Drugs of the Future 2003, 28(11): 1059-1063

CCC: 0377-8282/2003

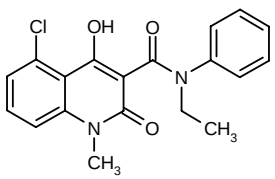
Laquinimod

Prop INN

Treatment of Multiple Sclerosis
Immunomodulator

ABR-215062
SAIK-MS Compound

5-Chloro-*N*-ethyl-4-hydroxy-1-methyl-2-oxo-*N*-phenyl-1,2-dihydroquinoline-3-carboxamide



$C_{19}H_{17}ClN_2O_3$
Mol wt: 356.8073
CAS: 248281-84-7
EN: 282472

Abstract

Multiple sclerosis (MS) is an inflammatory, demyelinating disorder of the central nervous system (CNS) characterized by focal leukocyte inflammation resulting in nerve cell dysfunction. The objectives of MS therapy have been the treatment of relapse, the prevention or modulation of relapse, the prevention of disease progression and accumulation of deficit, and the control of symptoms. The disease-modifying agents currently available include antiinflammatory, immunomodulating and immunosuppressive agents that must be administered as weekly injections. Although these agents appear to prevent or delay long-term disability and may alter the course of the disease, long-term disease-suppressing effects have not been proven. In addition, they are associated with inflammatory reactions at the injection site and influenza-like side effects. Thus, the search for orally active, safer, more effective agents continues. Suppression of the activity of T-cells, B-cells and macrophages which attack the myelin sheath is a focus for development of immunomodulatory agents for the treatment of MS, and researchers are searching for effective agents that lack the typical adverse events associated with immunomodulators. One novel chemical entity to emerge is laquinimod (ABR-215062), an orally active immunoregulator that has exhibited inhibitory activity against disease development and inflammatory cell infiltration in the CNS in rodent models of human demyelinating diseases. Moreover, laquinimod has a favorable safety profile and has shown efficacy in phase I and II trials in patients with MS. Laquinimod continues to undergo phase II development for the treatment of MS.

Synthesis

Reaction of 2-amino-6-chlorobenzoic acid (I) with phosgene and $NaHCO_3$ in dioxane gives 5-chloroisatoic anhydride (II), which is methylated by means of iodomethane and NaH in DMF to yield 5-chloro-1-methylisatoic anhydride (III). Finally, anhydride (III) is condensed with the malonic monoamide (IV) by means of NaH in hot dimethylacetamide (1). Scheme 1.

Alternatively, condensation of anhydride (III) with ethoxy malonyl chloride (V) by means of NaOMe and triethylamine in dichloromethane affords 5-chloro-4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acid ethyl ester (VI), which is finally condensed with *N*-ethylaniline (VII) in refluxing toluene (1). Scheme 1.

Alternatively, ester (VI) is hydrolyzed by means of concentrated HCl in hot Ac_2O to give the carboxylic acid (VIII), which is finally condensed with *N*-ethylaniline (VII) by means of $SOCl_2$ and TEA in dichloromethane (1). Scheme 1.

Introduction

Multiple sclerosis (MS) is an inflammatory, demyelinating disorder of the central nervous system (CNS) that is characterized by focal leukocyte inflammation resulting in nerve cell dysfunction, muscle weakness and visual disturbances, among other neurological impairments (*e.g.*, mild cognitive impairment, tremors, dizziness, hearing loss, spasticity, fatigue, pain and loss of touch, temperature and/or pain sensations). The inflammation occurs in random areas of white matter of the CNS and the resulting demyelination slows or completely blocks neuronal transmission. Hardened, sclerotic patches of scar tissue or plaques replace the myelin, and occasionally the nerve fiber itself is damaged (2).

