Drugs of the Future 2009, 34(2): 115-118

CCC: 0377-8282/2009
DOI: 10.1358/dof.2009.34.2.1339255

TOSEDOSTAT

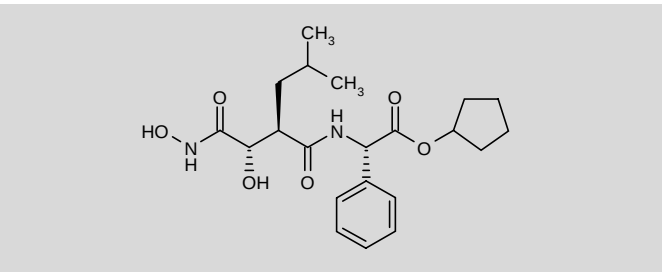
Prop INN

Aminopeptidase Inhibitor
Oncolytic

CHR-2797
BB-76163

2(*S*)-[2(*R*)-[1(*S*)-Hydroxy-1-(*N*-hydroxycarbamoyl)methyl]-4-methylpentanamido]-2-phenylacetic acid cyclopentyl ester

InChI=1/C21H30N2O6/c1-13(2)12-16(18(24)20(26)23-28)19(25)22-17(14-8-4-3-5-9-14)21(27)29-15-10-6-7-11-15/h3-5,8-9,13,15-18,24,28H,6-7,10-12H2,1-2H3,(H,22,25)(H,23,26)/t16-,17+,18+/m1/s1



$C_{21}H_{30}N_2O_6$
Mol wt: 406.4727
CAS: 238750-77-1
EN: 281076

ABSTRACT

Tosedostat (CHR-2797) is an orally active aminopeptidase inhibitor that is converted in vivo to a pharmacologically active metabolite, CHR-79888, and exerts an antiproliferative effect against a wide range of leukemic and epithelial tumor cell lines. A wide separation exists between its antiproliferative effects on myeloid and normal bone marrow cells and it shows synergistic activity with cytarabine, bortezomib and tretinoin in vitro. An inhibitory effect on CD13 protein was also demonstrated. It was active in murine models of breast carcinoma, both alone and in combination with carboplatin. The kinetics of tosedostat and its metabolite are linear, with excellent exposure in plasma and packed red blood cells. Tosedostat did not affect the pharmacokinetic profile of paclitaxel in the clinic. In the largest phase II study reported to date, tosedostat demonstrated encouraging efficacy in elderly and/or previously treated patients with refractory/relapsed acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS). The most common adverse events were fatigue, thrombocytopenia, pyrexia, peripheral edema and diarrhea. Few treatment-related interruptions occurred.

SYNTHESIS

Tosedostat can be prepared by condensation of 3(*R*)-isobutyl-L-malic acid pentafluorophenyl ester acetonide (I) with (*S*)-phenylglycine cyclopentyl ester (II) in CH_2Cl_2 to give amide (III), which is then submitted to ring opening of the dioxolane moiety with hydroxylamine in methanol (1). Scheme 1.

BACKGROUND

Metalloenzyme inhibitors, such as matrix metalloproteinase (MMP) inhibitors and aminopeptidase inhibitors, have been implicated in tumor growth regulation (2). However, MMP inhibitors have not shown direct antiproliferative effects in the clinic, with the exception of batimastat (3). Further research in the area of metalloenzymes led to the development of a novel class of aminopeptidase inhibitors, including tosedostat (CHR-2797), which shows potent and selective antiproliferative effects against hematopoietic and epithelial tumor cell lines.

PRECLINICAL PHARMACOLOGY

Tosedostat has potent antiproliferative effects across a range of leukemic and solid tumor cell lines (Table I). The acid metabolite (CHR-79888, Fig. 1) derived from tosedostat has a much weaker antiproliferative effect in vitro and has optimal antiproliferative effects only when formed inside the cell (4).

Further work on acute myeloid leukemia (AML) blasts and normal bone marrow progenitors demonstrated that AML blasts were much more sensitive to tosedostat than their normal progenitors. The

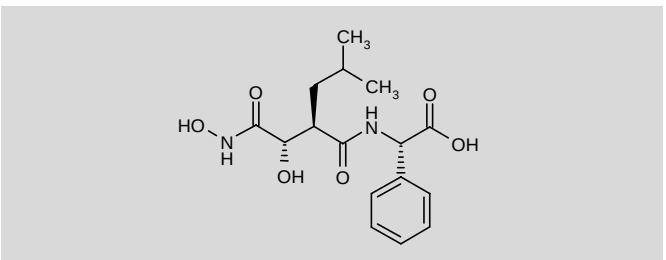


Figure 1. Structure of CHR-79888.

