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Enzastaurin Hydrochloride

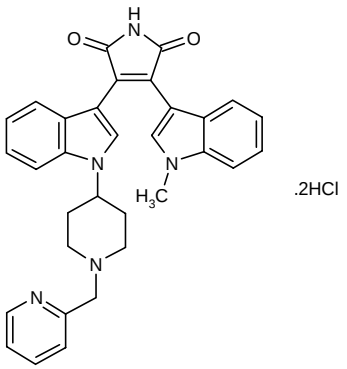
Protein Kinase C β Inhibitor
Antiangiogenic Agent

Rec INNM; USAN

317615.2HCl
LY-317615.2HCl

3-(1-Methyl-1*H*-indol-3-yl)-4-[1-[1-(pyridin-2-ylmethyl)piperidin-4-yl]-1*H*-indol-3-yl]-1*H*-pyrrole-2,5-dione dihydrochloride

InChI=1/C32H29N5O2.2ClH/c1-35-19-25(23-9-2-4-11-27(23)35)29-30(32(39)34-31(29)38)26-20-37(28-12-5-3-10-24(26)28)22-13-16-36(17-14-22)18-21-8-6-7-15-33-21;;/h2-12,15,19-20,22H,13-14,16-18H2,1H3,(H,34,38,39);2*1H



.2HCl

$C_{32}H_{31}Cl_2N_5O_2$
Mol wt: 588.5264

CAS: 365253-37-8
CAS: 170364-57-5 (free base)
CAS: 359017-79-1 (monoHCl)
EN: 306147

Abstract

Protein kinase C (PKC) overexpression has been linked to several types of cancer, with the PKCβ isoform suspected to be involved in vascular endothelial growth factor (VEGF)-induced tumor development and angiogenesis and in the apoptosis-regulating phosphatidylinositol 3-kinase (PI3K)/Akt pathway. Targeting of PKCβ therefore represents a potentially effective strategy for the treatment of cancer. Enzastaurin (LY-317615) is an acyclic bisindolylmaleimide that potently and selectively inhibits the PKCβ isoform. Enzastaurin displayed anticancer efficacy in several preclinical cancer models and in clinical trials in patients with advanced cancers. It is currently undergoing phase III development for relapsed glioblastoma multiforme and diffuse B-cell lymphoma.

Synthesis

Enzastaurin can be prepared by several related methods. The key intermediate 4-(1-indolyl)-1-(2-pyridylmethyl)piperidine (I) is acylated by oxalyl chloride to produce the glyoxylyl chloride (II) (1-4), which is optionally converted to the glyoxylate ester (III) upon quenching with methanol (3, 4). The title bis(indolyl)maleimide compound is either obtained by cyclization of glyoxylyl chloride (II) with the indolyl acetimidate (IV) in the presence of triethylamine, followed by acidic dehydration (1, 2), or by cyclization of the glyoxylate ester (III) with (1-methylindol-3-yl)acetamide (V) in the presence of potassium *tert*-butoxide (3, 4). In a related procedure, enzastaurin is obtained by condensation of the piperidinyl indoleacetamide (VI) with methyl 1-methyl-3-indolylglyoxylate (VII) in the presence of potassium *tert*-butoxide (3). Scheme 1.

The intermediate 4-(1-indolyl)-1-(2-pyridylmethyl)-piperidine (I) can be prepared following several synthetic strategies. Condensation of *o*-nitrotoluene (VIII) with dimethylformamide dimethyl acetal in the presence of pyrrolidine gives the pyrrolidino enamine (IXa) along with minor amounts of the *N,N*-dimethyl analogue (IXb). Subsequent treatment of the mixture of enamines (IXa,b) with methanol and an acidic catalyst provides *o*-nitrophenylacetaldehyde dimethyl acetal (X) (3, 5). Alternatively, treatment of the diazonium salt derived from *o*-nitroaniline (XI) with 1,3-butadiene in the presence of $CuCl_2$ and NaOAc affords 1-(2-nitrophenyl)-4-chloro-2-butene (XII), which is subjected to ozonolysis in MeOH to give the nitro acetal (X) (6). Amino acetal (XIII) is then obtained by catalytic hydrogenation of (X) in the presence of Pd/C (3, 5, 6). The required pyridylmethyl piperidone (XVII) can be prepared by two different methods. Michael addition of ethyl acrylate (XV) to 2-(aminomethyl)pyridine (XIV), followed by Dieckmann cyclization utilizing potassi-

