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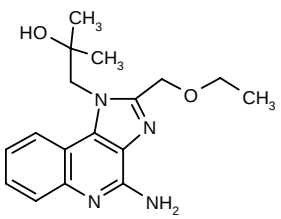
CCC: 0377-8282/99

S-28463

Treatment of Hepatitis C
Interferon Inducer

R-848
VML-600

2-[4-Amino-2-(ethoxymethyl)-1*H*-imidazo[4,5-*c*]quinolin-1-yl]-1,1-dimethylethanol
4-Amino-2-(ethoxymethyl)-α,α-dimethyl-1*H*-imidazo[4,5-*c*]quinoline-1-ethanol



$C_{17}H_{22}N_4O_2$ Mol wt: 314.3868

CAS: 144875-48-9

EN: 221036

Synthesis

S-28463 has been synthesized by two related ways:

1) The cyclization of the diaminoquinoline (I) with ethoxyacetic acid (II) by heating at 120 °C gives the imidazoquinoline (III), which is oxidized to the 5-*N*-oxide (IV) by treatment with peracetic acid. Finally, compound (IV) is aminated by means of ammonium hydroxide in *p*-toluenesulfonyl chloride in dichloromethane (1). Scheme 1.

2) The esterification of 3-nitroquinoline-2,4-diol (I) with trifluoromethanesulfonic anhydride (II) gives the disulfonate (III), which is condensed with 2-hydroxyisobutylamine (IV) and triethylamine in dichloromethane, yielding the aminoquinoline (V). The reaction of (V) with dibenzylamine by means of triethylamine in refluxing toluene affords the quinolinediamine (VI), which by reduction of its nitro group with $NaBH_4$ provides the quinolinetriamine (VII). The cyclization of (VII) with 2-(ethoxymethyl)acetyl chloride (VIII) by means of *p*-toluenesulfonic acid in refluxing acetonitrile gives the protected imidazoquinoline (IX), which is finally deprotected by treatment with Pd/C and formic acid (2). Scheme 2.

Description

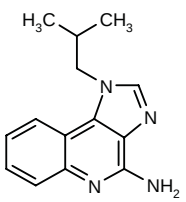
Colorless solid, m.p. 190-3 °C (1).

Introduction

The immune response modifier imiquimod (Aldara®) [I], discovered and developed by 3M Pharmaceuticals, was launched in the U.S. in 1997 for the treatment of genital and perianal warts/condyloma acuminata in adults. The precise topical mechanism of action of imiquimod is unclear, although numerous studies have shown that this and related compounds act via the induction of cytokines, especially interferon-α. Imiquimod has no direct antiviral or antitumor activity, although it exerts potent antiviral and antitumor effects due to its cytokine-inducing mechanism of action (3).

Based on the success of imiquimod, scientists at 3M dedicated further research efforts to the synthesis of related imidazoquinoline compounds which culminated in the discovery of the imiquimod analog S-28463 (R-848, VML-600). Early studies indicated that S-28463 is approximately 100 times more potent than its predecessor in terms of cytokine-inducing effects and the inhibition of viral infection (4). S-28463 has been targeted for the treatment of hepatitis C, a viral infection that causes inflammation of the liver.

First called non-A, non-B hepatitis, hepatitis C was discovered in the mid-1970s and affects approximately 35,000 Americans each year – more than those infected with hepatitis B. According to the International Hepatitis Foundation, more than 80% of all cases of hepatitis C become chronic, with some patients developing cirrhosis and a relatively low number eventually progressing to



[I]

