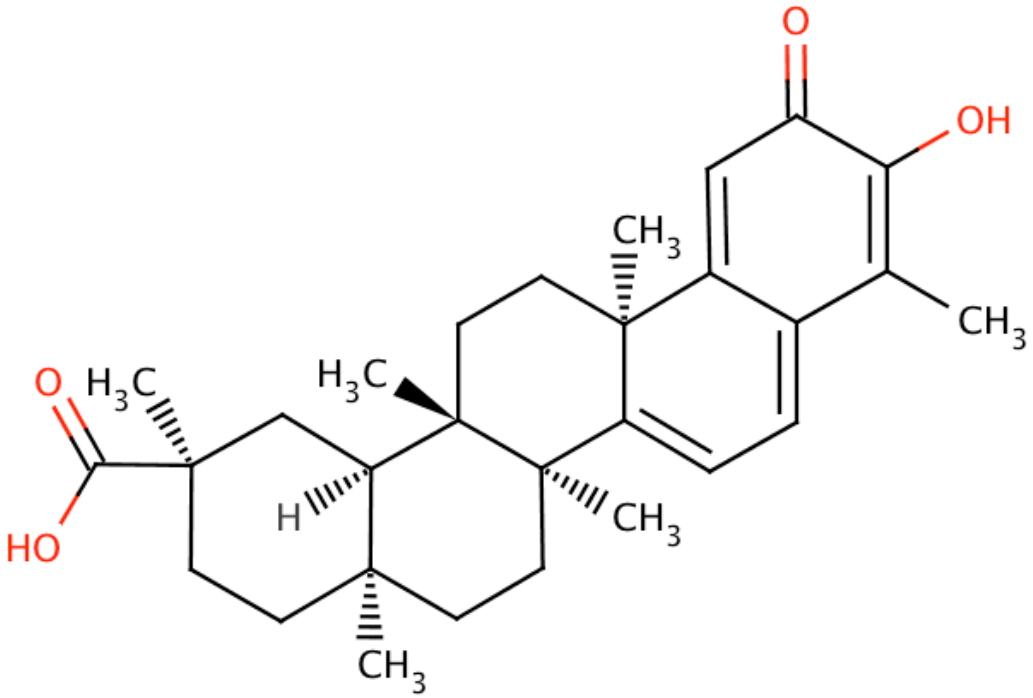
CINAPS Compound Dossier

Celastrol



5/6/2008

National Institute of Neurological Disorders and Stroke

Table of Contents

I. Compound information 1

II. Rationale 3

Scientific Rationale / Mechanism

Consistency

III. Efficacy (animal models of Parkinson's disease) 5

Animal Models: Rodent

Animal Models: Non-human primates

IV. Efficacy (clinical and epidemiological evidence) 6

Clinical Studies

. Epidemiological Evidence

V. Relevance to Other Neurodegenerative Diseases 7

VI. Pharmacokinetics 8

General ADME

CNS Penetration

Calculated logBB

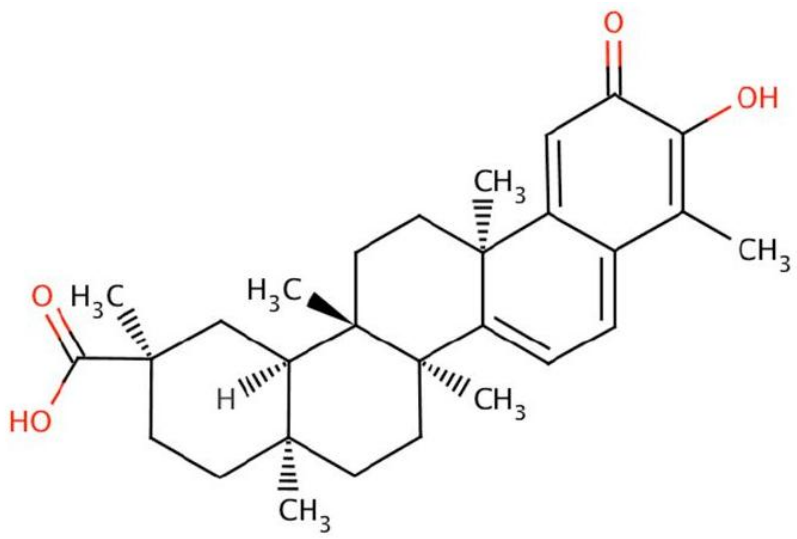
VII. Safety, Tolerability, and Drug Interation Potential 9