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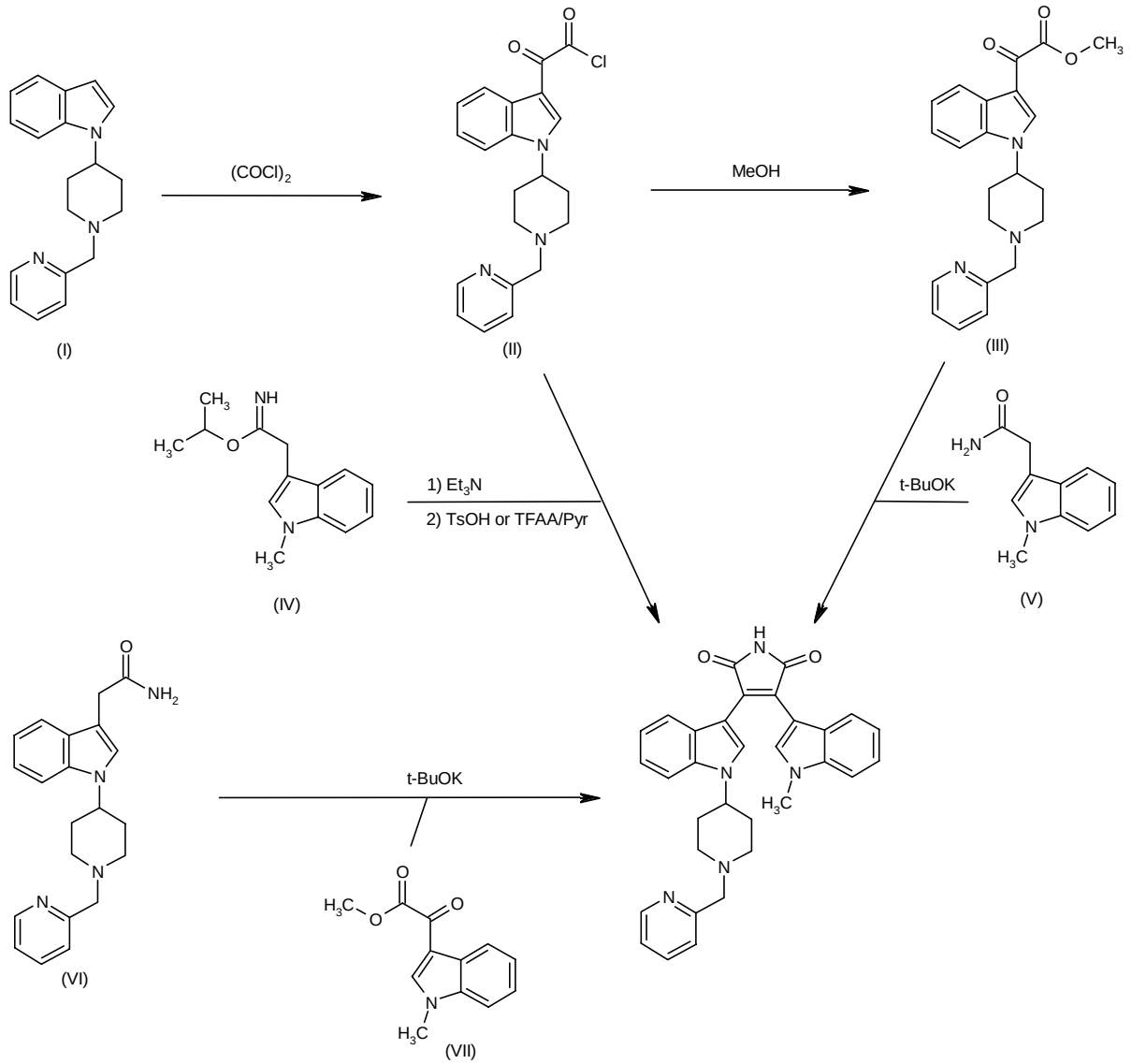