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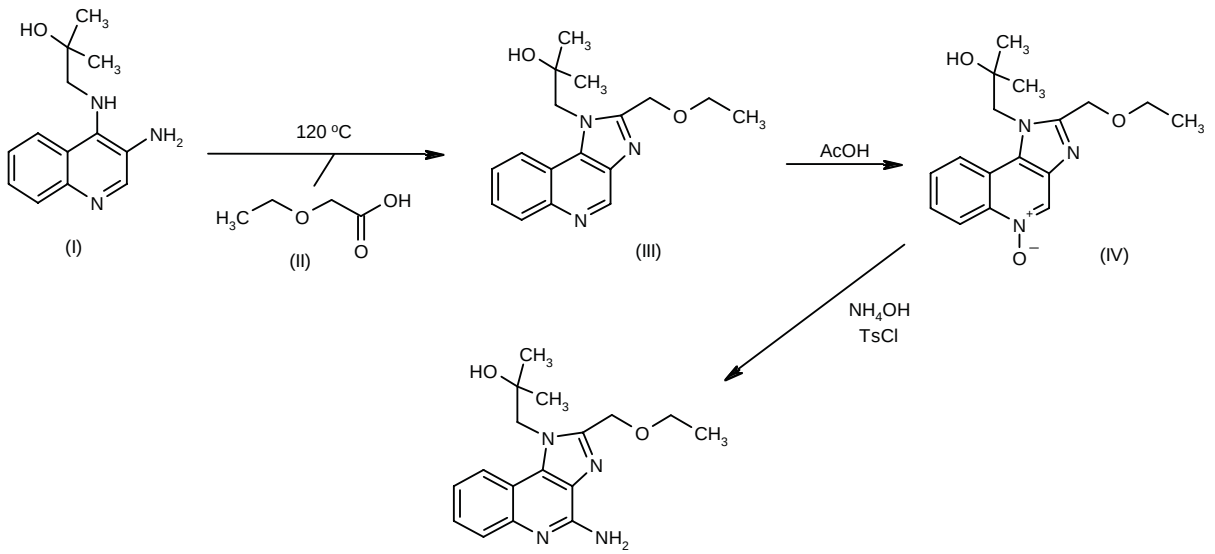
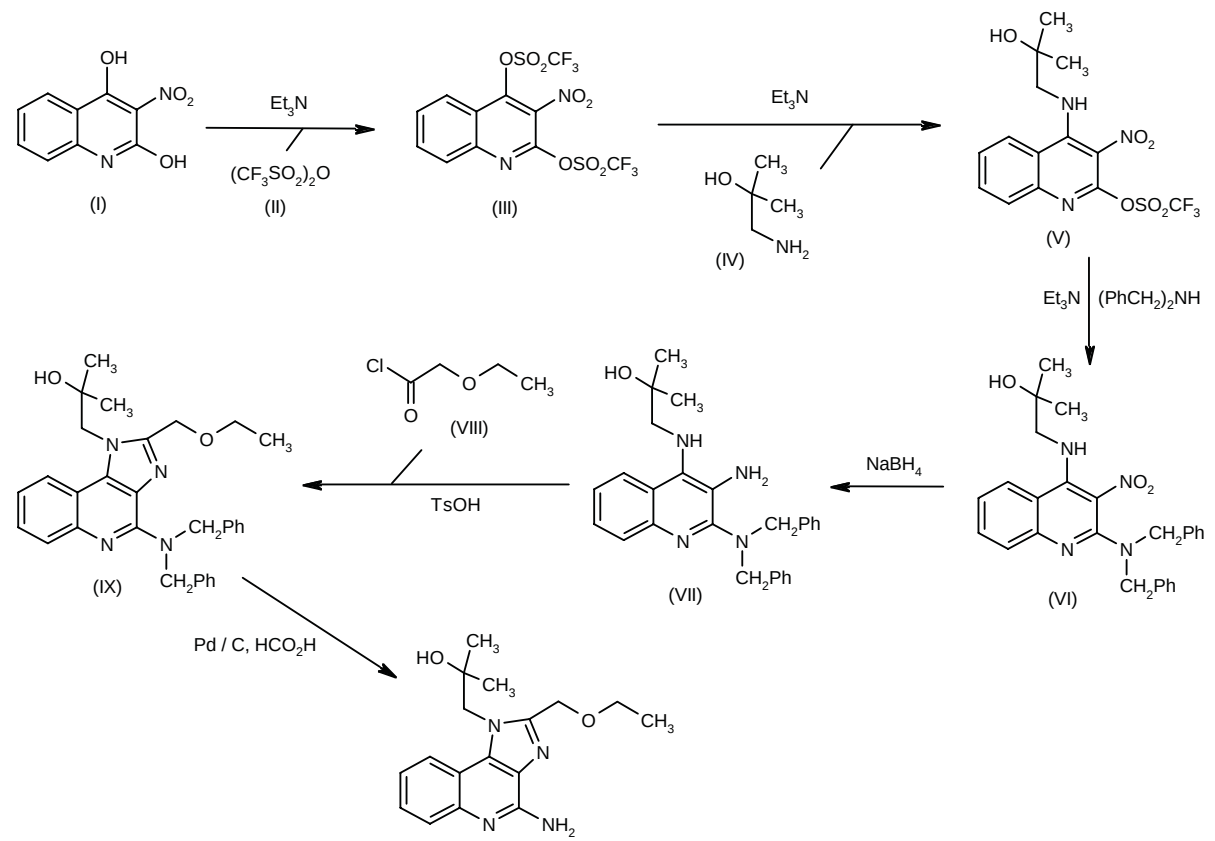
Scheme 1: Synthesis of S-28463



Scheme 2: Synthesis of S-28463



liver cancer. Risk factors for infection with hepatitis C include receipt of blood transfusions or blood products prior to July 1992, use of intravenous drugs, hemodialysis and hemophilia, as well as body piercing, tattooing and cocaine snorting. Current and potential strategies for the treatment and prevention of hepatitis are summarized in Table I.