An estimated 2.5 million individuals suffer from MS worldwide. The disorder is more frequently diagnosed in

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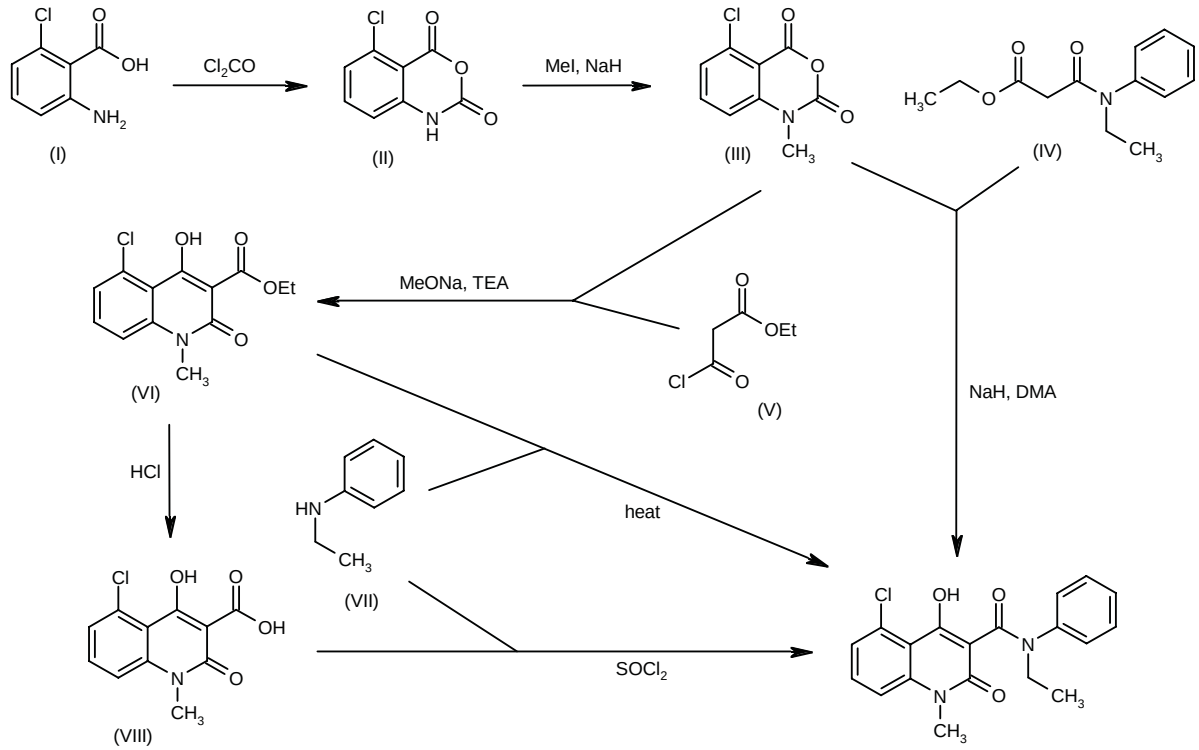
Scheme 1: Synthesis of Laquinimod

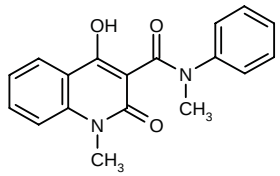


young adults and occurs twice as often in women as in men (2-4). There are several subclasses of MS. Relapsing-remitting MS (RRMS) is the most frequent form at first diagnosis and is characterized by unpredictable relapses or attacks where new symptoms appear or existing symptoms become more severe. Secondary progressive MS accounts for 40% of all cases and occurs in patients initially diagnosed with RRMS who later experience progressive disability, often with superimposed relapses. Primary progressive MS accounts for about 10% of all cases and occurs more frequently in men than in women. It is characterized by a lack of distinct attacks so that the individual experiences a slow onset and steady worsening of symptoms without remission. Benign MS occurs in about 10% of all diagnosed patients and is characterized by 1 or 2 attacks followed by complete recovery. Finally, there is an extremely rare subtype of MS known as progressive relapsing MS, which is progressive from onset and is characterized by obvious acute attacks (2-4).

