world.

In contrast to the major developments in other areas of drug research in recent decades, very little progress has been made worldwide in the investigation of new antitrypanosomals. Difluoromethyl ornithine (DFMO, eflornithine), an inhibitor of ornithine decarboxylase, the only new trypanocide approved in the last half century, is only effective against gambiense sleeping sickness but not against the rhodesiense type (2). Pentamidine, melaminophenyl arsenical compounds and suramin remain the mainstay of chemotherapy in African trypanosomiasis, but they are either highly toxic (*e.g.*, toxicity caused by melarsoprol in 2-10% of all patients manifests as fatal encephalopathy (3)) or are often rendered ineffective by resistant strains due to more than 50 years of use (4). It is therefore important that new and safe antitrypanosomal agents be developed for both human and animal diseases.

In the 1940s, chloroguanide hydrochloride was found to have antimalarial effects and its metabolite *in vivo*, the dihydro-*s*-triazine compound cycloguanide hydrochloride (1a), has come into clinical use as an antimalarial agent (5). Further study led to the discovery of the benzyloxydihydrotriazine compound BRL-50216 (1b), which was found to be more active against *Plasmodium berghei* in rodent models (6). Some bis-triazine analogs were also found to possess activity against trypanosomes belonging to the same family (protozoa) as *Plasmodium*. In a study in mice, 80% of animals infected with *T. brucei rhodesiense* were cured at a dose of 6.6 mg/kg of compound 2a (7); all mice infected with *T. brucei* or *T. congolense* were cured by compound 2b at doses of 1.0 or 2.5 mg/kg. Activity of 2b against an established infection of *T. congolense* in Zebu cattle was evident with a single subcutaneous dose of 5 mg/kg but relapse of infection occurred within 20 days after clearance (8).

The chemical structures of some triazine compounds are shown in Figure 1.

Two series of triazine derivatives, 1,2-dihydro-2,2-dimethyl-4,6-diamino-1-(ω-haloalkyloxy)-*s*-triazines and

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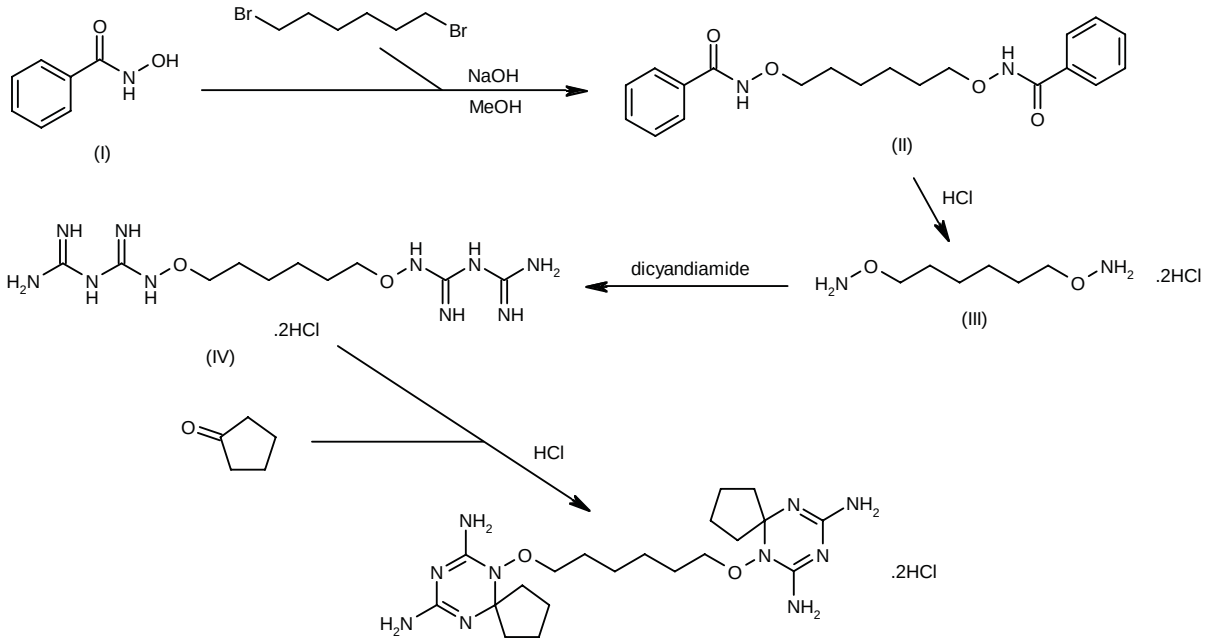
Scheme 1: Synthesis of Trybizine Hydrochloride