Safety and Tolerability

Drug Interaction Potential

VIII. Bibliography 10

I. Compound Information

Common name	Celastrol
Structure	

PubChem ID	122724	**MF**	C29 H38 O4	**MW**	450.61
CASRN	34157-83-0	**Polar surface area**	74.60	**logP**	5.41

IUPAC name	(2*S*,4a*S*,6a*S*,12b*R*,14a*S*,14b*R*)-10-Hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydropicene-2-carboxylic acid
Other names	tripterine; 3-hydroxy-24-*nor*-2-oxo-1(10),3,5,7-friedelatetraen-29-oic acid
Drug class	Natural product; Antioxidant

Notes

Celastrol is a pentacyclic triterpenoid compound extracted from the Chinese Thunder of God vine, *Tripterygium wilfordii*, Hook f. The plant is a perennial twining vine in the family of Celestraceae and is similar in appearance and habit as the native North American bittersweet, *Celastrus scandens* L. Known in Chinese as *lei kung teng* or *lei gong teng*, Thunder of God vine was introduced into the U.S. in 1935 to investigate the traditional Chinese use of the powdered root as an agricultural insecticide, particularly against insects that feed on the leaves of numerous vegetables (**Swingle, 1941**).

Celastrol is one of two compounds isolated from Thunder of God vine that exhibit *in vitro* and *in vivo* biological activity. The other compound, triptolide, is a structurally unrelated diterpenoid epoxide with diverse anti-inflammatory and anticancer activities. While triptolide has been synthesized by several routes, there are no reports on the synthesis of celastrol, requiring that the compound be isolated from its natural source for subsequent studies (**Corson, 2007**). Differentiation between these two compounds is relevant because the whole *T. wilfordii* extract is undergoing clinical

trials for rheumatoid arthritis, sponsored by the National Institute of Arthritis and Musculoskeletal Diseases. Therefore, reports on the biological action of celastrol must be parsed carefully to assure that the pure compound is being investigated and not the combination of celastrol and triptolide as present in *T. wilfordii* extracts.

Development status No development reported. (**ADIS R&D Insight, 7128**)

II. Rationale

IIa. Scientific Rationale / Mechanism

The rationale for investigating celastrol in the prevention of Parkinson's disease derives largely from the investigation of the pure compound and *T. wilfordii* extracts as anti-inflammatory agents (**Allison, 2001**; **Pinna, 2004**). Efficacy in rheumatoid arthritis was first confirmed in a controlled, double-blind trial in China reported in 1987 using an ethanolic extract of *T. wilfordii* known as T2, but the extract appeared to have been optimized for content of polyglycosides and not celastrol or triptolide (**Tao, 1987**). The antioxidant and anti-inflammatory activities of celastrol specifically have led to investigation of its efficacy against another Alzheimer's disease, another progressive neurodegenerative disorder.

The potential for celastrol in the prevention of Parkinson's disease is implied by its antioxidant (**Sassa, 1990**; **Sassa, 1994**) and anti-inflammatory (**Allison, 2001**; **Pinna, 2004**) activities as well as its potent activity in inducing the heat-shock response *in vitro* (**Westerheide, 2004**). In addition, one study in mice demonstrated that celastrol could prevent both MPTP dopaminergic neurotoxicity and striatal lesions induced by 3-nitropropionic acid (**Cleren, 2005**).