Pharmacological Actions

In vitro in antigen-stimulated murine splenic cultures and mitogen-stimulated human peripheral blood mononuclear cell cultures, S-28463 inhibited the production of IL-5 and increased that of interferon-γ. Antibodies to mouse IL-12 completely blocked the latter effect of the compound but not the former. Like imiquimod, S-28463 increased the release of Th1 cytokines from $CD4^+$ and $CD8^+$ lymphocytes and inhibited Th2 cytokine release (5-7).

In cultured human keratinocytes prepared from newborn foreskin incubated with S-28463 (1 or 10 μg/ml) for 6 h, the compound significantly increased IFN-α mRNA at both concentrations. mRNAs of IL-1α and IL-8 were upregulated by 1.4- and 2.5-fold, respectively, only at the highest concentration. IL-1α, IL-8 and TNF-α protein expression remained high 24 h after treatment, but that of IFN-α was below levels of detection. Thus, the immunomodulating activity of S-28463 in the skin may be due to the induction of IL-1α, IL-8 and TNF-α production (8).

In another *in vitro* study, both S-28463 and imiquimod were shown to stimulate B-cell proliferation in murine B-cells in a dose-dependent fashion; the mitogenic activity of S-28463 in this assay was more potent than that of imiquimod. In subsequent assays, S-28463 induced the secretion of IgM, IgE, IgG1 and IgG2a from resting B-cells; this effect was potentiated by the addition of IFN-γ, IL-4, IL-4 and IFN-γ, respectively (9).

In vitro in keratinocytes transformed by human papillomaviruses, *e.g.*, SiHa and CaSki squamous cell carcinoma, both S-28463 and imiquimod demonstrated significant effects – either general or HPV-specific – on target cells. These effects were in some cases the same as those obtained from treatment with IFN-α. Differentiation status was found to be a key factor in the antiviral and immunomodulatory effects of the compounds (10).

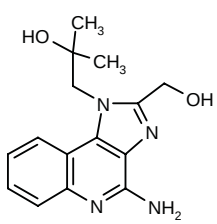
S-28463 demonstrated potent antiviral and immunomodulating activity in various preclinical models. Potent anti-HSV activity was seen following topical, intravaginal or subcutaneous administration to guinea pigs; this activity correlated with induction of serum 2',5'-oligoadenylate synthetase activity. When administered 24 h prior to infection, S-28463 (0.03-0.3 mg/kg s.c.) provided nearly complete protection. Consistent with the results obtained *in vitro*, S-28463 increased serum levels of IFN and TNF *in vivo* in rats and levels of IFN, TNF-α, IL-1RA and IL-6 *in vivo* in cynomolgus monkeys (4, 11). Topical application of a gel formulation of S-28463 (0.01, 0.1 and 1.0% wt/vol) to hairless mice produced significant increases in IFN-α and IFN levels in the skin as compared to vehicle (3, 12).

In a guinea pig model of recurrent genital herpes simplex virus (HSV-2) infection, S-28463 (0.1 mg/kg s.c.) was administered either daily, every other day or weekly for 3 weeks. The number of lesions recurring during the treatment period was reduced by more than 80% using all three dosing regimens, with the lowest incidence of recurrence in the once-weekly treatment group. The number of lesion recurrences continued to be reduced significantly for up to 8 weeks after discontinuation of treatment (13).

The anti-HSV potential of S-28463 was further confirmed in another preclinical model in which the compound was administered as an adjuvant to an immunotherapeutic HSV gD DNA vaccine to latently infected guinea pigs. Soon after recovery from primary genital HSV-2 disease, animals were randomized to treatment with the gD vaccine or a saline control vaccine, followed by administration of S-28463 (0.03 mg/kg) or a saline adjuvant on days 7 and 28 postvaccination. Weekly lesion scores and mean weekly lesion days were decreased in all three active treatment groups as compared to the group receiving the saline vaccine plus saline adjuvant, but the difference was significant only in the group receiving gD vaccine plus S-28463. In this group, a significant decrease was also seen in the cumulative number of lesion days, with efficacy lasting through day 65 postvaccination (14, 15).

