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MONOGRAPH

VORELOXIN

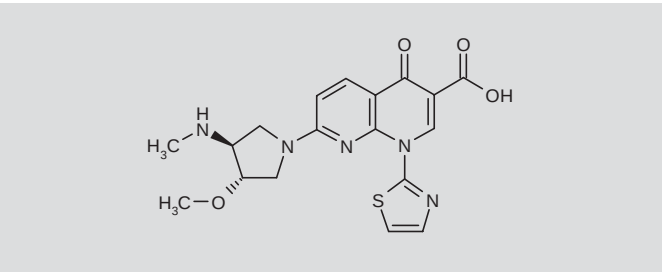
USAN

DNA-Intercalating Drug
Topoisomerase II Inhibitor
Oncolytic

AG-7352
SNS-595
SPC-595

(+)-7-[3(*S*)-Methoxy-4(*S*)-(methylamino)pyrrolidin-1-yl]-4-oxo-1-(2-thiazolyl)-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid

InChI=1/C18H19N5O4S/c1-19-12-8-22(9-13(12)27-2)14-4-3-10-15(24)11(17(25)26)7-23(16(10)21-14)18-20-5-6-28-18/h3-7,12-13,19H,8-9H2,1-2H3,(H,25,26)/t12-,13-/m0/s1



$C_{18}H_{19}N_5O_4S$
Mol wt: 401.44
CAS: 175414-77-4
CAS: 175519-16-1 (hydrochloride)
EN: 237721

ABSTRACT

Voreloxin (SNS-595) is being developed by Sunesis Pharmaceuticals under license from Dainippon Sumitomo Pharma for the treatment of a variety of solid and hematological malignancies. Voreloxin is a naphthyridine derivative that acts partly by inhibition of topoisomerase II, causing replication-dependent DNA damage in the S-phase of the cell cycle and leading to apoptosis via irreversible G2 arrest. The drug has activity as monotherapy in recurrent acute myelogenous leukemia (AML) and in platinum-refractory ovarian cancer. The predominant dose-limiting toxicity is neutropenia. Voreloxin is now being tested in combination with other cytotoxic agents in patients with AML.

SYNTHESIS*

Voreloxin can be prepared by several different ways.

Treatment of 2,6-dichloropyridine (I) with butyllithium in THF followed by quenching with CO_2 affords 2,6-dichloronicotinic acid (II) (1). After chlorination with boiling $SOCl_2$, the resulting acid chloride (IIIa) is condensed with diethyl malonate by means of magnesium ethoxide in Et_2O to give the nicotinoyl acetate (IV) (1, 2). Alternatively, activation of 2,6-dichloronicotinic acid (II) with CDI provides imidazolide (IIIb) which, without isolation, is condensed with the potassium salt of diethyl malonate in the presence of $MgCl_2$ and Et_3N to yield the keto ester (IV) (3). Treatment of compound (IV) with triethylorthoformate and acetic anhydride followed by reaction with 2-aminothiazole (V) gives the enamino ester (VI), which is cyclized to the 1,8-naphthyridine derivative (VII) upon heating with K_2CO_3 in dioxane (1-3). Condensation of chloronaphthyridine (VII) with the chiral pyrrolidine (VIII) by means of triethylamine in acetonitrile yields the protected voreloxin (IX), which is finally hydrolyzed with hot aqueous HCl (1, 2). In a related method, chloronaphthyridine (VII) is coupled with the unprotected pyrrolidine (X) to afford voreloxin ethyl ester (XI), which is finally hydrolyzed with NaOH in H_2O/EtOH (3). Scheme 1.

The chiral pyrrolidine intermediates (VIII) and (X) can be prepared by several different methods.

Protection of 3-pyrroline (XII) with Boc_2O in MeOH followed by oxidation of the resulting *N*-Boc-pyrroline (XIII) with *m*-CPBA in CH_2Cl_2 provides epoxide (XIV), which is also alternatively prepared by reaction of 1-Boc-3-pyrroline (XIII) with NBS in aqueous DMSO and subsequent cyclization of the obtained bromohydrin (XV) in 1 N NaOH. A further method to prepare pyrroline (XIII) is by condensation of *cis*-1,4-dichloro-2-butene (XVI) with *tert*-butyl carbamate in the presence of NaH in dry DMF. Ring opening of epoxide (XIV) with NaN_3 in dioxane/H_2O leads to the *trans*-azidoalcohol (XVII), which is alkylated with iodomethane and NaH in THF to give the methyl ether (XVIII). After catalytic hydrogenation of azide (XVIII) over Pd/C, the *N*-Boc protecting group in the resulting amine (XIX) is replaced with a benzyl group by acidic hydrolysis followed by alkylation with benzyl chloride and Et_3N to give the protected pyrrolidine (XX) (4). Opti-