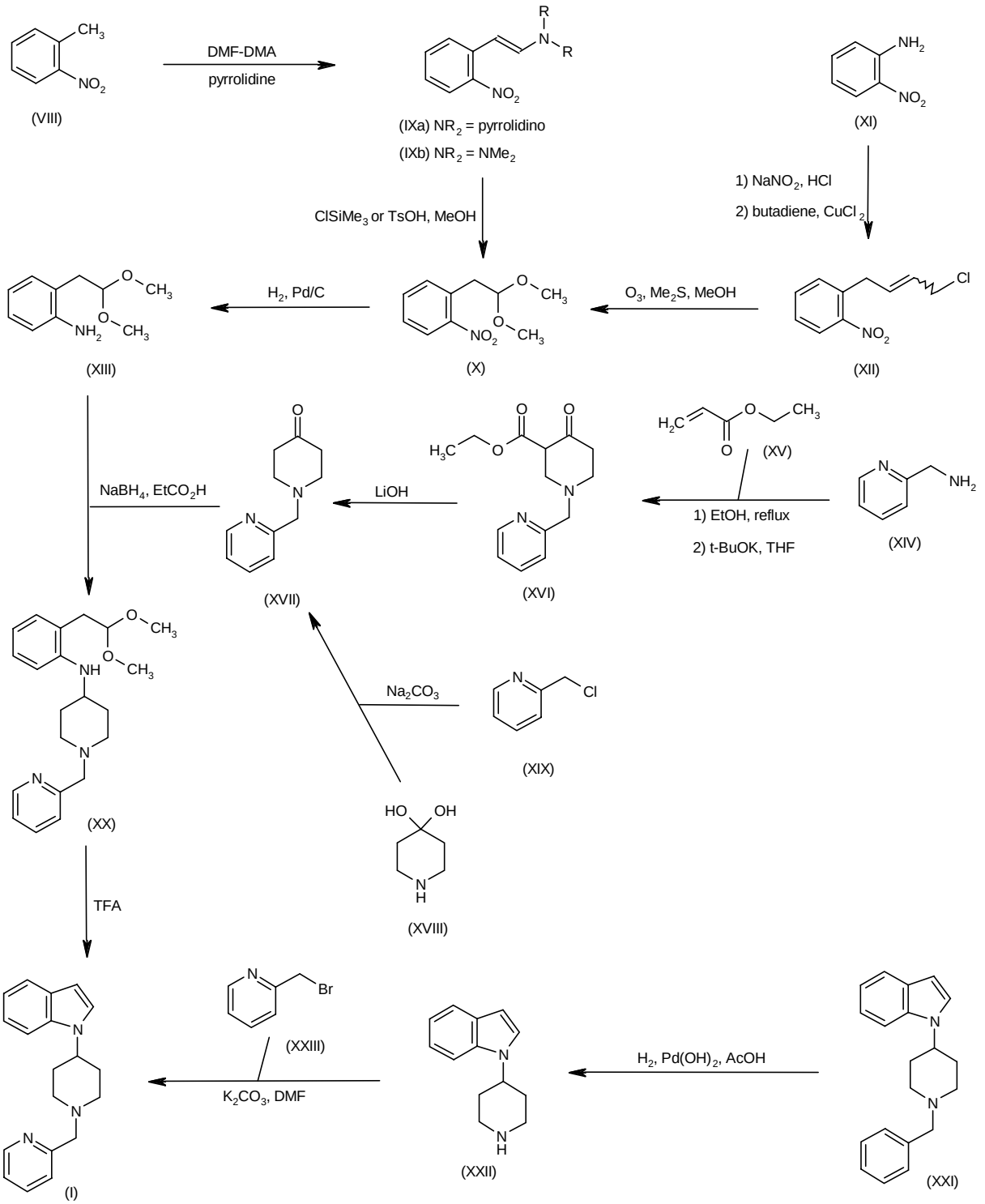
Scheme 1: Synthesis of Enzastaurin