Pharmacokinetics and Metabolism

Oral administration of imiquimod has been a problem due to its extensive presystemic biotransformation; thus a study was conducted to determine the potential metabolic advantages of S-28463. [^{14}C]-Labeled compound (0.5 mg/kg) was administered to male CD rats intravenously, orally or topically (as a 0.25% gel) and animals were sacrificed at various time points in order to analyze concentrations of the study drug and of a putative metabolite, S-28371 [II], in tissues and serum. Following intravenous administration, distribution was extensive and both parent drug and carbon-14 were eliminated biexponentially. Serum concentrations of the metabolite, in contrast, declined in a monoexponential fashion. Absolute oral bioavailability of S-28463 was 90.4%, and serum drug levels again declined biexponentially. S-28463 was the predominant analyte in serum after both



[II]

Table I: Therapeutic strategies for the treatment and prevention of viral hepatitis (from Prous Science Ensemble database).

Hepatitis C			
Intron A	Recombinant IFN-α2b	Schering-Plough	L-1985
Infergen (consensus interferon)	Nonnaturally occurring, recombinant type 1 IFN	Amgen	L-1997
Rebetron	Recombinant IFN-α2b plus the antiviral nucleoside analog ribavirin	Schering-Plough	L-1998
Zadaxin (Thymosin α1)	Immune system enhancer	SciClone	L-1998
Alferon N Injection	Highly purified, natural source multispecies IFN-α	Interferon Sciences	NDA filed
PEG-Intron A	Polyethylene glycol-modified form of recombinant IFN-α2b	Schering-Plough/Enzon	Phase III
Pegasys	Pegylated 40K-IFN-α2	Roche	Phase III
VML-600	Immune response modifier	3M Pharm./Vanguard Medica	Phase II
VX-497	IMPDH inhibitor	Vertex	Phase II
Maxamine (histamine diHCl)	Histamine analog, immunoenhancing agent	Maxim	Phase I/II
rhIL-12	Recombinant human IL-12	Genetics Institute/Wyeth-Ayerst	Phase I/II
H-CIG	Hepatitis C immune globulin	Nabi	Phase I
AM-365	N/A	Amrad	Preclinical
GD-0039	Carbohydrate processing enzyme inhibitor	GlycoDesign	Preclinical
ISIS-14803	Antisense oligodeoxynucleotide	Isis	Preclinical
Nabi-Civacir	Human polyclonal antibody product	Nabi	Preclinical
Omniferon	Second-generation multispecies natural IFN-α	Viragen	Preclinical
VP-14637	Antiviral	ViroPharma	Preclinical
Heptazyme	Ribozyme selectively targeting HCV RNA	Ribozyme/Lilly	Research
	IFN-α2b gene therapy	Schering-Plough/ Immune Response	Research
Ro-32-6167	HCV NS3-4A protease inhibitor	Roche	Research
VRT-21493	HCV NS3 protease inhibitor	Vertex	Research
WO 9743310	HCV NS3 protease inhibitors	Schering-Plough	Patent literature
WO 9817679	HCV NS3 protease inhibitors	Vertex	Patent literature
WO 9822496	HCV protease inhibitors	Roche	Patent literature
	HCV protease inhibitors	AxyS/Bristol-Myers Squibb	Research
	HCV replication inhibitors	Merck & Co./Isis	Research
Hepatitis B			
Intron-A	Recombinant IFN-α2b	Schering-Plough	L-1985
Thymosin α1 (Zadaxin)	Immune system enhancer	SciClone	L-1996
Nabi-HB	Hepatitis B immune globulin (human), reformulated	Nabi	L-1999
Lamivudine	Nucleoside analog	BioChem Pharma/Glaxo Wellcome	L-1999
Adefovir dipivoxil	Nucleoside analog	Gilead	Phase III
Lobucavir	Nucleoside analog	Bristol-Myers Squibb	Phase III (suspended)
BMS-200475	Guanosine antiviral agent	Bristol-Myers Squibb	Phase II
Tuvirumab	Monoclonal anti-HBV antibody	Protein Design Labs	Phase II
Emtricitabine	Nucleoside analog	Triangle	Phase I/II
β-L-Fd4C	Nucleoside analog	Vion	Preclinical
Clevudine	Nucleoside analog	Triangle	Preclinical
DAPD	Nucleoside analog	Triangle	Preclinical
Nabi-3700.001	Nucleoside analog	Nabi	Preclinical
Robustaflavone	Naturally occurring biflavanoid	MediChem Res.	Preclinical
XTL-001	Fully human monoclonal antibody	XTL Biopharmaceuticals	Preclinical
	IFN-α2b gene therapy	Schering-Plough/Immune Response	Research
Hepatitis vaccines			
Engerix-B	Hepatitis B vaccine, recombinant	SmithKline Beecham	L-1987
Havrix	Hepatitis A vaccine, inactivated	SmithKline Beecham	L-1992
Vaqta	Hepatitis A vaccine, inactivated	Merck & Co.	L-1996
Twinrix	Combination hepatitis A/B vaccine	SmithKline Beecham	L-1997
Hepatyrix	Combination hepatitis A/thyroid vaccine	SmithKline Beecham	L-1999
Hepagene	Third-generation HBV vaccine	Medeva	NDA filed
Bio-Hep	Mammalian cell-derived recombinant HBV vaccine	Bio-Technology General	Phase III
CpG-7909	CgP DNA-based vaccine immune stimulant, adjuvant to hepatitis B vaccines	CpG Immunopharmaceuticals	Phase I
	Prophylactic HBV DNA vaccine	PowderJect/Glaxo Wellcome	Phase I
	HCV vaccine based on noninfectious HCV-like particles containing HCV structural proteins	NIDDK/NIH	Preclinical
	Therapeutic vaccine against HCV	Innogenetics	Preclinical
	Edible plant-based HBV vaccine	Axis Genetics	Research
	Hepatitis E vaccine	Genelabs/SmithKline Beecham	Research