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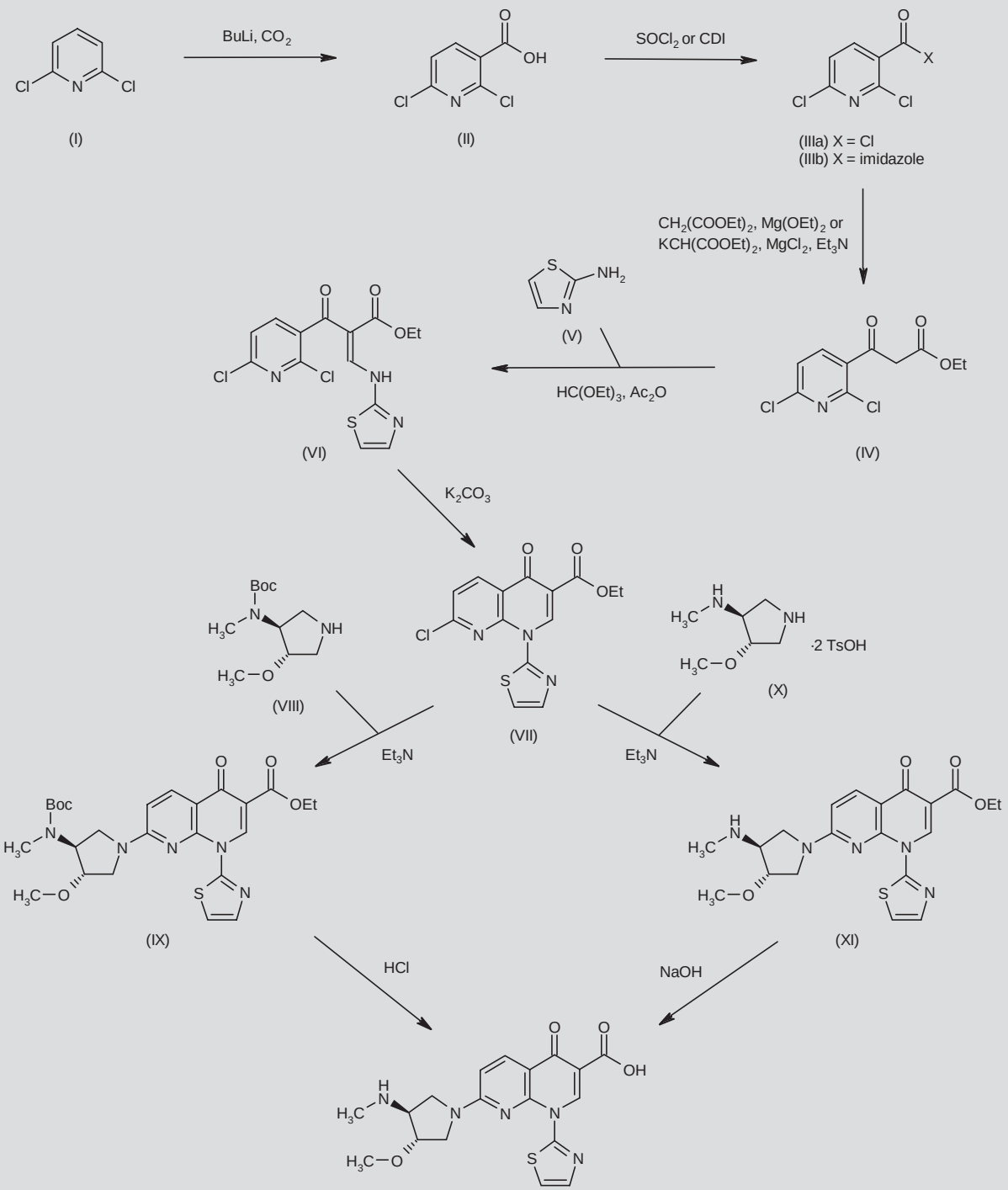
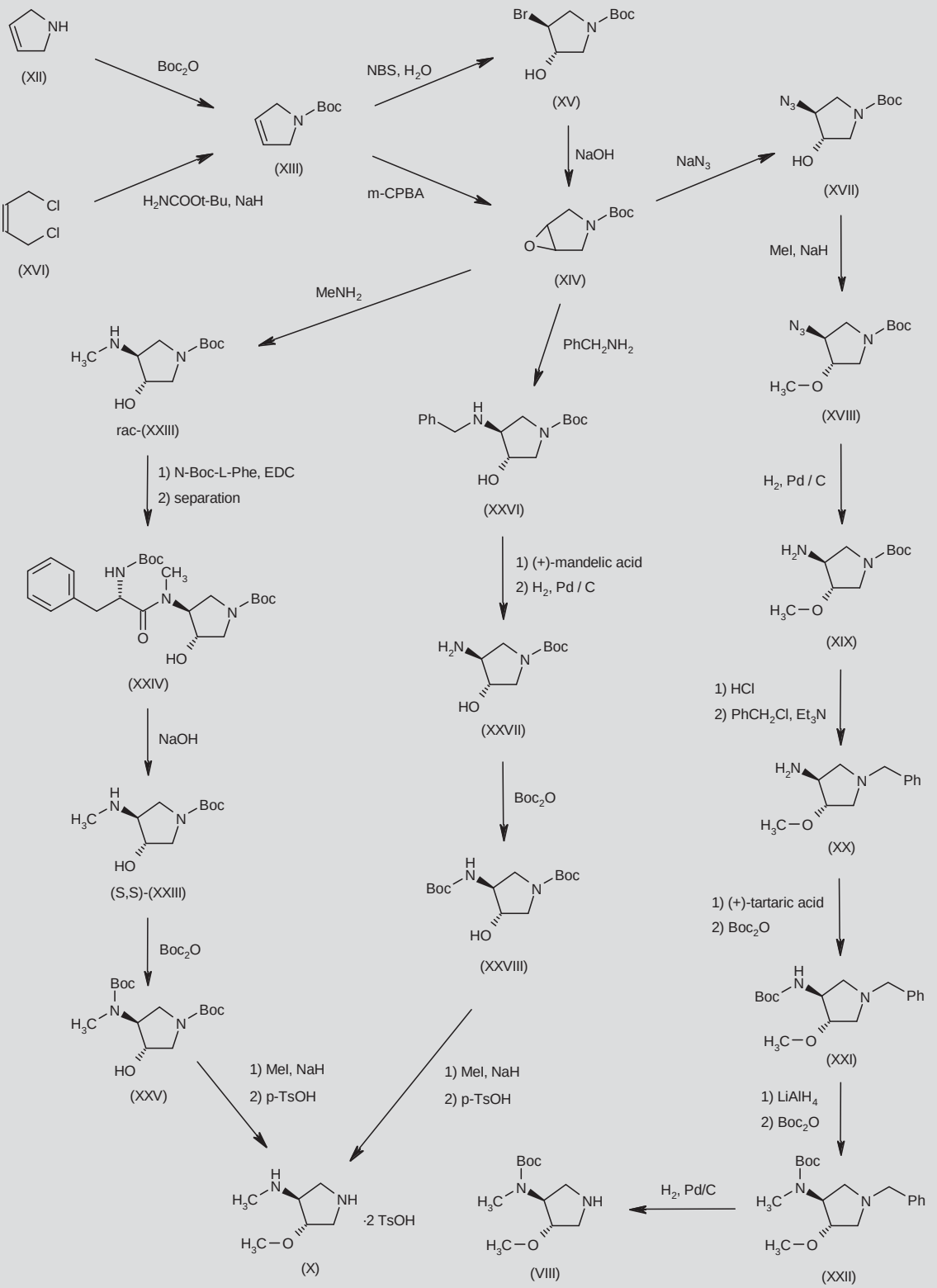
Prous Science, Provenza 388, 08025 Barcelona, Spain.