The antioxidant activity of celastrol has been demonstrated by investigating lipid peroxidation in a rat liver mitochondria assay initiated by ADP and divalent iron. The IC50 value for celastrol was 7 µM, or 15 times less than the positive control, tocopherol (**Sassa, 1990**). Analog studies revealed that the antioxidant activity was due largely to the combination of the dienonephenol moiety and the anionic carboxyl group (**Sassa, 1994**). The phenol scavenges radicals, preventing the production of reactive aldehydes such as 4-hydroxy-2-nonenal, and the carboxyl prevents the attack of oxygen radicals on the inner mitochondrial membrane by increasing its negative surface charge.

Celastrol has been shown to act as anti-inflammatory agent by inhibiting *E. coli* lipopoysaccharide-induced secretion of TNF-α and IL-1β from human monocytes and human lung macrophages by preventing activation of the transcription factors NF-κB and AP-1 (**Allison, 2001**). This represents one of the most potent biological effects of celastrol: the IC50 values for inhibition of TNF- α and IL-1β secretion were 70 nM and 30 nM, respectively, and 50-100 nM in macrophages (**Allison, 2001**). Similar to these results, celastrol also inhibited secretion of TNF-α, IL-1β, IL-6, and IL-8 from intestinal tissue biopsied from patients with Crohn's disease (**Pinna, 2004**). However, in the specimens from this chronic intestinal inflammatory disease, concentrations of 1 µM were required to achieve at least a 50% reduction in each of the four cytokines.

Celastrol also inhibits the production of NO induced in macrophages and endothelial cells, minimizing the denaturing of proteins due to conversion of tyrosine residues to nitrotyrosine (**Allison, 2001**).

In support of a role in cytoprotection against misfolded proteins, celastrol emerged from a screen of FDA-approved and other biologically-active compounds for stimulating the activity of the hsp70 heat shock protein gene promoter (**Westerheide, 2004**). The effects of celastrol were not cell-type dependent as 10- to 35-fold activation was observed in HeLa, MCF-7, BT474, H157, and SH-SY5Y human carcinoma cell lines at 3 µM. The kinetics and dose-response for hsp70 promoter activation by celastrol was remarkable for its similar to that of elevated temperature, as hsp70 induction in HeLa cells was first observed at 2 µM, peaked at 7 µM, then fell to near-control levels at higher concentrations. Hsp70 promoter activity was functionally significant in that celastrol pretreatment of HeLa and SH-SY5Y cells conferred cytoprotection against a 20 or 40 min heat stress at 45 °C. The magnitude of cytoprotection against this protein-unfolding stress was similar to the well-documented protective effect of previous exposure to a 1 hr heat-shock at 42 °C.

IIb. Consistency

Studies with celastrol as a pure compound are largely consistent with regard to potency against several biological endpoints. The compound uniformly exhibits half-maximal potencies against several *in vitro* targets within the range of 0.1 to 3 µM. Again, caution must be stressed with regard to interpretation of any study employing crude *T. wilfordii* extracts as celastrol is only one of two or more biologically-active constituents.

III. Efficacy (animal models of Parkinson's disease)

IIIa. Animal Models: Rodent

To date there has been only one published study on the efficacy of celastrol in protecting rodents from chemically-induced parkinsonism. Cleren et al. (**Cleren, 2005**) exposed 12-week old, male Swiss-Webster mice (8-12 per group) to 15 mg/kg MPTP, i.p., q 2h x 4. Animals were pretreated with DMSO or celastrol (3 mg/kg, i.p.) at 12 hr prior to the first MPTP injection, then again at 12 hr following the first MPTP injection. Neurochemical parameters were measured 7 days later.

MPTP alone decreased tyrosine hydroxylase-expressing neurons in the substantia nigra pars compacta by 48% and striatal dopamine concentrations by 37%. Celastrol attenuated both of these deficits, with reductions of only 10% in TH-expressing neurons and 18% in striatal dopamine. Celastrol treatment was also associated with an induction of hsp70 and nearly a two-fold increase in its translocation to the nucleus of dopaminergic neurons. The pro-inflammatory effect of MPTP, as measured by TNF-α and NF-κB immunoreactivity, were qualitatively attenuated by celastrol relative to DMSO control (i.e., the report did not indicate the quantitative change in immunocytochemical staining across the treatment groups.).