R.T. Owen, R. Castañer, J. Bolós.
Prous Science, Provenza 388, 08025 Barcelona, Spain.

Scheme 1. Synthesis of Tosedostat

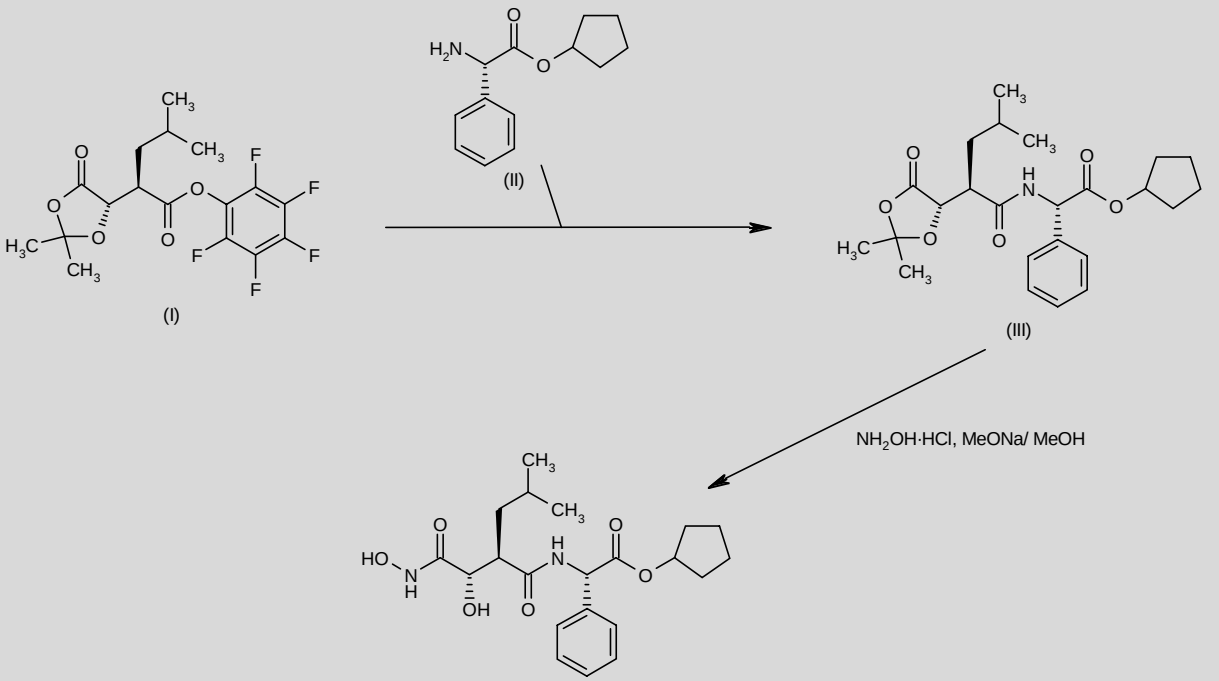


Table I. *Selected cell lines and inhibition of cell growth by tosedostat (data from Refs. 4 and 5).*

Cell line	Tumor type	IC_{50} (nmol/L)
U-937	Histiocytic lymphoma	10
HL-60	Promyelocytic leukemia	30
KG-1	Acute myelocytic leukemia	15
HNT-34	Acute myelogenous leukemia	35
GDM-1	Myelomonoblastic leukemia	15
MDA-MB-468	Breast adenocarcinoma	55
NCI-H23	Non-small cell lung carcinoma	75

median IC_{50} of tosedostat in AML blasts was 1.07 μM compared to 9.02 μM in normal cells, a 9-fold difference (5) (these data are from the poster not the abstract, as the latter was said to be incorrect; Chroma personal communication). Synergy was also demonstrated in combination with cytarabine, bortezomib and tretinoin in 69%, 78% and 62% of cells, respectively, even in nonpromyelocytic leukemia cell types. Tosedostat also inhibited CD13, a cell-surface protein selectively expressed on myeloid cells, a concentration of 10 μM causing 48% inhibition after 5-h incubation (5).

Human promyelocytic leukemia HL-60 cells incubated with tosedostat 6 μmol/L showed upregulation of a number of genes whose primary function appears to be to increase intracellular amino acid levels. These comprise, among other genes, amino acid transporters and biosynthetic enzymes, e.g., *SLC7A11, SLC38A2, ASNS, CBS* and *CTH*. Other genes upregulated by tosedostat include many not directly related to amino acid transport or biosynthesis, e.g., *TRIB3, ATF* and *VEGF*. One of the most upregulated genes was stanniocalcin-2 (*STC2*), a secreted protein and thus a potential plasma marker (4).