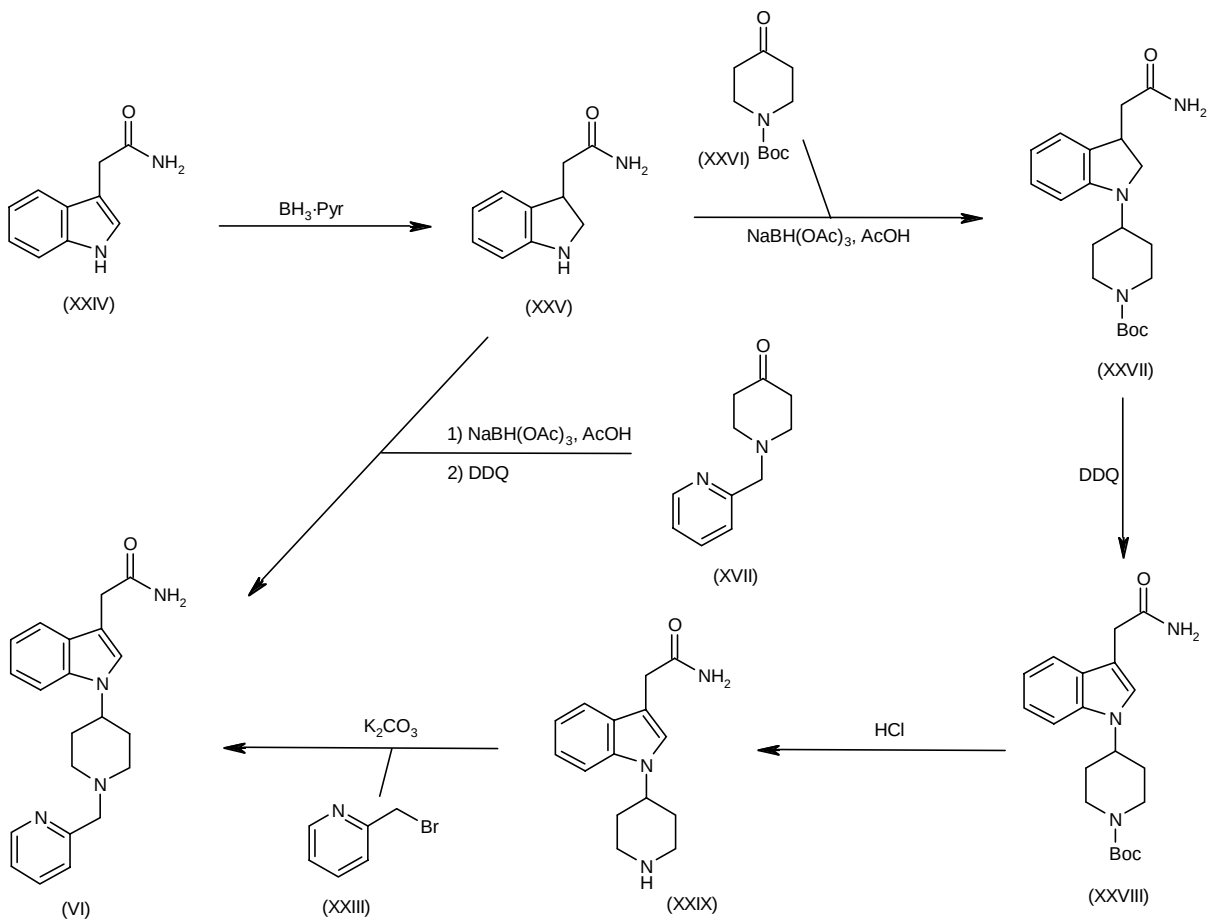
um *tert*-butoxide, provides the carbethoxy piperidone (XVI). Subsequent base-catalyzed decarboxylation of keto ester (XVI) generates the piperidone (XVII) (3). Alternatively, 1-(2-pyridylmethyl)-4-piperidone (XVII) is produced by alkylation of 4-piperidone hydrate (XVIII) with 2-picolyl chloride (XIX) in the presence of sodium carbonate. The reductive amination of piperidone (XVII) with amino acetal (XIII) using *in situ*-generated sodium tri(propionyloxy)borohydride furnishes the piperidinyl aniline (XX), which is cyclized to the target indole (I) upon treatment with trifluoroacetic acid (3-5). In a different strategy, 4-(1-indolyl)-1-(2-pyridylmethyl)piperidine (I) is prepared by catalytic hydrogenolysis of the *N*-benzyl piperidine (XXI) over $Pd(OH)_2$, followed by alkylation of the deprotected piperidine (XXII) with 2-(bromomethyl)-pyridine (XXIII) (1, 3). Scheme 2.