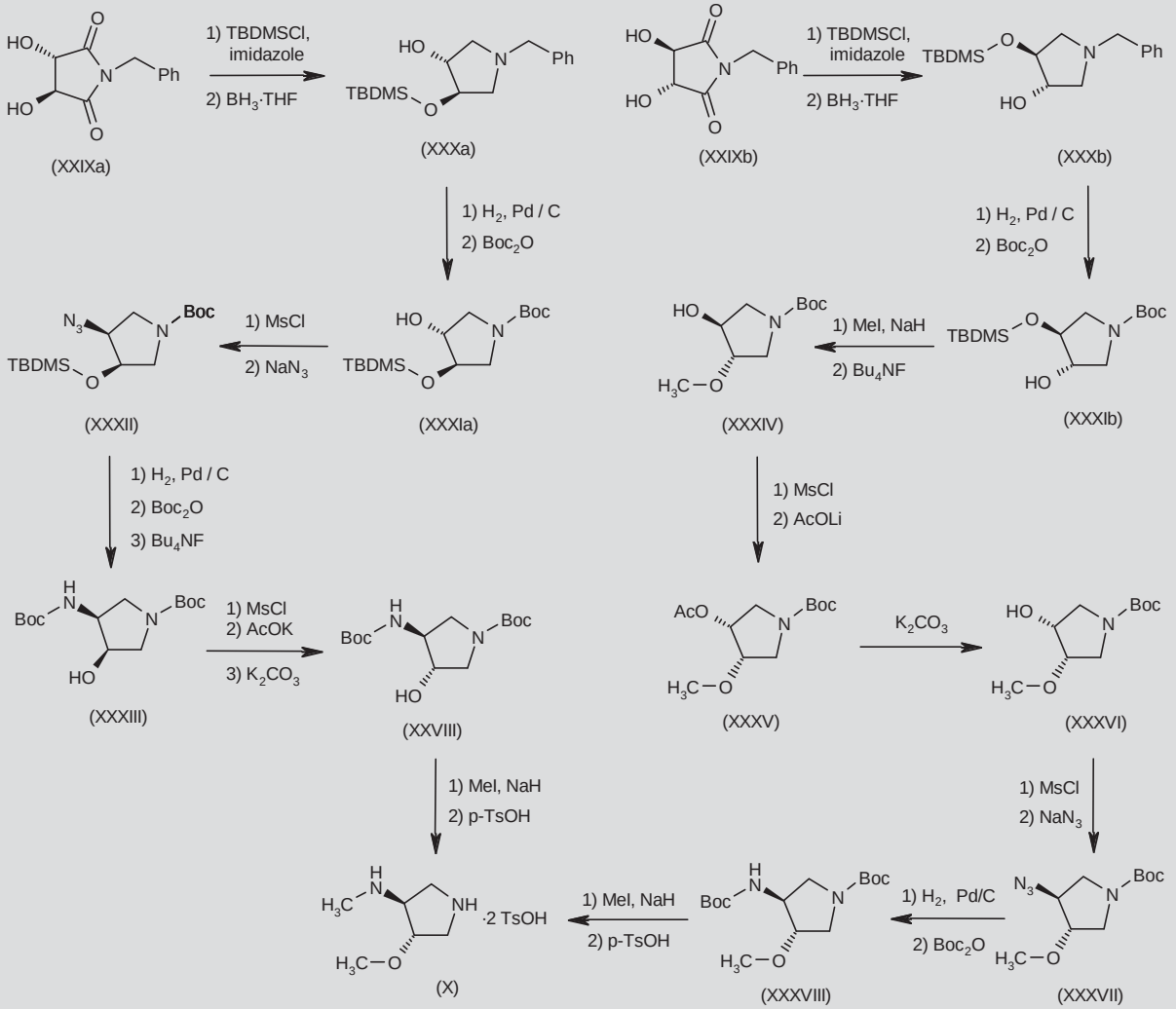
Scheme 1. Synthesis of Voreloxin



Scheme 2. Synthesis of Intermediates (VIII) and (X)



Scheme 3. Stereospecific Synthesis of Intermediate (X)

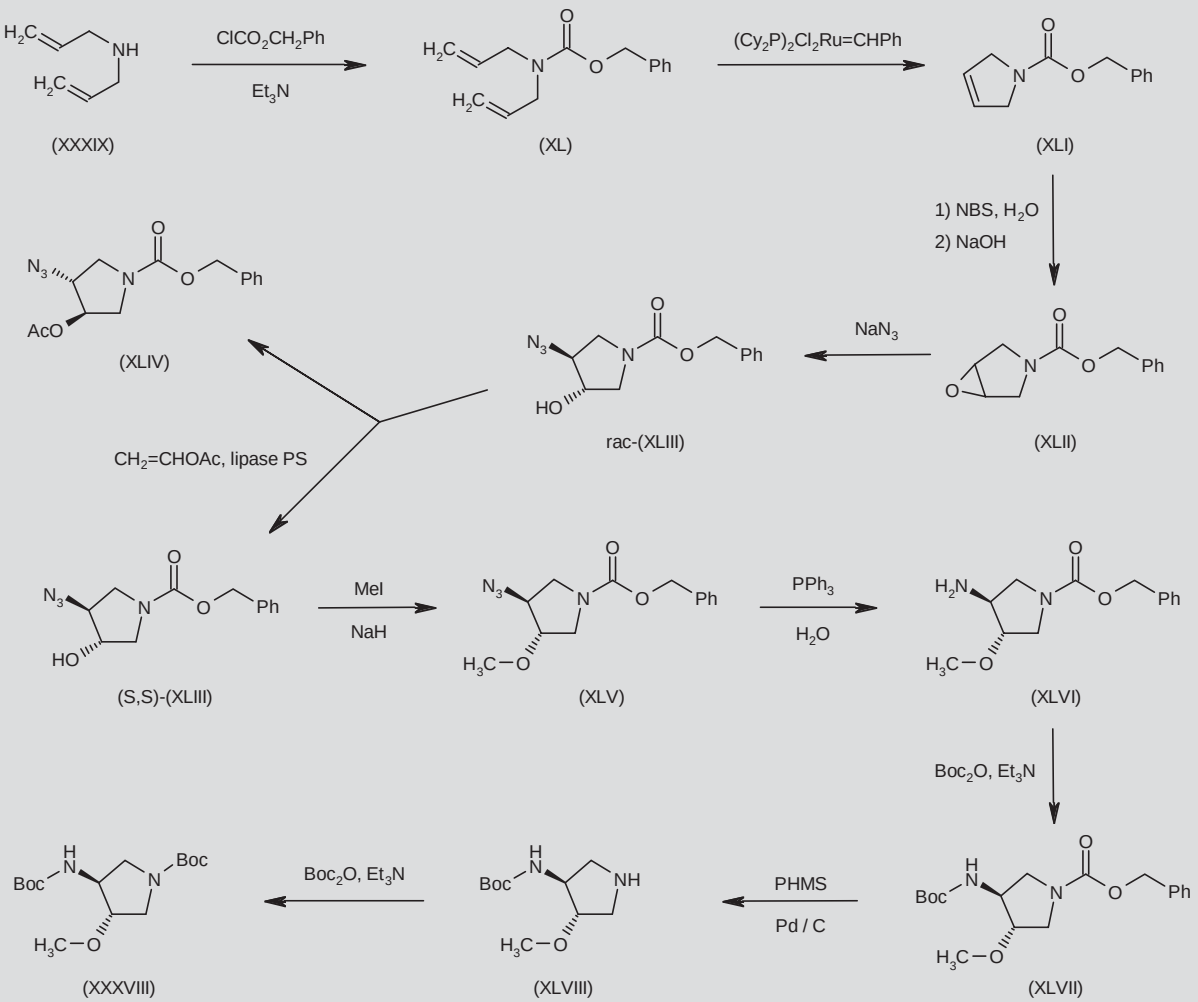


cal resolution of racemic *trans*-3-amino-1-benzyl-4-methoxypyrrolidine (XX) using D-(+)-tartaric acid provides the target (*S*,*S*)-enantiomer, which is then protected as the *tert*-butyl carbamate (XXI) by treatment with Boc_2O in MeOH. Subsequent reduction of carbamate (XXI) with $LiAlH_4$ in THF followed by reprotection of the resulting *N*-methylamine with Boc_2O in CH_2Cl_2 provides compound (XXII), which is finally debenzylated by catalytic hydrogenation over Pd/C in EtOH to the desired pyrrolidine (VIII) (2, 4). Scheme 2.