Experiments using multiple myeloma cells demonstrated inhibition of cell proliferation and induction of apoptosis and that cell death occurred in a non-caspase-dependent manner. This was associated with activation of the unfolded protein response. Synergy with dexamethasone (100 nM) was shown and an additive effect with bortezomib (6). Apoptosis induced by tosedostat in leukemia cell lines is thought to occur via upregulation of Noxa and a decrease in Mcl-1 (7).

Tosedostat showed a dose–response relationship in a murine HOSP1 mammary carcinoma model at 3, 10 and 30 mg/kg/day p.o. Inhibition of tumor burden, tumor weight and colony number was seen. At the highest dose, statistically significant differences were seen on all parameters; at 3 mg/kg/day only tumor burden was significantly reduced ($P < 0.001$ vs. controls) (8).

Tosedostat showed synergistic activity with carboplatin in a murine xenograft model using human breast cancer MDA-MB-468 cells. Tosedostat alone (100 mg/kg/day p.o.) caused an 18% inhibition of tumor growth, whereas in combination with carboplatin (40 mg/kg/week) tumor regression was seen, without an increase in carboplatin toxicity (9). The antiproliferative effects of tosedostat were also demonstrated in another MDA-MB-468 xenograft model. Mice were randomized on day 7 postinoculation to receive vehicle or tose-

Table II. *Key pharmacokinetic parameters for tosedostat and its active metabolite CHR-79888 after 28 days of daily dosing (data from Ref. 12 abstract and poster).*

Dose (mg/day)	n	C_{max} (ng/mL)	t_{max} (h)	AUC_{0-t} (ng.h/mL)	$t_{1/2\beta}$ (h)
10	1	109 [60]	1 [6]	224 [1106]	ND [ND]
20	1	176 [108]	0.5 [4]	392 [1654]	1.2 [11]
40	3	218 [246]	2.7 [6]	695 [3431]	1.2 [9.5]
60	3	348 [312]	0.8 [4]	765 [4338]	1.1 [9.2]
90	3	434 [535]	1.7 [5.3]	1350 [7006]	2.8 [8.2]
130	4	741 [786]	1.4 [4.5]	2232 [11,229]	1.3 [9.25]
180	3	1658 [1728]	2.0 [4.7]	1920 [14,709]	1.0 [8.5]
240	3	1265 [1326]	2.0 [5.3]	3958 [21,745]	3.7 [11.1]

Values for CHR-79888 are presented in brackets; ND, not determined. Values are means when n > 1. C_{max}, peak plasma concentration; t_{max}, time to peak plasma levels; AUC, area under the curve; $t_{1/2\beta}$, elimination half-life.

dostat (100 mg/kg/day i.p.). Half the animals were treated between days 21 and 49 and the remainder at 39-70 days postinoculation. Tumor weight was decreased by 30% when dosing commenced on day 21 and by 22% when treatment began on day 39. No significant toxicity occurred except for a small, occasional decrease in body weight gain (10).

Using a human breast carcinoma MDA-MB-435 tumor xenograft model in nude mice, oral administration of tosedostat 100 mg/kg/day was found to significantly inhibit lung tumor burden by 98%, as well as spontaneous lung metastases, and was well tolerated (11).

PHARMACOKINETICS AND METABOLISM

The pharmacokinetics of tosedostat and its active metabolite (CHR-79888) are linear, with excellent drug exposure in plasma and packed red cells (Table II). Peak blood levels of tosedostat are reached in approximately 1-2 h, with a terminal half-life ($t_{1/2}$) of approximately 1-4 h. The metabolite reaches peak levels in approximately 4-6 h and has a $t_{1/2}$ of approximately 8 h (12).

SAFETY

The first phase I study of tosedostat was conducted in 37 patients (median age of 61.5 years) with advanced solid tumors refractory to standard therapy. Twelve cohorts received between 10 and 320 mg/day p.o. The first 4 patients received a dose of 10 mg for 7, 14, 21 or 28 days. Subsequent cohorts received 28 days of continuous dosing, with dose-doubling in single patient cohorts until dose-limiting toxicity (grade 2). Thereafter the study followed a 3+3 design with dose steps of 25-40%. Common (grade 1-2) toxicity included diarrhea and fatigue (47% each), dizziness (24%), constipation, vomiting and abdominal pain (all 21%) and thrombocytopenia (18%). The maximal tolerated dose was 320 mg/day. Two patients were unable to complete 28 days of treatment due to syncope/anemia and dizziness/visual disturbance/thrombocytopenia, respectively (13).