The intermediate piperidinyl indoleacetamide (VI) is prepared by the following sequence. Indole-3-acetamide (XXIV) is reduced to the corresponding indoline (XXV) employing borane-pyridine complex. Reductive alkylation of (XXV) with 1-Boc-4-piperidone (XXVI) in the presence of sodium triacetoxyborohydride gives the piperidinyl indoline (XXVII), which is further converted to indole (XXVIII) utilizing DDQ as the oxidizing reagent. After acidic cleavage of the *N*-Boc group of (XXVIII), the deprotected piperidine (XXIX) is alkylated with 2-(bromomethyl)pyridine (XXIII) to furnish the target intermediate (VI). In a related procedure, compound (VI) is

Scheme 2: Synthesis of Intermediate (I)



Scheme 3: Synthesis of Intermediate (VI)



prepared by reductive alkylation of indoline (XXV) with 1-(2-pyridylmethyl)-4-piperidone (XVII), followed by oxidation with DDQ (3). Scheme 3.

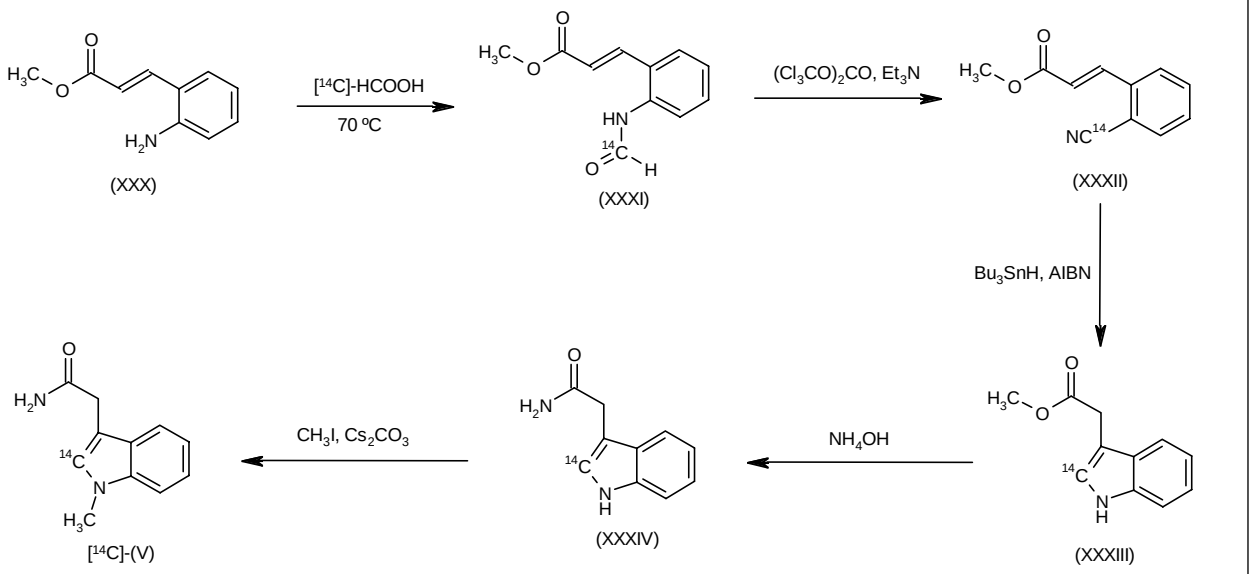
The preparation of radiolabeled (1-methylindol-3-yl)acetamide [^{14}C]-(V), with application as a synthetic precursor for [^{14}C]-enzastaurin, has also been described. Treatment of methyl *o*-aminocinnamate (XXX) with hot [^{14}C]-formic acid affords the radiolabeled formamide (XXXI), which is dehydrated to the isonitrile (XXXII) by means of triphosgene and triethylamine. Radical cyclization of (XXXII) in the presence of tributyltin hydride and AIBN, followed by reaction of the resulting indolylacetate ester (XXXIII) with concentrated NH_4OH in toluene in a sealed tube at 110 °C gives the indolylacetamide (XXXIV). The target radiolabeled intermediate (V) is then obtained by methylation of indole (XXXIV) with iodomethane and cesium carbonate (7). Scheme 4.