Epoxide (XIV) is converted to the aminopyrrolidine (X) by two additional strategies. Ring opening of epoxide (XIV) with aqueous methylamine yields the racemic *trans*-aminoalcohol (XXIII), which is resolved by acylation with *N*-Boc-L-phenylalanine and EDC in CH_2Cl_2 followed by separation of the diastereomers by column chromatography. Hydrolysis of the desired diastereomer (XXIV) with 20% NaOH in EtOH affords the (*S*,*S*)-enantiomer of amino alcohol (XXIII), which is protected as the bis-carbamate (XXV) using Boc_2O in MeOH. Finally, methylation of alcohol (XXV) with iodomethane and NaH in DMF, followed by removal of both Boc groups with *p*-TsOH in *i*-PrOH provides the target (*S*,*S*)-3-methoxy-4-(methylamino)pyrrolidine ditosylate (X) (4). Scheme 2.

Alternatively, reaction of epoxide (XIV) with benzylamine provides the racemic amino alcohol (XXVI), which can be resolved by recrystallization of the diastereoisomeric salts with (+)-mandelic acid in acetonitrile/water. Subsequent debenzylation of the target enantiomer with H_2 and Pd/C in BuOH provides the (*S*,*S*)-amino alcohol (XXVII). Protection of the primary amino group of compound (XXVII)

Scheme 4. Synthesis of Intermediate (XXXVIII)

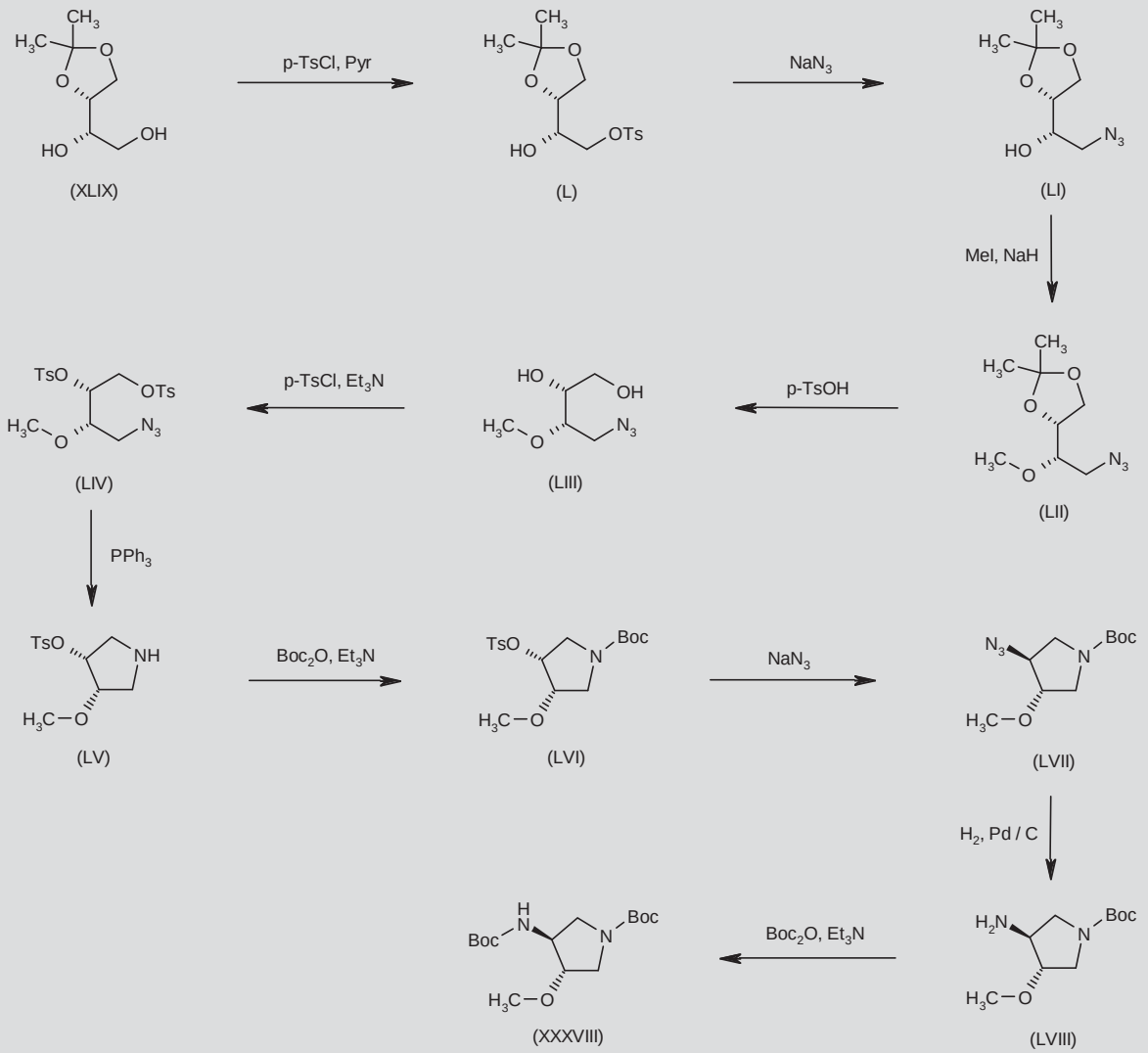


with Boc_2O in EtOH gives the bis-carbamate (XXVIII), which is finally submitted to *N,O*-dialkylation with iodomethane and NaH, followed by Boc group cleavage with *p*-TsOH in THF/MeOH to yield the key intermediate (X) (4). Scheme 2.