IIIb. Animal Models: Non-human primates

No studies are available

IV. Efficacy (Clinical and Epidemiological Evidence)

IVa. Clinical studies

Celastrol has not yet been tested in clinical trials as a pure compound. The ethanolic T2 extract of *T. wilfordii* has been investigated for rheumatoid arthritis (**Tao, 1987**) and a trial of a similar extract is ongoing at the National Institute of Arthritis and Musculoskeletal Diseases. Any extrapolation of results from these studies as to the effectiveness of celastrol itself would be premature, thereby justifying its clinical investigation as a pure compound.

IVb. Epidemiological evidence

Extracts of *T. wilfordii* have been used in traditional Chinese medicine for hundreds if not thousands of years to treat fever, chills, joint pain, and edema. Our search did not identify any systematic retrospective studies which investigated a link between use of this traditional medicine and the incidence of any neurodegenerative disorder.

V. Relevance to other neurodegenerative diseases

The antioxidant, anti-inflammatory activities combined with the potential to trigger the heat shock response are all consistent with activity against Huntington's disease and Alzheimer's disease. Protection by celastrol of 3-nitropropionic-acid-induced degeneration of striatal medium-sized spiny GABAergic neurons in mice is consistent with activity against Huntington's disease (**Cleren, 2005**).

Similarly, the ability of celastrol to improve motor coordination (rota-rod), learning and memory (passive avoidance foot-shock) in normal, female Sprague-Dawley rats has been taken as support for investigating celastrol in Alzheimer's disease. Remarkably, these latter effects were observed with a single celastrol dose as low as 7 µg/kg, i.p. (**Allison, 2001**).

VI. Pharmacokinetics

VIa. General ADME

No systematic studies are available regarding celastrol bioavailability *in vivo* or metabolism *in vitro* or *in vivo*. The oral activity of celastrol in rodents would imply that the compound and/or its active metabolites are sufficiently bioavailable. The oral activity of T2 *T. wilfordii* extract in human subjects with rheumatoid arthritis does not necessarily imply celastrol bioavailability as other compounds in the extract (e.g., triptolide or polyglycosides) may be responsible for the clinical efficacy reported.

The phenolic hydroxyl group on celastrol would be an attractive site for glucuronidation and drug inactivation. The existence or kinetics of such activity have not yet been investigated.

VIb. CNS Penetration

The *in vivo* efficacy of celastrol against MPTP- and 3-nitropropionic acid- induced neurotoxicity implies that celastrol or its metabolite(s) achieve CNS concentrations consistent with those observed to have in vitro effects (**Cleren, 2005**). However, there has been no systematic study of celastrol concentrations in CNS tissue or cerebrospinal fluid.

VIc. Calculated logBB (Clark Model) -0.14

VII. Safety, Tolerability, and Drug Interaction Potential

VIIa. Safety and Tolerability

The current body of *in vivo* literature is suggestive that celastrol has an excellent safety profile. Celastrol is well-tolerated in rodents (oral and intraperitoneal) and human subjects (oral). Mice (male immunodeficient NCRNU-M mice) given celastrol at 1-3 mg/kg, i.p., for up to 31 days in a prostate cancer xenograft study exhibited no untoward effects (**Yang, 2006**).

A significant consideration of celastrol safety comes from one in vitro report that the compound may prolong the QT interval. In patients predisposed to cardiac arrhythmias, prolongation of QT interval can be symptomatic and, in rare cases, fatal. The studies with celastrol that raise these concerns derive from *in vitro* work with non-cardiac cells (HEK-293 human embryonic kidney and SH-SY5Y human neuroblastoma) overexpressing cardiac potassium channels (Kir2.1 and hERG) and patch-clamp studies with cells that are models for, but unrelated to, cardiac tissues. However, the potential gravity of such an effect suggests that it should be monitored carefully in any *in vivo* study (**Sun, 2006**).