The etiology of MS is unknown. The destruction to myelin characteristic of the disease appears to result from an abnormal immune response which could be triggered by a dormant virus or other pathogen. Human herpesvirus type 6 (HHV-6), Epstein-Barr virus (EBV) and Chlamydia pneumoniae have been frequently associated with MS, although other viruses and pathogens may also be responsible for development of the disorder. Environmental factors have also been associated with an increased risk of MS since higher incidences are reported in more temperate climates. Moreover, genetic susceptibility, possibly due to mutations of several interacting genes (*e.g.*, ApoE-ε4, HLA-DR2 and HLA-DR53 alleles), may also be involved in the pathology of MS (2, 4-9).

The objectives of MS therapy have been the treatment of relapse, the prevention or modulation of relapse, the prevention of disease progression and accumulation of deficit, and the control of symptoms. To date, the disease-modifying agents available, *i.e.*, antiinflammatory, immunomodulating and immunosuppressive agents, include recombinant interferon (IFN) β_{1b} and β_{1a}, glatiramer acetate (Copolymer-1) and mitoxantrone hydrochloride. These agents are administered as frequent injections to prevent or delay long-term disability and they may alter the course of the disease. However, long-term disease-suppressing effects have not been proven, and treatment is associated with inflammatory reactions at the injection site and influenza-like side effects. Thus, the search for safer, more effective, orally active agents continues (2, 10, 11).

Immunomodulation is one of the most attractive approaches for the treatment of MS. Suppression of the activity of T-cells, B-cells and macrophages which attack



[I]

the myelin sheath is a focus for development of immunomodulatory agents for the treatment of MS. Roquinimex (Linomide®) [I] is an orally active immunomodulator that was effective in inhibiting MS in clinical trials. However, phase III trials were terminated due to the considerable incidence and intolerability of adverse events (12-14). Researchers have therefore focused on developing more effective agents that lack the typical adverse events associated with immunomodulators, such as fever, muscle and joint pain and stiffness.

One such novel chemical entity to emerge is laquinimod (ABR-215062), an orally active immunoregulator that resulted from chemical modifications made to the roquinimex structure, including introduction of substituents to the quinolone ring together with chain elongation of the amidic methyl group. Laquinimod is chemically distinct from roquinimex and has shown inhibitory activity against disease development and inflammatory cell infiltration in the CNS in rodent models of human demyelinating diseases. Moreover, the agent was more potent than roquinimex and showed a good safety profile in dog models. Laquinimod was chosen for further development as a treatment for MS (15, 16).

Pharmacological Actions

Laquinimod completely inhibited the development of murine acute experimental autoimmune encephalomyelitis (EAE), an *in vivo* model of human demyelinating disease (*i.e.*, MS). Laquinimod (0.04, 0.2, 1 and 5 mg/kg/day p.o. on days 3-7 and 10-12 postimmunization) dose-dependently inhibited the development of acute EAE in SJN/N mice immunized with C57BL/6 mouse spinal cord homogenate and injected with pertussis toxin (PTX) to induce acute EAE, with complete protection seen at the highest dose. Analysis of brain tissue from laquinimod-treated animals revealed that treatment with the agent at doses of 1 and 10 mg/kg p.o. significantly reduced and abolished, respectively, leukocyte ($CD45^+$ cells) inffiltration into the CNS. Roquinimex (0.2, 1, 5 and 25 mg/kg/day) also inhibited the development of acute EAE, although it was approximately 20 times less potent than laquinimod. Both agents inhibited the reduction in body weight observed with acute EAE induction. Moreover, laquinimod was shown to significantly inhibit the development of acute EAE in both IFN-β knockout and wild-type C57BL/6 mice, indicating that the mechanism of action of the agent is independent of IFN-β expression (15, 17).

Laquinimod was also shown to inhibit relapse in a chronic relapsing murine EAE model (B10.RIII mice immunized with MBP89-101 peptide and *Mycobacterium tuberculosis* in complete Freund's adjuvant and injected with PTX), indicating that the agent can be effective against established disease. Laquinimod (5 mg/kg p.o. on the first day of observed clinical manifestations) markedly decreased the number of relapses (0.2 *vs.* 1.1 in controls). In addition, treatment with the agent decreased the number of days with severe disease (clinical score of 4 or more: 5.8 *vs.* 15.9 days in controls) (15).

