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Incyclinide

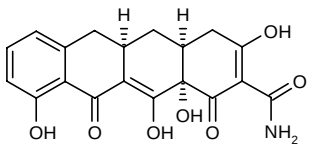
Rec INN; USAN

Dual MMP-2/MMP-9 Inhibitor
Treatment of Acne
Treatment of Rosacea

CMT-3
COL-3
NSC-683551

(4a*S*,5a*R*,12a*S*)-3,10,12,12a-Tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydronaphthacene-2-carboxamide

InChI=1/C19H17NO7/c20-18(26)14-11(22)6-9-5-8-4-7-2-1-3-10(21)12(7)15(23)13(8)16(24)19(9,27)17(14)25/h1-3,8-9,21-22,24,27H,4-6H2,(H2,20,26)/t8-,9-,19-/m0/s1



$C_{19}H_{17}NO_7$
Mol wt: 371.3408

CAS: 015866-90-7

EN: 271939

Abstract

The chemically modified tetracycline (CMT) incyclinide (COL-3, CMT-3) has been extensively studied as a potential new therapeutic agent for allergic conditions, inflammatory (*i.e.*, arthritis, acute respiratory distress syndrome [ARDS], septic shock syndrome, acne and rosacea), neoplastic (*i.e.*, Kaposi's sarcoma, colon carcinoma, melanoma, prostate cancer) and infectious (fungal) diseases. The most prominent characteristic of CMTs is their lack of antibacterial properties, accompanied by retention, or even enhancement, of metalloproteinase (MMP) inhibition. Studies have demonstrated that incyclinide is a modulator of MMP-2, MMP-9 and serine proteases, as well as cytokines and interleukins that are crucial in the development of inflammation, angiogenesis and tumorigenesis. Furthermore, toxicology studies have demonstrated that incyclinide is relatively safe.

Synthesis*

Incyclinide can be obtained by two different ways (Scheme 1):

1) The reaction of 6-demethyl-6-deoxytetracycline (I) with methyl iodide in acetone gives 6-demethyl-6-deoxytetracycline methiodide (II), which is treated with Zn in Ac-OH to obtain the target 4-desdimethylamino-6-demethyl-6-deoxytetracycline (1, 2).

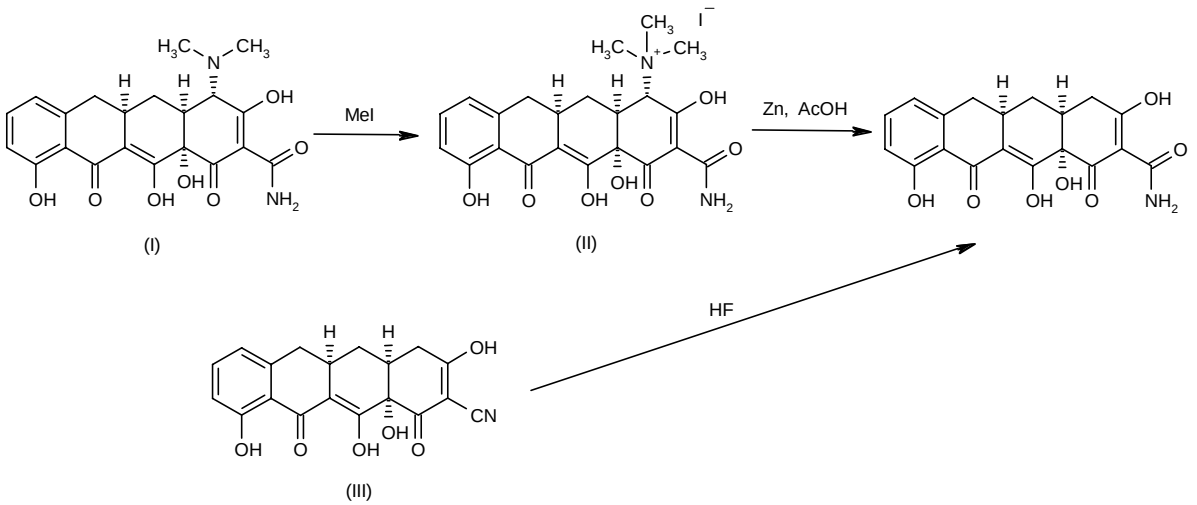
2) The controlled hydrolysis of 2-decarboxamido-4-desdimethylamino-6-demethyl-6-deoxytetracycline-2-nitrile (III) by means of HF and traces of water yields the target 4-desdimethylamino-6-demethyl-6-deoxytetracycline (3).