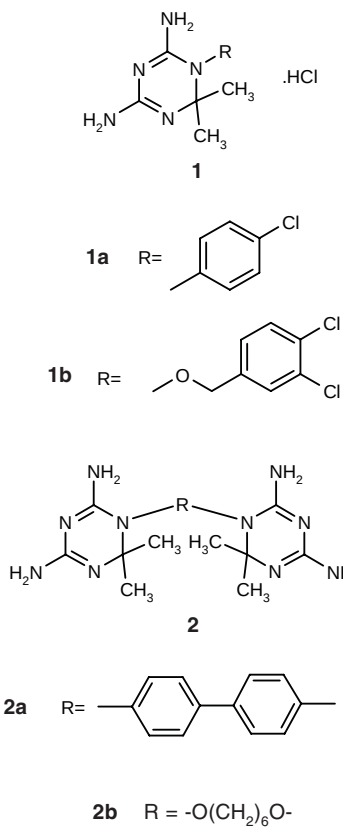


Fig. 1.

O,*O*'-bis(4,6-diamino-1,2-dihydro-2,2-disubstituted-*s*-triazin-1-yl)alkanediols, were synthesized by the Shanghai Institute of Pharmaceutical Industry as part of their research on new antiprotozoal agents (1). After extensive studies, SIPI-1029 (tribizine hydrochloride) was selected as a novel antitrypanosomal agent.

Pharmacological Actions

In vitro

In vitro, SIPI-1029 was 50% inhibitory for growth of blood-stream trypomastigotes of one strain of *T. b. brucei* and three strains of *T. b. rhodesiense* at 0.15-2.15 nM. The IC_{50} was about equal to that of melarsen oxide for arsenical-sensitive *T. b. brucei* but 20- to 160-fold lower for resistant *T. b. rhodesiense* (9). Another study showed that the MICs of SIPI-1029 against *T. b. rhodesiense* STIB900 and *T. b. gambiense* STIB930 were 0.4 and 2.7 ng/ml, respectively. The multidrug-resistant *T. b. brucei* were less susceptible and the difference in susceptibility between the susceptible and multidrug-resistant organisms was 10-fold. *T. evansi* and *T. equiperdum* were very susceptible to SIPI-1029; their MICs (0.2 and 0.1 ng/ml, respectively) were the lowest obtained for all trypanosome species. *T. congolense* was 50- to 100-fold less susceptible. Overall, the MIC and the IC_{50} values for all trypanosome were at least 10-fold lower than those of the reference compound, diminazene aceturate (10).

Investigations of the time-dose response in *T. b. brucei* STIB920 revealed that an exposure of 10 μg/ml over 16 h was necessary to eliminate all trypanosomes. When the exposure time was extended to 48 h, a concentration of 10 ng/ml was sufficient (10).

In vivo

Results of an experiment in mice infected with *T. b. brucei* Lab 110 EATRO showed that SIPI-1029 was extremely active at a dose range of 0.5-10 mg/kg/day for 3 days, with 100% cure at 0.5, 1.0, 2.5 and 5 mg/kg. Single doses in the same range were also effective, yielding a dose-response curve, with cure rates of 60% and 100% for the 2.5 and 10 mg/kg doses, respectively. In the models of *T. b. rhodesiense* clinical isolate infections, SIPI-1029 was curative for 12 of 13 isolates at less than or equal to 10 mg/kg for 3 days. A number of these isolates were resistant to melarsoprol and/or diminazene aceturate and pentamidine. The strain for which SIPI-1029 was not curative was completely refractory to melarsen oxide, melarsoprol and pentamidine and highly refractory to diminazene aceturate (9).

In another study, SIPI-1029 was able to eliminate *T. b. rhodesiense* and *T. b. gambiense* in an acute rodent model with 4 doses of 0.25 and 1 mg/kg i.p., respectively, or with 4 oral doses of 20 mg/kg in the former model. The oral activity was particularly important because all currently available trypanocides have to be administered parenterally or intravenously with the exception of DFMO, which unfortunately is only effective against gambiense trypanosomiasis. SIPI-1029 also exhibited activity against suramin-resistant *T. evansi* strains in mice. However, a late-stage rodent model with CNS involvement could not be cured, indicating that the compound may not pass the blood-brain barrier in sufficient quantities. This could be a drawback because in the progress of the disease, trypanosomes invade the CNS. Of all trypanocides thus far investigated, only melarsoprol and DFMO are able to cross the blood-brain barrier (10).

Other experiments revealed that SIPI-1029 cured all or half of mice infected with *T. evansi* at a single dose of 3.28 or 1.14 mg/kg s.c., respectively, and the curative dose in rats was 2 mg/kg i.m. The mean effective dose (ED_{50}) against *T. evansi* was 0.28 ± 0.03 mg/kg i.p. (1).