The efficacy of laquinimod in inhibiting inflammatory cell migration into peripheral nerve tissue was demonstrated in an *in vivo* study using a rat model of experimental autoimmune neuritis (EAN) induced by immunization with peripheral nerve P0 protein peptide 180-199 and Freund's adjuvant, a $CD4^+$ T-cell-mediated model of the human demyelinating disease Guillain-Barré syndrome. Treatment with laquinimod (1.6 and 16 mg/kg/day s.c. starting on the day of immunization and continuing until day 35) significantly and dose-dependently delayed the onset of clinical EAN by 2-8 days and decreased its severity as compared to controls. Treatment with the agent inhibited P0 protein peptide 180-199-specific T-cell responses and reduced inflammation and demyelination in peripheral nerves. Suppression of EAN in laquinimod-treated animals was associated with dose-dependent reductions in the inflammatory cytokines IFN-γ and TNF-α in lymph nodes, and increases in the antiinflammatory cytokine IL-4 in peripheral nerve tissue. From these results, it was suggested that the efficacy of laquinimod may be due to regulation of the Th1/Th2 cytokine balance (16).

Pharmacokinetics

A coupled-column HPLC method was developed and described for the determination of laquinimod in human and animal plasma samples. The calibration graph was linear over the range of 0.75-967 μmol/l. The method was validated using laquinimod-spiked human plasma and the limit of quantitation, intermediate precision and accuracy obtained were 0.75 μmol/l, 1.8-3.6% (C.V.) and 97.7-114.7%, respectively (18).

The pharmacokinetics of laquinimod and roquinimex (5 mg/kg p.o.) were compared in SJL/N mice. Similar values for total exposure were obtained for the agents. Plasma C_{max} and AUC values were 26 μmol/l and 152 μmol·h/l, respectively, for laquinimod and 30 μmol/l and 125 μmol·h/l, respectively, for roquinimex. However, binding to plasma proteins differed for the agents. The free fraction of laquinimod and roquinimex in mouse plasma was 2.2 and 21.5%, respectively, and when the C_{max} and AUC were calculated for the unbound agents, the AUC value for roquinimex was 8 times higher as compared to laquinimod (15).

A study compared the systemic exposure to free laquinimod-inducing inflammatory side effects to the systemic exposure to free laquinimod-inducing therapeutic effects obtained from humans and animals models. Analysis of results predicted a therapeutic window for laquinimod that was improved as compared to roquinimex. A pharmacokinetic study in beagle dogs reported that laquinimod was well tolerated at doses up to 1.2 mg/kg/day, with little induction of inflammatory side effects. The AUC value for the unbound drug (AUC_{unb}) at a dose of 1.2 mg/kg/day was 2.9 μmol·h/l; in humans, a similar AUC_{unb} was obtained with a dose of 5 mg/day. In a study examining the pharmacokinetics of laquinimod in mice with acute EAE, doses of 0.1-1 mg/kg/day resulted in AUC_{unb} values of 0.06-0.6 μmol·h/l; a similar AUC_{unb} was obtained in humans for doses of 0.1-1 mg/day. In human studies, inflammatory side effects were observed at doses of 2.4-2.5 mg/day, suggesting that humans are more sensitive than predicted from animals models. From analysis of these results, a therapeutic window for laquinimod in humans was predicted with a 102 span between therapeutic and side effect dose-response curves. It was predicted that initial therapeutic efficacy could be expected at a dose of 0.03 mg/day in patients with MS, with maximum therapeutic activity occurring at about 0.3 mg/day (15, 16, 19, 21).

The safety, tolerability and pharmacokinetics of laquinimod (50 μg in an aqueous solution) were examined in a study in 3 healthy male volunteers. Laquinimod was safe and well tolerated. A compartmental model was fitted to the plasma concentration-over-time data. The expected plasma concentrations-over-time profiles for various dosing regimens could be predicted from this model (20, 21).