Background

The first chemically modified tetracycline (CMT) was described in 1987 (4). Soon thereafter, it was discovered that tetracyclines (TCNs) can inhibit mammalian-derived matrix metalloproteinases (MMPs) *in vitro* and *in vivo* (5, 6) by mechanisms which are independent of their antibacterial properties. Since then, more than 30 different CMTs in which the 4-dimethylamino group has been deleted have been developed. The most prominent characteristic of these CMTs is their lack of antibacterial activity, accompanied by retention or even enhancement of their MMP-inhibitory effects (7, 8). CMTs have therefore been tested for their efficacy as inhibitors of connective tissue breakdown, including the preservation of bone and cartilage, in a variety of animal models of disease, including arthritis, osteoporosis, aortic aneurysm, periodontitis and cancer (9). One of these nonantibacterial TCN analogues, incyclinide (CMT-3, COL-3), has been extensively studied as a potential new therapeutic agent for oral administration (8).

Martha H. Viera, Oliver A. Perez, Brian Berman. University of Miami, Miller School of Medicine, Department of Dermatology and Cutaneous Surgery, 1600 NW 10th Avenue, RSMB, Room 2023A (R250), Miami, FL 33136, USA. *Synthesis prepared by N. Serradell, E. Rosa, J. Bolós. Prous Science, P.O. Box 540, 08080 Barcelona, Spain.

Scheme 1: Synthesis of Incyclinide



Preclinical Pharmacology

Incyclinide is an inhibitor of serine proteases such as elastase and has been shown to inhibit cyclooxygenase type 2 (COX-2) activity and associated prostaglandin E_2 (PGE_2) production. Incyclinide also inhibits the production of inducible nitric oxide synthase (iNOS), tumor necrosis factor-α (TNF-α) and the interleukins IL-1, IL-6 and IL-8, and it induces apoptosis in certain tumor cell lines (10-12). The results of early studies demonstrated that incyclinide is a modulator of MMP-2, MMP-9 and serine proteases, as well as cytokines and interleukins crucial to the development of inflammation, angiogenesis and tumorigenesis (9, 10, 12-14). The pharmacokinetics, metabolism, toxicology and therapeutic applications of incyclinide are under active investigation (15-22).

Incyclinide inhibits mast cell and macrophage function. As mast cells release histamine, eicosanoids, proteases, prostaglandin and cytokines when activated, inhibition of mast cell function has implications for the treatment of allergic and inflammatory diseases. *In vitro* studies have shown that incyclinide inhibits the release of histamine from rat serosal mast cells, with a reduction of 47 ± 7.3% compared to controls reported (10). Similarly, incyclinide has been shown to decrease the activation-induced secretion of TNF-α and IL-8 by 75% and 40%, respectively, in human leukemic mast cells (23). Moreover, incyclinide significantly inhibited protein kinase C (PKC), which plays a central role in mast cell activation. Also, the inhibitory effect of incyclinide on MMPs results in inhibition of mast cell degranulation (10).