Stereospecific methods to prepare aminopyrrolidine (X) have been developed starting from both enantiomers of *trans*-*N*-benzyl-2,3-dihydroxysuccinimide (XXIXa) and (XXIXb), generated respectively from D- and L-tartaric acid. Monoprotection of the (*S,S*)-diol (XXIXa) with one equivalent of TBDMSCl in the presence of imidazole in cold DMF followed by imide reduction with borane-THF complex leads to the pyrrolidine (XXXa), which after debenzylation with H_2 and Pd/C and reprotection with Boc_2O provides the *tert*-butyl carbamate (XXXIa). Alcohol (XXXIa) is then converted to the corresponding mesylate, which undergoes nucleophilic displacement by NaN_3 in DMF to give the *cis*-azide (XXXII). Azide (XXXII) is then reduced by catalytic hydrogenation over Pd/C to afford the corresponding amine, which is protected with Boc_2O, followed by desilylation with tetrabutylammonium fluoride to give the *cis*-alcohol (XXXIII). Inversion of the configuration of alcohol (XXXIII) to the *trans*-isomer (XXVIII) is then accomplished by mesylation with MsCl and Et_3N followed by displacement with potassium acetate in DMF and acetate hydrolysis with K_2CO_3 in MeOH. Finally, compound (XXVIII) is methylated with iodomethane and NaH and subsequently deprotected with *p*-TsOH in *i*-PrOH (5). Scheme 3.

Alternatively, the (*R,R*)-imide (XXIXb) is converted to the *trans*-diol derivative (XXXb) as before, and the *N*-benzyl group is then

Scheme 5. Synthesis of Intermediate (XXXVIII)



exchanged for a Boc group, giving alcohol (XXXIb). Alkylation of compound (XXXIb) with iodomethane and NaH affords the corresponding methyl ether, which is desilylated to alcohol (XXXIV) by means of TBAF in THF. Mesylation of alcohol (XXXIV), followed by displacement with lithium acetate produces the methoxy acetate (XXXV), which is hydrolyzed to the *cis*-alcohol (XXXVI) using K_2CO_3 in MeOH. Mesylation of alcohol (XXXVI) followed by displacement with NaN_3 provides the azide (XXXVII). After reduction with H_2 and Pd/C, the resulting amine is protected as the Boc derivative (XXXVIII) with Boc_2O in EtOH. Finally, amine (XXXVIII) is alkylated with iodomethane and NaH, followed by deprotection with *p*-TsOH (X) (5). Scheme 3.

Intermediate (XXXVIII) can be prepared by several alternative methods. Protection of diallylamine (XXXIX) with benzyl chloroformate and Et_3N in CH_2Cl_2 yields the benzyloxycarbonyl derivative (XL), which undergoes ring-closing metathesis in the presence of Grubbs' catalyst to give 1-Cbz-3-pyrroline (XLI). Reaction of compound (XLI) with NBS in aqueous DMSO followed by treatment of the resulting bromohydrin with 1 N NaOH leads to epoxide (XLII), which undergoes ring opening with NaN_3 in aqueous dioxane to afford the racemic *trans*-azidoalcohol (XLIII). Resolution of *rac*-(XLIII) is then effected by incubation with lipase PS in the presence of vinyl acetate in *i*-Pr_2O to give the (*R*,*R*)-acetate (XLIV) along with unreacted (*S*,*S*)-alcohol (XLIII). Methylation of (*S*,*S*)-alcohol (XLIII) with iodomethane and NaH affords ether (XLV), which is submitted to azide group reduction by means of PPh_3 in THF/H_2O to give amine (XLVI). Protection of

amine (XLVI) with Boc_2O and Et_3N followed by selective removal of the Cbz group in the resulting bis-carbamate (XLVII) using poly(methylhydrosiloxane) and Pd/C in EtOH leads to the pyrrolidine (XLVIII), which is finally treated with Boc_2O and Et_3N (6). Scheme 4.

Treatment of the known diol (XLIX) with tosyl chloride in cold pyridine gives the primary tosylate (L), which is reacted with NaN_3 in DMF to give azidoalcohol (LI). Subsequent alkylation of the secondary alcohol (LI) with MeI and NaH followed by acidic ketal hydrolysis of the resulting methyl ether (LII) affords the diol (LIII), which is converted to ditosylate (LIV) using *p*-TsCl and Et_3N. Reduction of azide (LIV) using PPh_3 in refluxing MeOH occurs with concomitant cyclization to give the pyrrolidine (LV), which is then protected as the *N*-Boc derivative (LVI) under standard conditions. The remaining tosylate group of (LVI) is then displaced with NaN_3 in DMF to furnish azide (LVII). After reduction by catalytic hydrogenation over Pd/C, the obtained amine (LVIII) is finally protected with Boc_2O and Et_3N in CH_2Cl_2 (7). Scheme 5.

BACKGROUND

Topoisomerase II is a nuclear enzyme that induces transient breaks in double-stranded DNA, which allows DNA to uncoil for cellular activities including replication, transcription, recombination and chromosome condensation/decondensation. Following these activities, topoisomerases also religate the cut DNA (8). Topoisomerase inhibitors cause replication-dependent DNA damage in the S-phase of the cell cycle, which leads to apoptosis via irreversible G2 arrest. There are two mechanisms of topoisomerase inhibition. The first mechanism is by DNA intercalation, which prevents the activity of topoisomerase, and the second is by direct topoisomerase II inhibition. Etoposide is a direct topoisomerase II inhibitor that is active in many malignancies, including lymphomas, leukemia and various carcinomas and sarcomas (9), although it is only indicated for refractory testicular tumors and small cell lung cancer. Doxorubicin is an example of a DNA intercalator. Doxorubicin and other anthracyclines are widely used for the treatment of many malignancies as single agents or in combination with other agents (10). Voreloxin belongs to a new class of DNA-intercalating molecules that inhibit topoisomerase II. Interestingly, other mechanisms appear to contribute to the anticancer effects of voreloxin, because the drug maintains activity against cancer cells even when the topoisomerase II enzyme is knocked down (11). Voreloxin is being developed by Sunesis Pharmaceuticals under license from Dainippon Sumitomo Pharma for the treatment of a variety of solid and hematological malignancies (12). At the time of publication, voreloxin is being tested in phase II clinical trials in patients with platinum-resistant ovarian cancer and acute leukemias.

PRECLINICAL PHARMACOLOGY

Voreloxin induces DNA damage, G2 arrest and apoptosis by selective intercalation of DNA and poisoning of topoisomerase II (11). Voreloxin's targeting of human topoisomerase II in cancer cells parallels the mechanism of action of quinolones on bacterial type II topoisomerases. Voreloxin is a poor topoisomerase II poison in comparison to etoposide, but it enhanced DNA cleavage by approximately 5-fold at specific G/C sites with topoisomerase II A and B. Similar to doxorubicin, voreloxin acts by DNA intercalation and topoisomerase II inhibition. Full intercalation occurred at concentrations of 10 μM. Studies using fixed-ring (predicted to be a good intercalator) and phenyl (predicted to be a poor intercalator) analogues demonstrated an increased cytotoxicity of 5.5-fold and a decreased cytotoxicity of 100-fold, respectively, compared with voreloxin. For example, cultured human T-cell lymphoblastic leukemia (CCRF-CEM) cells exposed to 1 μM voreloxin display approximately a 3- to 5-fold increase in physiological levels of DNA cleavage mediated by topoisomerase II (A and B). The impact of topoisomerase II knockdown is greater in studies with etoposide and doxorubicin, suggesting that voreloxin is less dependent on topoisomerase II for its cytotoxicity (11). Topoisomerase I does not appear to be inhibited (13).