An open-label phase I salvage study assessed the tolerance of tosedostat in elderly and/or relapsed patients with AML, myelodysplastic syndrome (MDS) or multiple myeloma receiving escalating doses of 60-180 mg/day tosedostat for up to 84 days; 13 patients completed the dose-finding phase of 28 days and 6 continued for at least 84 days. Dose-limiting toxicity of platelet count reduction was seen in two patients on the highest dose and this was considered the maximum tolerated dose (MTD) for maintenance therapy. Thrombocytopenia (6.7%), diarrhea (4.5%), dizziness and fatigue (both 3.9%) were the most common adverse events (14).

A phase Ib dose-escalation study of oral tosedostat and i.v. paclitaxel was conducted in 22 patients (median age of 59 years) with advanced solid tumors refractory to current therapy. Patients received paclitaxel 135-175 mg/m^2 and escalating doses of tosedostat of 90-240 mg for up to 6 cycles of 21 days' duration. Common grade 1-3 toxicities included alopecia and fatigue (95% each), sensory neuropathy (59%), myalgia (50%), anorexia and dizziness (45% each), rash (32%) and paclitaxel infusion reactions (59%). Six patients continued tosedostat after discontinuation of paclitaxel (15).

A multicenter, open-label phase II study of tosedostat (130 mg/day p.o.) was conducted in 41 patients (mean age of 67 years) with refractory or relapsed AML or MDS. Patients were treated for up to 84 days. The most frequently reported adverse events were fatigue (61%), thrombocytopenia (49%), pyrexia (39%), peripheral edema (39%) and diarrhea (34%). The adverse event discontinuation rate was 22% (16).

CLINICAL STUDIES

In the phase I study described earlier (12, 13), one patient achieved a partial response (PR) at 130 mg/day and six patients had confirmed

stable disease (SD) after three months at doses ranging from 40 to 180 mg/day. Twenty-three patients had progressive disease (these data are from the poster not the abstract, as the latter was said to be incorrect; Chroma personal communication).

Monotherapy with tosedostat (60-180 mg/day) was associated with complete bone marrow responses in 3 of 12 AML patients (14).

In the paclitaxel/tosedostat combination therapy study, PR was achieved by 3 patients (14%) and SD by 12 patients (57%). Five (24%) patients had progressive disease (15).

In the multicenter phase II study (16), CR and PR were achieved by 7.9% and 18.4% of AML patients, respectively. Of the three MDS patients one had a PR, one achieved SD and one had resistant disease.

A six-month, single-arm phase IIb study to evaluate the efficacy, safety and tolerability of tosedostat in elderly subjects with treatment-refractory or relapsed AML will be conducted. The primary outcome measure will be the efficacy of tosedostat by measuring CR and CR with incomplete platelet recovery (CRp). Secondary outcomes will include the safety and tolerability of tosedostat and evaluation of efficacy measures other than CR and CRp for type and duration of response (17).

DRUG INTERACTIONS

When i.v. paclitaxel was administered with tosedostat the AUC, C_{max}, t_{max} and $t_{1/2}$ values were essentially the same whether paclitaxel was given alone on day 1 (135-175 mg/m^2/day) or with tosedostat on day 22 at doses between 90 and 180 mg/day (15).

SOURCES

Chroma Therapeutics (GB); licensed from Vernalis (GB).