Incyclinide has been shown to inhibit several aspects of the inflammatory cascade, specifically MMP-2, MMP-9 and neutrophil elastase (NE), suggesting its potential in the treatment of conditions in which various inflammatory mediators are implicated, such as acute respiratory distress syndrome (ARDS), septic shock syndrome (SSS), acne, rosacea and arthritis (12, 21, 24, 25). Using the cecal ligation and puncture (CLP) rat model, incyclinide was found to decrease MMP-2 and MMP-9 levels and improved survival in a dose-dependent fashion when given at the time of lung injury and before the development of ARDS (26). Using a porcine model, it was demonstrated that incyclinide inhibited MMP-2, MMP-9 and NE, as well as IL-6, IL-8 and IL-10, and prevented sepsis-induced ARDS and the development of SSS, despite positive blood cultures (12). The compound also inhibited collagen degradation in an experimental animal model of arthritis (27).

Fife *et al.* used human umbilical cord vascular endothelial cells (HUVEC) to demonstrate that incyclinide inhibits angiogenesis by inhibiting endothelial cell microtubule formation (28).

Human breast carcinoma MDA-MB-231 cells expressing MMP-9 treated with incyclinide *in vitro* showed decreases in MMP-9 protein production (45%) and gelatinase activity (60%), with a concomitant reduction in levels of TNF-α. In this study, incyclinide also prevented extracellular matrix degradation without inducing general cytostasis or cytotoxicity (11).

The human colon carcinoma-derived COLO 205 cell line demonstrates high extracellular matrix-degrading activity and MMP overexpression. A recent *in vitro* study using the COLO 205 cell line demonstrated that incyclinide inhibits invasiveness by inhibiting MMP-2 release, MMP-2 gelatinolytic activity, and more importantly, by preventing extracellular matrix degradation (29).

Prostate cancer cells have been shown to secrete high levels of MMPs, which are involved in metastasis to bone and the lungs. Among several anti-MMP agents examined, incyclinide showed the greatest activity against prostate cancer cell proliferation and invasion (30-33). In

prostate cancer cells, incyclinide induces apoptosis and necrosis, mitochondrial depolarization and the production of reactive hydroxyl radicals, and it inhibits the production of MMP-2 and tissue inhibitor of MMP TIMP-1 and TIMP-2 (30). An *in vivo* study using the Dunning rat prostate cancer (CaP) model demonstrated that incyclinide (20 or 40 mg/kg/day p.o. for 21 days) reduced CaP lung metastasis by more than 50% and tumor incidence by 55 ± 9%, with minimum systemic toxicity (31).

Incyclinide has been shown to decrease melanoma cell metastasis by inhibiting MMP activity, as well as vasculogenic mimicry, defined as the generation of microvascular channels by genetically deregulated aggressive tumor cells, and vasculogenic mimicry-associated gene expression (34, 35). Further analysis revealed that incyclinide inhibits the formation of laminin 5 gamma 2 chain promigratory fragments in aggressive melanoma cells and the induction of laminin 5 gamma 2 chain gene expression in poorly aggressive melanoma cells. These results suggest that incyclinide, as well as the related CMTs, may be useful for targeting molecular cues in the microenvironment of aggressive tumors, particularly when used with other therapies that specifically target and kill aggressive tumor cells (34).

MMPs are also important mediators of tumor metastasis to bone, contributing significantly to morbidity in breast and prostate cancer patients. Studies have shown that the anti-MMP activity of incyclinide impacts the bone metastatic potential of cancer cells, and it is being evaluated in patients with metastatic bone cancer (36).

CMTs have also demonstrated antifungal properties. Liu *et al.* determined that *Candida albicans* was sensitive to incyclinide, with a 50% inhibitory concentration of 1 μg/ml. Incyclinide exhibited fungicidal activity against most of the fungi examined, especially filamentous species, inducing 90% inhibition of viability in 84% of the filamentous fungi tested (37).