Clinical Studies

A multiple-dose, dose-escalation phase I trial was conducted in both healthy volunteers and patients with MS to determine the safety, tolerability and maximum tolerated dose (MTD) of laquinimod (0.05-2.4 mg/day p.o.). In the first part of the study, healthy volunteers received a loading dose (0.05-2.4 mg/day p.o.) to achieve steady-state plasma levels, while in the second part, patients with MS were treated daily for up to 4 weeks. Laquinimod was concluded to have a favorable safety and tolerability profile. In patients, dose-limiting inflammatory activation was observed with a dose of 2.4 mg/day and the MTD was concluded to be 1.2 mg/day (21).

The safety, tolerability and efficacy of laquinimod (0.1 or 0.3 mg/day in tablet form for 6 months) were examined in a multicenter, randomized, placebo-controlled, parallel-group phase II trial that included a 2-month posttreatment follow-up period and involved more than 200 patients with MS. Primary endpoints included assessment of active inflammation of the brain using MRI and determination of the number of flare-ups and the time to the first flare-up. Secondary endpoints were related to clinical outcome and included the number of exacerbations and use of quantitative assessment scales (EDSS and MSFC) to evaluate the level of patient disability. Preliminary analysis of results showed that laquinimod had a favorable safety profile. Patients treated with the highest dose had a significant 30% reduction in MRI disease activity as compared to placebo; patients treated with the lower dose also had decreases in disease activity, although the results did not reach significance as compared to placebo. In addition, only about 25% of the patients receiving laquinimod had an exacerbation during the treatment period, with EDSS scores remaining stable. Analysis of the results from this trial continues (22).

Laquinimod is currently undergoing phase II development for the treatment of MS.

Source

Active Biotech AB (SE).

References

1. Björk, A., Jönsson, S., Fex, T., Hedlund, G. (Active Biotech AB). *Quinoline derivs.* EP 1073639, JP 2002513006, US 6077851, WO 9955678.

2. Prous Science Drug R&D Backgrounders: *Multiple sclerosis (online publication).* Updated November 11, 2003.

3. Noonan, C.W., Kathman, S.J., White, M.C. *Prevalence estimates for MS in the United States and evidence of an increasing trend for women.* Neurology 2002, 58: 136-8.

4. Noseworthy, J.H., Lucchinetti, C., Rodriguez, M., Weinshenker, B.G. *Multiple sclerosis.* New Engl J Med 2000, 343: 938-52.

5. Álvarez-Lafuente, R., Martín-Estefanía, C., de las Heras, V., Castrillo, C., Picazo, J.J., Varela de Seijas, E., Arroyo González, R. *Active human herpesvirus 6 infection in patients with multiple sclerosis.* Arch Neurol 2002, 59: 929-33.

6. Ascherio, A., Munger, K.L., Lennette, E.T., Spiegelman, D., Hernán, M.A., Olek, M.J., Hankinson, S.E., Hunter, D.J. *Epstein-Barr virus antibodies and risk of multiple sclerosis: A prospective study.* JAMA - J Am Med Assoc 2001, 286: 3083-8.

7. Levin, L.I., Munger, K.L., Rubertone, M.V., Peck, C.A., Lennette, E.T., Spiegelman, D., Ascherio, A. *Multiple sclerosis and Epstein-Barr virus.* JAMA - J Am Med Assoc 2003, 289: 1533-6.

8. Chapman, J., Vinokurov, S., Achiron, A., Karussis, D.M., Mitosek Szewczyk, K., Birnbaum, M., Michaelson, D.M., Korczyn, A.D. *APOE genotype is a major predictor of long-term progression of disability in MS.* Neurology 2001, 56: 312-6.

9. Luomala, M., Elovaara, I., Ukkonen, M., Koivula, T., Lehtimäki, T. *The combination of HLA-DR1 and HLA-DR53 protects against MS.* Neurology 2001, 56: 383-5.

10. Goodin, D.S., Frohman, E.M., Garmany, G.P. Jr., Halper, J., Likosky, W.H., Lublin, F.D., Silberberg, D.H., Stuart, W.H., van den Noort, S. *Disease modifying therapies in multiple sclerosis. Report of the Therapeutics and Technology Assessment*

Subcommittee of the American Academy of Neurology and the MS Council for Clinical Practice Guidelines. Neurology 2002, 58: 169-78.