In cancer cell populations grown in synchronous fashion, voreloxin (10 μM) causes a rapid increase in caspase-3 activation when these cells reach the S-phase, but not the G1- or M-phases. Voreloxin is different from the other G2 arrestors in that it causes p21 expression early in S-phase and a significant S-phase lag (14). For example, in human colon carcinoma HCT 116 cells, apoptosis occurs within 5 h of exposure, which is 2- to 10-fold faster than the comparator cytotoxic compounds studied (cisplatin, docetaxel, etoposide, gemcitabine, doxorubicin, irinotecan, bleomycin and mitomycin C). After exposure to voreloxin, cells keep cycling normally until they reach S-phase, which is then 30% longer than in untreated cells and, upon entering S-phase, a rapid appearance of checkpoint markers (Chk kinases, CDC25 phosphatases, CDK1 and p21) occurs, ending in a sustained G2 arrest with 4N DNA content. Induction of DNA damage in S-phase cells is biphasic over the concentration range. There is a first concentration-dependent increase in DNA damage with concentrations up to 10 μM, but above 20 μM less damage is observed. This DNA damage activates ataxia telangiectasia mutated (ATM) and ATM- and Rad3-related protein (ATR), which was reflected by sustained phophorylation of checkpoint kinases Chk2 and Chk1, respectively (15). Furthermore, this interaction occurs via p53-independent and -dependent mechanisms (16), including p73, c-ABL and p21. At that point, there is a rapid activation of the DNA damage-sensing ATM kinase, which phosphorylates histone H2AX, an indication of double-strand break formation. Histone H2AX is a ubiquitous member of the H2A histone family that differs from the other H2A histones by the presence of an evolutionarily conserved *C*-terminal motif, –KKATQASQEY. The serine residue in this motif becomes rapidly phosphorylated in cells and animals when DNA double-strand breaks are introduced into their chromatin by various physical and chemical means. Phosphorylated H2AX recruits DNA repair proteins to the double-strand break sites.

Voreloxin-mediated damage is repaired in part by the DNA-dependent protein kinase (DNA-PK) non-homologous end-joining (NHEJ) pathway, consistent with topoisomerase II A poisons. Hence, inhibition of DNA-PK causes a 10-fold increase in DNA damage (17). NHEJ is referred to as "non-homologous" because the break ends are directly ligated without the need for a homologous template, in contrast to homologous recombination, which requires a homologous sequence to guide repair. Homologous recombination also plays a role in repair of voreloxin-induced double-strand breaks. The RAD51 protein (required for homologous recombination repair), which co-localizes in nuclear foci with γ-H2AX, may also be important for modulating the sensitivity to voreloxin. In vitro, CHO cells deficient in

RAD51 are more sensitive, with an IC_{50} of 6 nM compared to 142 nM in nondeficient cells. In sensitive cells, such as HCT 116, voreloxin decreases RAD51 protein levels within 24 h of exposure in a concentration-dependent fashion, whereas in more resistant cells, such as human colon adenocarcinoma HT-29, levels of RAD51 protein increase in response to voreloxin (18).

Similar to other topoisomerase II A poisons, there is no correlation between sensitivity to voreloxin and levels of ERCC-6 (a protein required for nucleotide excision repair) or XRCC1 (a protein required for single-strand break repair) (18).

In vitro, voreloxin is cytotoxic to a wide variety of human tumor cell lines, including non-small cell lung cancer NCI-H460, lung carcinoma Calu-6, ovarian carcinoma PA-1, ovarian adenocarcinoma SK-OV-3, colon carcinoma HCT 116, stomach carcinoma Hs 746T and breast adenocarcinoma MDA-MB-231 cells. Voreloxin also demonstrates in vitro activity against tumor cells resistant to doxorubicin, etoposide, cisplatin and camptothecin (13). In vitro voreloxin inhibits proliferation with IC_{50} values of 53, 23, 18 and 10 ng/mL, respectively, in promyelocytic leukemia HL-60, Jurkat T-cell leukemia, CCRF-CEM and camptothecin-resistant CCRF-CEM/C2 human cell lines (19). In the extreme drug resistance cell proliferation assay, voreloxin (0.1-20 μM) causes concentration-dependent killing of primary cancer cells harvested from 17 ovarian tumors, with a median IC_{50} of 1 μM. None of the biopsies appeared to be resistant to voreloxin (20, 21).

Voreloxin exhibits strong antitumor activity in murine solid tumor models, with some animals showing complete tumor regression. Inhibition rates vary from 80% to 100%, which is significantly higher than irinotecan and paclitaxel (0-32% and 19-22%, respectively) (19, 22, 23). Voreloxin also increases survival significantly more than cisplatin, etoposide, doxorubicin, irinotecan and paclitaxel in murine metastatic liver tumor models (22). The activity of voreloxin at maximum doses does not appear to be schedule-dependent in animal models, but at lower doses more frequent administration is more active (24).

In combination, voreloxin is synergistic (combination indices < 0.85) with the DNA-damaging agents etoposide, daunorubicin, dactinomycin, mitomycin C, cisplatin and carboplatin and the antimetabolite drugs pemetrexed, methotrexate, cytarabine, 5-fluorouracil, the DNA-dependent protein kinase (DNA-PK) inhibitor wortmannin and the heat shock protein HSP90 inhibitor geldanamycin. Conversely, an antagonistic effect is observed when voreloxin is added to cells along with the microtubule inhibitor docetaxel or the antimetabolite agent gemcitabine. However, when docetaxel or gemcitabine is added prior to voreloxin, synergy is observed (25). Table I shows the xenograft models used to test the activity of voreloxin.