Pharmacokinetics and Metabolism

The pharmacokinetics and metabolism of incyclinide have been studied in different clinical and preclinical experiments where the mechanism of absorption, route of elimination and half-life of incyclinide have been examined (11, 18, 30, 38, 39). Studies have shown that incyclinide is highly hydrophobic and that gastric emptying is not the rate-limiting step in its absorption. A preclinical study was undertaken to elucidate the mechanism of absorption of incyclinide after a single dose to rats. In this study, it was found that the absorption of incyclinide is limited by its dissolution rate. A fine suspension of incyclinide was better absorbed than a course suspension of the drug. Absorption was enhanced by the presence of food and endogenous bile in the gastrointestinal tract. Therefore, variability in bile concentration, food content and other physiological factors affecting the dissolution of incyclinide in the gastrointestinal tract may lead to an irregular absorption rate and variable serum concentrations (15, 40).

Incyclinide is eliminated via the gastrointestinal, hepatobiliary and urinary tracts. The principal route of incyclinide elimination in rats is via the gastrointestinal tract. However, incyclinide exhibits nonlinear gastrointestinal pharmacokinetics due to dissolution rate-limited absorption. Environmental, pathophysiological and drug-related factors may influence the gastrointestinal elimination rate of incyclinide (15, 20). The hepatobiliary excretion of incyclinide 24 h after oral administration was calculated to account for 1.36 ± 0.66% of the administered dose. The role of the hepatobiliary tract in the elimination of incyclinide is therefore minimal, due to its relatively small molecular weight and extreme lipophilicity (15, 40). Therefore, phase I metabolism via cytochrome P-450 and phase II metabolism via glucuronidation are expected to play a minor role in the metabolism of incyclinide (15, 20). However, *in vitro* and *in vivo* studies demonstrated the presence of glucuronides in urine (median of 13.6% of the total administered dose) (40).

A study by Rudek *et al.* demonstrated that incyclinide has a long terminal half-life (median = 59.8 h), a large apparent volume of distribution (median = 50.2 l) and a low apparent clearance (median = 9.93 ml/min) in humans (20).

Safety

Incyclinide-induced phototoxicity, the major adverse event reported, as well as skin rash and malaise, have been determined to be dose-dependent (16, 20). Other toxicities that do not appear to be dose-related are fever, fatigue, dizziness, headache, mucositis, elevated liver function tests, heartburn, nausea, vomiting, anorexia, constipation, diarrhea, peripheral and central neurotoxicities, anemia, leukopenia, thrombocytopenia and drug-induced lupus (16, 18, 19, 40).

A prospective clinical study examined the effect of incyclinide at 36, 50, 70 and 98 mg/m^2/day in 33 subjects. Dose-limiting phototoxicity was observed at doses exceeding 50 mg/m^2/day without sun protection. An unacceptably high incidence of photosensitivity skin reactions and malaise was noted in the first 28-day course of incyclinide in subjects treated with doses exceeding 50 mg/m^2/day. At this dose, severe toxicity occurred in 2 of 12 subjects after administration of the first course of incyclinide. Therefore, the maximum recommended daily oral incyclinide dose for phase II studies is 50 mg/m^2/day, accompanied by the use of sunscreen and other photoprotective agents (17). Incyclinide at 36 mg/m^2/day appeared to be well tolerated without sunscreen (19).

Although phototoxicity is the most commonly reported adverse event with administration of incyclinide, and the most common reason for discontinuation from clinical trials, drug-induced lupus is also a concern. Cases of drug-induced lupus have been reported specifically after administration of incyclinide for the treatment of cancer (22). A phase I clinical trial at the National Institutes of Health (NIH) reported a drug-induced lupus reaction in subjects who were given incyclinide for the treatment of