oral and i.v. dosing, while approximately 40% of the serum total radiolabel was attributed to unknown metabolites. Radiolabel distributed rapidly to tissues following oral and i.v. dosing, reaching peak mean levels 1 h postdosing and declining promptly thereafter. The highest C_{max} values were obtained in cecum, kidneys, liver, small intestine, stomach and urinary bladder, while the highest mean percentages of administered dose were detected in gastrointestinal tract contents, kidneys, liver, skeletal muscle, small intestine, skin and stomach. After topical dosing, more than 83% of the dose was recovered in the skin wash. Topical absorption of the radiolabel from the gel formulation was approximately 8.5%. The most extensive urinary excretion following oral and i.v. dosing of S-28463 occurred in the first 12 h postdosing, while fecal excretion was nearly consistent over the first 24 h. After topical administration, excretion of radiolabel was minimal through 72 h postdosing. S-28463 was identified in radiomonitored LC and LC/MS analyses, which revealed *in vivo* biotransformation to S-28371. Excretion of unchanged drug and metabolite in the urine during the first 12 h was estimated to be 16% and 26%, respectively, following i.v. dosing; other radioactive fractions were minor. Thus it appears that the biotransformation profile of S-28463 is relatively simple, in contrast to that of the parent drug (16).

Clinical Studies

S-28463 was administered to healthy volunteers in a preliminary clinical trial. The cytokine-inducing agent was applied as a single topical dose to a 50-cm^2 area on the upper arm at various doses and concentrations (200 mg, 0.01% gel; 1 g, 0.01% gel; 1 g, 0.05% gel; or 1 g, 0.25% gel), and the systemic and cutaneous pharmacodynamics of the compound were evaluated. Results were also studied following multiple applications of S-28463 using various doses and concentrations (0.25% for 8 h, 2x weekly; 0.05% for 8 h, 2x weekly; 0.05% for 8 h, 3x weekly; 0.01% for 24 h, 3x weekly). Following a single application of the compound, serum interferon (IFN), 2',5'-oligoadenylate synthase (2',5'-AS), neopterin and IL-1RA increased only slightly, with no significant difference in mRNA response for IL-6, IFN-α or myeloperoxidase in skin biopsies as compared to baseline or to placebo. In the multiple-dose study, a significant dose-response relationship was observed for increase in serum IFN and neopterin, but not for 2',5'-AS or IL-1RA responses. Immunohistology showed that $CD3^+$ cells also increased in a dose-dependent fashion, consistent with T-lymphocyte infiltration, while $CD1a^+$ cells decreased, consistent with Langerhans cell emigration from treated skin. No clear dose-response relationship was established for IL-6, IL-8, IFN-α or myeloperoxidase responses in skin biopsies, although increases after the 0.25% dosing regimen were greater than with any other dose. Maximal change from baseline for all four mRNA markers, as well as maximal change for all four serum markers, correlated with the measurable amount of drug excreted in the urine following the last application of S-28463. These preliminary clinical findings indicate that S-28463 is a potent immunomodulator that significantly enhances the immune response of the skin (17).

The development of S-28463, which has been licensed to Vanguard Medica and subsequently renamed VML-600, is being targeted for the oral treatment of liver infection caused by hepatitis C virus. Phase II trials are scheduled to begin in the second half of 1999 (18, 19).

Manufacturer

3M Pharmaceuticals (US), licensed to Vanguard Medica Ltd. (GB).

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