PHARMACOKINETICS AND METABOLISM

Voreloxin is relatively stable after 60 minutes of incubation, with ≥ 75% unchanged drug in three different species. Voreloxin is less than 75% bound to serum proteins and does not inhibit the human ether-a-go-go-related gene (hERG) potassium channel. No significant off-target activity has been detected towards a panel of approximately 60 enzymes or receptors (26). Incubation of voreloxin with human, monkey and rat liver microsomes yields three metabolites, suggesting the involvement of cytochrome P450 enzymes in its metabolism. Incubation with Supersomes™ containing individually expressed cytochrome enzymes showed that CYP3A4, 2D6 and 1A2 play a role in the cytochrome P450-mediated metabolism of voreloxin. Incubation of voreloxin with baculovirus-expressed human UGT isoforms indicates several UDP-glucuronosyltransferase (UGT) conjugates (UGT1A1, 1A4, 1A3, 2B7, 2B15 and 1A8) capable of conjugating voreloxin. Plasma concentration–time profiles for voreloxin and total radioactivity pharmacokinetics are similar. The drug declines in a biphasic fashion, with terminal half-lives of 6.3 and 5.4 h, respectively. The maximum plasma concentration at time zero is 4.2 μgEq/mL, the area under the concentration–time curve (AUC) 17 μgEq.h/mL, the clearance 10 mL/min/kg and the volume of distribution at steady state 4 L/kg. Forty-eight hours after intravenous injection 37.9%, 32.5% and 19.6%, respectively, of the administered radioactivity is excreted into the bile, feces and urine. High-performance liquid chromatography (HPLC) of voreloxin from plasma (4 h), urine (6-8 h) and bile (4-6 h) shows that the intact drug makes the largest peaks in plasma, urine and bile. There is one metabolite in plasma (M4), six metabolites in urine and seven in bile. Voreloxin metabolites in rat bile were structurally characterized using triple quadruple liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS). M1 is an ester glucuronide. Metabolites M1a and M1b have identical molecular and product ions to M1, also suggesting glucuronide metabolites. The identity of metabolite M2a is dihydrodecarboxylic acid voreloxin. *O*-Desmethyl-voreloxin has the same retention time as M3. Metabolite M4 is *N*-desmethyl-voreloxin. In all species the *N*-desmethyl metabolite M4 is the predominant species observed based on AUC of the ionized species. Metabolite M5 is a nonpolar metabolite. *N*-Desmethyl-voreloxin (M4) shows similar cytotoxic activity to voreloxin, whereas the *O*-desmethyl and dihydrodecarboxylic acid metabolites are inactive at the highest concentrations tested (27).

Table I. *Activity of voreloxin in animal models (23).*

Human cell line	Schedule	Dose (mg/kg)	Combined drug	Inhibition (%)	
				Combination	Control*
Ovarian carcinoma A2780	Weekly i.v.	10	Cisplatin	64.2	49.0
NSCLC NCI-H460	Weekly i.v.	10	Carboplatin	74.6	31.6
NSCLC NCI-H460	Weekly i.v.	10	Gemcitabine	81.7	36.6
Pancreatic adenocarcinoma BxPC-3	Weekly i.v.	15	Gemcitabine	71.5	20.2

Control is the single-agent activity of the other drug used in the combination

Table II. *Pharmacokinetic parameters of voreloxin in humans.*

Study	No. of patients	Dosing	C_{max} (μg/mL)	Mean clearance (L/m^2)	AUC (μg.h/mL)	Terminal half-life (h)	Volume at steady state (L/h/m^2)	Ref.
SPO-0001	41	Single i.v. doses of 3-75 mg/m^2	0.139-5.05	2.0	1.14-46.09 (proportional to dose)	21.3	53.2	24
SPO-0002	21	Weekly i.v. doses of 3-24 mg/m^2	0.577-1.84	2.22	1.71-15.20	18.5	48.9	21
SPO-0004	67	Weekly for 3 weeks (18-90 mg/m^2)	0.4-4.4	2.0	6-102	25	61	26
		Twice weekly for 2 weeks (9-50 mg/m^2)	0.2-3.1	2.2	3-48	27	73	
SPO-0006	82	Every 3 weeks (48 mg/m^2)		1.8	28	18	45	28

Human pharmacokinetics have been examined in four studies. Table II summarizes the pharmacokinetic parameters. No evidence for drug-dependent alterations in pharmacokinetic parameters was observed with weekly dosing (28), indicating a lack of accumulation upon repeated administration.

SAFETY

In animal models, i.v. injections of voreloxin (15 or 20 mg/kg) on days 0 and 4 did not cause weight loss through day 16 (29). Bone marrow cellularity at day 6 was reduced to 15% and 7.5%, respectively, for the 15 and 20 mg/kg doses. Absolute neutrophil counts subsequently returned to normal levels by day 16 (19).

In combination studies with the cytotoxic agents carboplatin, gemcitabine and cisplatin body weight decreased from baseline by 8%, 8% and 14%, respectively (30).

In clinical trials in patients with solid tumors (studies SPO-0001 and SPO-0002), the dose-limiting toxicity (DLT) was neutropenia. Grade 4 thrombocytopenia was observed in two patients in these studies (28, 31, 32). Other side effects include grade 1 or 2 nausea/vomiting, diarrhea and mucositis. In the phase I clinical trial in patients with acute leukemia (SPO-0004), DLTs included myelosuppression, bowel obstruction and grade 3 mucositis. Other side effects included neutropenic fever, nausea, vomiting, anorexia, fatigue, alopecia and diarrhea (33). Voreloxin appears to be generally well tolerated in patients with leukemia, independent of age (34). The phase II clinical trials in patients with lung and ovarian cancers confirmed the toxicity profile of neutropenia, fatigue, nausea, vomiting, alopecia, dysguesia, headache and arthralgia (21, 35, 36).

CLINICAL STUDIES

Voreloxin is injected over 10 min i.v. Table III shows the studies of voreloxin that have been conducted or are ongoing.

Phase I trials

In the phase I clinical trial SPO-0001, escalating doses of voreloxin were administered i.v. once every 3 weeks to patients with various solid tumors. The maximum tolerated dose (MTD) was 48 mg/m^2. A partial response (PR) was observed in 1 patient and stable disease (SD) was observed in 12 patients (31, 32). In the second phase I clinical trial, SPO-0002, voreloxin was administered i.v. every week for 3 times every 4 weeks at escalating doses of 3-24 mg/m^2. The MTD was 15 mg/m^2 for a once-weekly dosing regimen. An unconfirmed PR was observed in one patient with mesothelioma (treated at 15 mg/m^2) and SD was achieved for at least four cycles in five patients who received doses of 6 mg/m^2 and above (28, 32). In the phase I clinical trial SPO-0004 in patients with acute leukemia, voreloxin was administered i.v. either weekly for 3 weeks on days 1, 8 and 15 (doses of 18-38, 50, 60, 72 or 90 mg/m^2; n = 40) or twice weekly for 2 weeks on days 1, 4 and 8, and 11 (doses of 9-30, 40 or 50 mg/m^2; n = 27). In the weekly dosing cohort, complete remissions and blast reductions of > 95% were observed at voreloxin doses of 50 mg/m^2. DLTs were myelosuppression, bowel obstruction and mucositis. Non-DLTs included nausea, vomiting and diarrhea. In the twice-weekly dosing cohort, voreloxin doses of 40 mg/m^2 demonstrated antileukemic activity. Of the patients who received these doses, one patient had a CR and one patient had blast reduction to less than 5%. The antileukemic activity correlated with plasma voreloxin concentrations above 1 μM (33). Two extension clinical trials (SPO-0003 and SPO-0008) are currently ongoing in patients who have previously achieved either a tumor response or SD after treatment with voreloxin.