refractory metastatic cancer. Three of 35 subjects in this study had clinical and laboratory features of drug-induced lupus and developed sunburn-like eruptions accompanied by fever, arthralgia and a positive antinuclear antibody (ANA) titer as high as 1:640 within 8-29 days after starting incyclinide therapy. The rapid onset and phototoxic appearance of the accompanying eruption suggested that damage to keratinocytes caused the formation of neoantigens with resulting autoantibodies. Two of the 3 subjects with features of drug-induced lupus also had elevated antihistone antibody levels. Antihistone antibodies are present in 90% of patients with drug-induced lupus reactions. One of the 3 subjects had marked systemic manifestations of drug-induced lupus, including pulmonary infiltrates and an elevated erythrocyte sedimentation rate (ESR), for more than a year after discontinuation of incyclinide. The other 2 subjects demonstrated evidence of a rash that abated within 2 weeks of discontinuing incyclinide therapy, although antihistone antibody titers remained elevated in these subjects during the duration of follow-up (22).

Clinical Studies

A randomized, placebo-controlled phase II proof-of-concept trial demonstrated significant clinical improvement and reduction of inflammatory lesions in subjects with rosacea administered incyclinide. The study enrolled 14 patients, 8 administered incyclinide 10 mg and 6 placebo once daily for 28 days. The primary endpoints of this study were inflammatory lesion count and clinician's erythema score at day 42. Erythema, as measured by the Investigator's Global Assessment (IGA) score, was decreased by 2.5 points in the incyclinide treatment group compared to 1.7 points in the placebo group. Furthermore, 80% improvement was noted in the lesion count by day 14 of treatment, and at day 42, lesion count was reduced by 69% compared to an increase of 12% on placebo. Incyclinide was well tolerated by all subjects, and no serious adverse events were reported (41, 42).

Based on its antiinflammatory properties and the clinical improvement seen in subjects with rosacea, a multicenter, randomized, double-blind, placebo-controlled trial was commenced to study its effects in subjects with acne. This phase II dose-finding study enrolled 302 patients who received placebo capsules or incyclinide capsules at doses of 5, 10 or 20 mg once daily for 12 weeks. Incyclinide was well tolerated; most adverse events were mild or moderate in severity and the incidence was similar across all study groups. In an intent-to-treat analysis of lesion count, dose-dependent effects were observed, with no effect on the lowest dose and a statistically significant reduction in lesion count on the highest dose compared to placebo at weeks 6 (36.0% *vs.* 17.5% on placebo) and 9 (36.1% *vs.* 23.8% on placebo). A secondary endpoint was the change in the IGA score for disease severity, which was unchanged on 5 mg and statistically significantly improved on 10 mg at week 9 and on 20 mg at weeks 6 and 9 (43, 44).

Based on its antiangiogenic properties, incyclinide has also been evaluated in patients with advanced solid tumors, as well as Kaposi's sarcoma, lymphoma, renal cell carcinoma and malignant melanoma (9, 11, 28, 38, 45-52).

In a phase II study in patients with AIDS-related Kaposi's sarcoma, incyclinide (50 or 100 mg once daily) gave an overall response rate of 33%, which was associated with a decrease in MMP-2 levels. Side effects of incyclinide in these subjects included photosensitivity and rash (53).

Conclusions

Incyclinide may prove to be an important drug in the near future for the treatment of inflammatory, neoplastic and infectious (fungal) diseases. Early studies demonstrated that incyclinide inhibits MMP-2, MMP-9, serine proteases, iNOS, TNF-α, IL-1, IL-6, IL-8 and extracellular matrix components which play a crucial role in the development of inflammatory and neoplastic diseases. Clinical studies demonstrated efficacy in the treatment of rosacea and acne, and clinical trials have also been conducted in patients with various types of cancer. It is interesting that incyclinide possesses not only antiinflammatory and antineoplastic properties, but also antifungal activity. Furthermore, toxicology studies have demonstrated that incyclinide is relatively safe. In the near future, incyclinide may have widespread use in medicine to effectively treat inflammatory, neoplastic and infectious diseases through its multiple mechanisms of action.

Source

CollaGenex Pharmaceuticals, Inc. (US); the U.S. National Cancer Institute is developing incyclinide for oncological indications.

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