11. Rudick, R.A. *Disease-modifying drugs for relapsing-remitting multiple sclerosis and future directions for multiple sclerosis therapeutics.* Arch Neurol 1999, 56: 1079-84.

12. Noseworthy, J.H., Wolinsky, J.S., Lublin, F.D. et al. *Linomide in relapsing and secondary progressive MS. Part I: Trial design and clinical results.* Neurology 2000, 54: 1726-33.

13. Wolinsky, J.S., Narayana, P.A., Noseworthy, J.H. et al. *Linomide in relapsing and secondary progressive MS. Part II: MRI results.* Neurology 2000, 54: 1734-41.

14. Tan, I.L., Lycklama à Nijeholt, G.J., Polman, C.H., Adèr, H.J., Barkhof, F. *Linomide in the treatment of multiple sclerosis: MRI results from prematurely terminated phase-III trials.* Multiple Scler 2000, 6: 99-104.

15. Brunmark, C., Runström, A., Ohlsson, L., Sparre, B., Brodin, T., Aström, M., Hedlund, G. *The new orally active immunoregulator laquinimod (ABR-215062) effectively inhibits development and relapses of experimental autoimmune encephomyelitis.* J Neuroimmunol 2002, 130: 163-72.

16. Zou, L.-P., Abbas, N., Volkmann, I., Nennesmo, I., Levi, M., Wahren, B., Winblad, B., Hedlund, G., Zhu, J. *Suppression of experimental autoimmune neuritis by ABR-215062 is associated with altered Th1/Th2 balance and inhibited migration of inflammatory cells into the peripheral nerve tissue.* Neuropharmacology 2002, 42: 731-9.

17. Runstöm, A., Axelsson, B., Hedlund, G.T., Nederman, T., Leandersson, T. *Laquinimod (ABR-215062) a candidate drug for treatment of multiple sclerosis inhibits the development of experimental autoimmune encephalomyelitis in IFN-β know-out mice.* Medicon Valley BioConf (Oct 8-10, Malmo) 2002, Abst.

18. Edman, K., Eriksson, B., Svensson, L., Gunnarsson, P.O. *Determination of laquinimod in plasma by coupled-column liquid chromatography with ultraviolet absorbance detection.* J Chromatogr B Anal Technol Biomed Life Sci 2003, 785: 311-6.

19. Nederman, T., Runström, A., Cort, N., Sparre, B. *Laquinimod, a candidate drug for oral treatment of MS, with improved therapeutic window, compared to the predecessor roquinimex, predicted for man.* Medicon Valley BioConf (Oct 8-10, Malmo) 2002, Abst.

20. Sparre, B., Ellman, M., Nordle, Ö., Olin, M., Höglund, P., Gunnarsson, P.O. *Pharmacokinetically guided design of a repeat dose safety and tolerance study of laquinimod in healthy volunteers.* Medicon Valley BioConf (Oct 8-10, Malmo) 2002, Abst.

21. Nederman, T., Runström, A., Sparre, B., Ellman, M., Nordle, O., Höglund, P., Sandberg, M., Leanderson, T. *Laquinimod - A drug candidate for oral treatment of MS.* Medicon Valley BioConf (Oct 8-10, Malmo) 2002, Abst.

22. *Phase II laquinimod study for MS meets primary endpoint according to first analysis.* DailyDrugNews.com (Daily Essentials) September 19, 2003.

Additional References

Runström, A., Brunmark, C., Ohlsson, L., Sparre, B., Hedlund, G. *The new orally active immunoregulator ABR-215062 effectively inhibits development and relapses of experimental autoimmune encephalomyelitis.* Benzon Symp (Aug 18-22, Conpenhagen) 2002, Abst IV-12.

Runström, A., Brunmark, C., Ohlsson, L., Sparre, B., Axelsson, B., Hedlund, G. *The new orally active immunoregulator laquinimod (ABR-215062) effectively inhibits development and relapses of experimental autoimmune encephalomyelitis.* Medicon Valley BioConf (Oct 8-10, Malmo) 2002, Abst.