Phase II trials

In the phase II clinical trial SPO-0005, voreloxin (48 mg/m^2) was administered i.v. every 3 weeks for up to 8 cycles to 31 patients with non-small cell lung cancer. Efficacy data are available for 28 patients, with 2 partial responses and 14 SD, while 12 patients progressed (PD) (35). The second phase II clinical trial SPO-0006 used the same dose and schedule in patients with refractory (relapsed < 90 days after end of initial therapy or never responded) or sensitive (relapsed > 90 days after end of initial therapy) small cell lung cancer (n = 25 and 26, respectively). Efficacy data are available from 23 refractory and 22 sensitive patients. Among the refractory cohort, 6 patients had SD and 17 patients had PD; among the sensitive cohort, 2 patients responded, 16 had SD and 4 patients had PD (35).

Table III. *Studies of voreloxin (available on www.clinicaltrials.gov).*

Study	Title	Status	Number of patients	Eligibility
SPO-0012	Safety and Tolerability Study of SNS-595 and Cytarabine Combination in Acute Myeloid Leukemia in Human	Recruiting		Acute myeloid leukemia
SPO-0014	Study of SNS-595 in Older Patients With Untreated Acute Myeloid Leukemia	Recruiting	21 of 55	Leukemia; acute disease; acute myeloid leukemia; nonlymphocytic leukemia; myelodysplastic syndromes
SPO-0004	Safety Assessment of Two Schedules of Intravenous Infusions of SNS-595 for the Treatment of Hematologic Malignancies	Active, not recruiting	67	
SPO-0010	Safety and Efficacy Clinical Study of SNS-595 in Patients With Platinum-Resistant Ovarian Cancer	Active, not recruiting	65	Epithelial ovarian cancer
SPO-0006	Safety and Efficacy Clinical Study of SNS-595 in Patients With Advanced Small Cell Lung Cancer	Active, not recruiting	51	Small cell lung cancer
SPO-0005	Safety and Efficacy Clinical Study of SNS-595 for Second-Line Therapy in Patients With Advanced NSCLC	Completed	31	Non-small cell lung cancer
SPO-0001	Initial Safety Assessment of SNS-595 for the Treatment of Solid Tumors.	Completed	41	Solid tumors
SPO-0002	Safety Assessment of Weekly Intravenous Infusions of SNS-595 for the Treatment of Solid Tumors	Completed	21	

Women were enrolled in a phase II trial of single-agent voreloxin (SPO-0010) for the treatment of platinum-resistant ovarian epithelial cancer (progressed during or relapsed within 6 months of platinum-based therapy). The median age was 60.5 years (range 33-82 years) and the performance status 1 or less. Patients had received a median of 2 (range 1-4) prior therapies, including 17 patients who had failed pegylated liposomal doxorubicin (PLD) therapy and 18 patients who were PLD-naïve. There were three dosing cohorts: 48 mg/m^2 every 3 weeks (cohort A, n = 65), 60 mg/m^2 every 4 weeks (cohort B, n = 35) and 75 mg/m^2 every 4 weeks (cohort C). Overall, disease control (CR + PR + SD for ≥ 90 days) was achieved in 46% of patients (2 CRs and 5 PRs were observed in cohort A for an overall response rate of 11%; 46 patients had SD and 12 had PD as best response). Data for cohort B patients enrolled at 60 mg/m^2 every 4 weeks appeared comparable. A low incidence of febrile neutropenia (< 10%) supported dose escalation to 75 mg/m^2 every 4 weeks (36).

Two studies, SPO-0012 and SPO-0014, are ongoing in patients with AML. Eligible patients for study SPO-0014 are at least 60 years old with newly diagnosed AML (either de novo or from an antecedent hematological disorder [AHD]) and a minimum of one additional adverse risk factor (age above 70, AML from AHD, intermediate- or poor-risk cytogenetics or ECOG performance status of 2). There are two cohorts of 72 mg/m^2 weekly for three doses (schedule A) or 72 mg/m^2 weekly for two doses (schedule B). Patients achieving a CR after induction or reinduction may receive up to two additional voreloxin courses as consolidation. Preliminary response data are available for all 29 patients enrolled into schedule A. Eleven patients achieved a CR (response rate 38%), with a median duration of response that has not been reached. The 30-day all-cause mortality rate was 17%. Infection was the most common cause of early mortality. The regimen was toxic, with febrile neutropenia, mucosal inflammation and pneumonia. Although stage 1 criteria were achieved, in order to reduce the duration of myelosuppression and improve treatment tolerability, the protocol was modified with elimination of the day 15 dose (schedule B) and the use of prophylactic anti-infectives was recommended. To date, 21 patients have been treated on schedule B. Early data for antileukemic activity are available for 18 patients. Eight patients failed treatment. The incidence of grade 3 or higher nonhematological adverse events is less than with schedule A and the 30-day all-cause mortality rate is only 6%. Early data suggest that schedule B is better tolerated while maintaining antileukemic activity, as demonstrated by blast reductions to ≤ 5% in postinduction bone marrow evaluations (34). Finally, preliminary results of the ongoing phase Ib SPO-0012 study of combination voreloxin plus cytarabine in relapsed or refractory AML patients were reported. Voreloxin was dose-escalated and given on days 1 and 4 in combination with two schedules of cytarabine: schedule A, cytarabine 400 mg/m^2/day by continuous i.v. infusion for 5 consecutive days; schedule B, cytarabine 1 g/m^2 by daily i.v. bolus for 5 consecutive days. The voreloxin starting dose was 10 mg/m^2 on days 1 and 4 for schedule A and 70 mg/m^2 on days 1 and 4 for schedule B. DLTs and pharmacokinetics were assessed during cycle 1. The MTD was 80 mg/m^2 on schedule A. CRs and PRs were observed at doses of ≥ 20 mg/m^2. Ex vivo activity assay results suggest that voreloxin is the

primary contributor to the majority of complete remissions. Enrollment in the phase II portion of schedule A and phase Ib dose escalation for schedule B are ongoing (37).

DRUG INTERACTIONS

There are no studies dealing with interactions or potential interactions with other drugs. However, given the metabolism through cytochrome P450, interactions are possible with many common drugs.

SOURCES

Sunesis Pharmaceuticals (US); licensed from Dainippon Sumitomo Pharma (JP).

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