REFERENCES

1. Pearson, L.A., Ayscough, A.P., Huxley, P., Drummond, A.H. (British Biotech Pharmaceuticals Ltd.). *Cytostatic agents*. CA 2323414, EP 1062202, JP 2002506057, WO 1999046241.
2. Overall, C.M., Lopez-Otin, C. *Strategies for MMP inhibition in cancer: Innovations in the post-trial era.* Nat Rev Cancer 2002, 2(9); 657-72.
3. Brown, P.D., Bowden, I.J., Miller, K.M. (British Biotech Pharmaceuticals Ltd.). *Use of MMP inhibitors*. US 5872152.
4. Krige, D., Needham, L.A., Bowden, L.J. et al *CHR-2797: An antiproliferative aminopeptidase inhibitor that leads to amino acid deprivation in human leukemic cells.* Cancer Res 2008, 68(16): 6669-79.
5. Jenkins, C., Mills, K., Pepper, C., Burnett, A. *Cellular aminopeptidase inhibition as a target for the therapy of AML by the novel agent CHR-2797.* Blood [49th Annu Meet Am Soc Hematol (Dec 8-11, Atlanta) 2007] 2007, 110(11): Abst 1608.
6. Davies, F.E., Moore, H.E., Davenport, E.L. et al. *Aminopeptidase inhibition as a targeted treatment strategy in myeloma.* Blood [49th Annu Meet Am Soc Hematol (Dec 8-11, Atlanta) 2007] 2007, 110(11): Abst 2505.
7. Drummond, A., Callaghan, J., Flores, N., Miles, L., Bawden, L., Farmer, H., Krige, D. *Antiproliferative aminopeptidase inhibitors inhibit mTOR and alter expression of Noxa and Mlc-1 in leukemic cells via amino acid deprivation.* Proc Am Assoc Cancer Res (AACR) 2007, 48: Abst 4069.
8. Drummond, A.H., Eccles, S.A., Box, G., Hooftman, L.W., Bone, E.A., Needham, L.A. *In vivo efficacy of CHR-2797, a novel aminopeptidase inhibitor, in a syngeneic rat HOSP.1 mammary carcinoma model.* Proc Am Assoc Cancer Res (AACR) 2005, 46: Abst 5850.
9. Hooftman, L.W., Bone, E.A., Drummond, A.H., Needham, L.A., Odedra, R., Wright, A., Laber, D. *Effect of the combination of the aminopeptidase inhibitor CHR-2797 and carboplatin on the growth of MDA-MB468 breast carcinoma in nude mice.* 17th AACR-NCI-EORTC Int Conf Mol Targets Cancer (Nov 14-18, Philadelphia) 2005, Abst B188.
10. Hooftman, L.W., Bone, E.A., Drummond, A.H., Needham, L.A., Laber, D.O., Odedra, R., Weight, A. *Antiproliferative effect of CHR-2797, a novel aminopeptidase inhibitor in the human breast cancer xenograft, MDA-MB 468.* 4th Int Symp Target Anticancer Ther (March 16-18, Amsterdam) 2006, Abst P.220.
11. Hooftman, L.W., Bone, E.A., Drummond, A.H., Needham, L.A., Laber, D.O., Odedra, R., Wright, A. *Antiproliferative effect of CHR-2797, a novel aminopeptidase inhibitor, in the human breast cancer xenograft, MDA-MB 435*. Proc Am Assoc Cancer Res (AACR) 2005, 46: Abst 637.
12. Protheroe, A., Reid, A., Attard, G. et al. *A phase I dose finding study of the aminopeptidase inhibitor CHR-2797 in patients with treatment-refractory metastatic cancer.* Ann Oncol [31st Eur Soc Med Oncol (ESMO) Congr (Sept 29-Oct 3, Istanbul) 2006] 2006, 17(9): Abst 412P.
13. Protheroe, A., Reid, A., Attard, G. et al. *First in-human phase I trial of a novel aminopeptidase inhibitor, CHR-2797.* J Clin Oncol [43rd Annu Meet Am Soc Clin Oncol (ASCO) (June 1-5, Chicago) 2007] 2007, 25(18, Suppl.): Abst 3537.
14. Davies, F., Ossenkoppele, G., Zachee, P. et al. *A phase I study of CHR-2797, an orally active aminopeptidase inhibitor in elderly and/or treatment refractory patients with acute myeloid leukemia or multiple myeloma*. Blood [49th Annu Meet Am Soc Hematol (Dec 8-11, Atlanta) 2007] 2007, 110(11): Abst 443.
15. van Herpen, C., Eskens, F., de Jonge, M. et al. *A phase 1b dose escalation study to evaluate safety and tolerability of the combination of the aminopeptidase inhibitor CHR-2797 and paclitaxel in patients with advanced or treatment refractory tumors.* 20th EORTC-NCI-AACR Symp Mol Targets Cancer Ther (Oct 21-24, Geneva) 2008, Abst 344.
16. Lowenberg, B., Davies, F., Müller-Tidow, C. et al. *A multicenter, phase II study of the aminopeptidase inhibitor, CHR-2797, in the treatment of elderly and/or previously treated patients with acute myeloid leukemia.* Blood [50th Annu Meet Am Soc Hematol (Dec 6-9, San Francisco) 2008] 2008, 112(11): Abst 961.
17. *Safety and anti-disease activity of oral tosedostat (CHR-2797) in elderly subjects with treatment refractory or relapsed AML (OPAL) (NCT00780598).* ClinicalTrials.gov Web site, February 7, 2009.