Mechanism of action

The mechanism of action of SIPI-1029 is not clearly known. Like diminazene aceturate, SIPI-1029 inhibited incorporation of [^{3}H]-hypoxanthine into DNA in *T. evansi*, with an IC_{50} of 1.33 μg/ml as compared to an IC_{50} of 1.73 μg/ml for diminazene aceturate (11). In experiments on inhibition of polyamine metabolism, which is related to the antitrypanosomal mode of action of DFMO, SIPI-1029 did not inhibit ornithine decarboxylase or *S*-adenosylmethionine synthetase at up to 500 μM but did inhibit *S*-adenosylmethionine decarboxylase with an IC_{50} of 38 μM (9).

Toxicology

In acute toxicity studies, the median lethal doses (LD_{50}) in mice and rats were 9.8 ± 0.7 mg/kg i.p. and 44.6 ± 2.8 mg/kg s.c., respectively. The minimum dose causing death of all tested rats (LD_{100}) was about 70 mg/kg s.c. (1).

In 14-day, subacute toxicity studies, no significant changes in clinical behavior, embryology and hematology biochemistry were observed when rats were given SIPI-1029 at a dose of 4 mg/kg i.m. (total 56 mg/kg, greater than LD_{50}); at a dose of 8 mg/kg (total 112 mg/kg, greater than LD_{100}), all tested rats survived, indicating that the drug has no significant accumulated toxicity. At a dose of 10 mg/kg, some severe pathologic changes were observed, especially in the liver, spleen and kidney (12).

No mutagenicity was found in the *in vitro* tests, including Ames, chromosome aberration in cultured Chinese hamster cells and unsequence DNA synthesis (13), or *in vivo* in micronucleus and sperm teratology tests (14).

Teratology studies were conducted in pregnant rats and mice administered SIPI-1029 at doses of 0, 2, 4 or 8 mg/kg from day 7-15 of gestation for rats and 0, 1.25, 2.5 or 5 mg/kg from day 6-14 of gestation for mice. Results showed that SIPI-1029 at all doses tested had no toxic effects on dams or fetuses (15).

Pharmacokinetics

An HPLC method was selected for determination of SIPI-1029 in biological samples with a chemical bonded C-18 reversed phase column as the supported phase and methanol:acetonitrile:water:diethylamine (25:20:55:0.33, pH 3.5 adjusted by phosphoric acid) as the mobile phase (16).

When mice were administered SIPI-1029 subcutaneously, the concentration-time curve exhibited a 2-compartment open model with t_{max} of 0.059-0.139 h and $t_{1/2\beta}$ of 122-165 h. SIPI-1029 was distributed in liver, kidney and muscle, but was not found in brain. This would partly explain its failure to cure the CNS-infected mice (13).

When the compound was administered to cattle (1 mg/kg i.m.), the same pharmacokinetic model as that in mice resulted, with the following parameters: $t_{1/2\alpha}$ of 100.9 min, $t_{1/2\beta}$ of 1291.4 min, $t_{1/2ka}$ of 7.825 min, AUC of 164.8 μg·min/ml, Tp of 27.41 min and C_{max} of 0.339 μg/ml. The rapid absorption, slow elimination and long retention in the plasma accounted for the good trypanocidal effect in cattle (16).

Studies in Animals

In an established infection of *T. evansi*, 8 cattle were divided into 4 groups and treated with a single dose of 0, 0.25, 0.5 or 1 mg/kg s.c.of SIPI-1029. The 4 animals in the 0.5 and 1 mg/kg groups and 1 animal in the 0.25 mg/kg group were cured; 2 control animals and 1 in the

0.25 mg/kg group were parasite-positive as determined by both pathogenic organism and immunology (13). In another experiment, 24 buffalo naturally infected with *T. evansi* were treated in 3 groups at a dose of 0.5, 1.0 or 1.5 mg/kg s.c., with respective cure rates of 85.7 (6/7), 71.4 (5/7) and 100% (10/10) (17). Thus, the single curative doses of SIPI-1029 were determined to be 0.5 mg/kg s.c. for cattle and 1.5 mg/kg s.c. for buffalo infected with *T. evansi*.

Over 100 cattle and buffalo infected with *T. evansi* have been treated with SIPI-1029 in China, with an overall efficacy rate of more than 94%. No significant adverse reactions have been observed. Another study in more than 400 cattle and buffalo is in progress.

Manufacturer

Xinchang Pharmaceutical Factory (CN).

Acknowledgements

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