Another theoretical safety concern relates to the presence of a quinone methide within the celastrol structure. Reactive quinone methides can alkylate DNA and have the potential for initiating carcinogenesis. However, there are no reports to date on the mutagenic or carcinogenic potential of celastrol.

VIIb. Drug Interaction Potential

The phase I and phase II metabolic profile for celastrol has not been elucidated in any mammalian species, *in vitro* or *in vivo*. Hence, pharmacokinetic interactions of celastrol with other pharmaceuticals is unknown at this time.

As a result of the numerous biological effects mediated by celastrol but no systematic studies of this agent with other drugs, pharmacodynamic interactions are unknown but should be studied as the compound progresses through preclinical and clinical studies.

VIII. Bibliography

ADIS, 7128	ADIS R&D Insight Database, Accession number 7128.
Allison, 2001	Allison A.C.; Cacabelos R.; Lombardi V.R.; Alvarez X.A.; Vigo C., *Celastrol, a potent antioxidant and anti-inflammatory drug, as a possible treatment for Alzheimer's disease*, Prog Neuropsychopharmacol Biol Psychiatry, **2001**, 25(7), 1341-57.
Cleren, 2005	Cleren C.; Calingasan N.Y.; Chen J.; Beal M.F., *Celastrol protects against MPTP- and 3-nitropropionic acid-induced neurotoxicity*, J Neurochem, **2005**, 94(4), 995 -1004.
Corson, 2007	Corson T.W.; Crews C.M., *Molecular understanding and modern application of traditional medicines: triumphs and trials*, Cell, **2007**, 130(5), 769-74.
Pinna, 2004	Pinna G.F.; Fiorucci M.; Reimund J.M.; Taquet N.; Arondel Y.; Muller C.D., *Celastrol inhibits pro-inflammatory cytokine secretion in Crohn's disease biopsies*, Biochem Biophys Res Commun, **2004**, 322(3), 778-86.
Sassa, 1990	Sassa H.; Takaishi Y.; Terada H., *The triterpene celastrol as a very potent inhibitor of lipid peroxidation in mitochondria*, Biochem Biophys Res Commun, **1990**, 172(2), 890-7.
Sassa, 1994	Sassa H.; Kogure K.; Takaishi Y.; Terada H., *Structural basis of potent antiperoxidation activity of the triterpene celastrol in mitochondria: effect of negative membrane surface charge on lipid peroxidation*, Free Radic Biol Med, **1994**, 17(3), 201-7.
Sun, 2006	Sun H.; Liu X.; Xiong Q.; Shikano S.; Li M., *Chronic inhibition of cardiac Kir2.1 and HERG potassium channels by celastrol with dual effects on both ion conductivity and protein trafficking*, J Biol Chem, **2006**, 281(9), 5877-84.
Swingle, 1941	Swingle W.T.; Haller H.L.; Siegler E.H.; Swingle M.C., *A Chinese Insecticidal Plant, Tripterygium Wilfordii, Introduced into the United States*, Science, **1941**, 93 (2403), 60-61.

Tao, 1987	Tao X.L.; Dong Y.; Zhang N.Z., *[A double-blind study of T2 (tablets of polyglycosides of Tripterygium wilfordii hook) in the treatment of rheumatoid arthritis]*, Zhonghua Nei Ke Za Zhi, **1987**, 26(7), 399-402, 444-5.
Westerheide, 2004	Westerheide S.D.; Bosman J.D.; Mbadugha B.N.; Kawahara T.L.; Matsumoto G.; Kim S.; Gu W.; Devlin J.P.; Silverman R.B.; Morimoto R.I., *Celastrols as inducers of the heat shock response and cytoprotection*, J Biol Chem, **2004**, 279(53), 56053 -60.
Yang, 2006	Yang H.; Chen D.; Cui Q.C.; Yuan X.; Dou Q.P., *Celastrol, a triterpene extracted from the Chinese "Thunder of God Vine," is a potent proteasome inhibitor and suppresses human prostate cancer growth in nude mice*, Cancer Res, **2006**, 66(9), 4758-65.