Background

The protein kinase C (PKC) family of serine/threonine kinases includes at least 11 isoforms that are involved in cell proliferation, cell differentiation, gene transcription, tumorigenesis and angiogenesis. The isoforms have differential tissue expression, subcellular locations and activator/substrate specificity, endowing each with distinct activities. Studies have demonstrated that PKC isozymes are involved in tumor initiation and progression via mediation of tumor cell survival and proliferation and tumor-induced angiogenesis. Moreover, PKC overexpression has also been linked to several types of cancer, including breast, colon, renal cell, hepatocellular, non-small cell lung (NSCLC) and prostate cancer and diffuse large B-cell lymphoma. Because the role of different isozymes varies in different diseases, researchers have focused on developing specific PKC isozyme inhibitors to target specific intracellular pathways (8-25).

The PKCβ isoform in particular has been implicated in several cancer types and is suspected to be involved in vascular endothelial growth factor (VEGF)-induced tumor development and angiogenesis and in the phosphatidylinositol 3-kinase (PI3K)/Akt pathway which regulates apoptosis, although the exact signaling cascade affected by this isozyme remains unclear. Nevertheless, targeting

Scheme 4: Synthesis of [^{14}C]-Labeled Intermediate (V)



of PKCβ represents a potentially effective strategy for the treatment of several cancer types (26, 27).

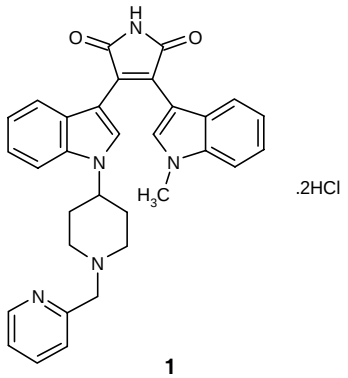
To date, there are only four PKC inhibitors under active development for the treatment of cancer, as shown in Table I. The most advanced agent is enzastaurin hydrochloride (LY-317615.HCl), an acyclic bisindolylmaleimide that competes with the adenosine triphosphate (ATP) binding site of PKC, thus preventing substrate phosphorylation. Enzastaurin exerts potent inhibitory activity against the PKCβ isoform and is relatively inactive against other cellular serine/threonine kinases or tyrosine kinases such as IκB, IκB kinase (IKK), c-Jun *N*-terminal kinase (JNK), mitogen-activated protein kinase (MAPK), MAPK kinase (MEK), stress-activated protein kinase (SAPK), AMP-activated protein kinase (AMPK), protein kinase C-related kinase 2 (PRK2, PKN2), protein kinase B β (PKBβ), PKBα, 3-phosphoinositide-dependent protein kinase 1 (PDPK1), epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), fibroblast growth factor receptor (FGFR), Bruton's tyrosine kinase (BTK), Src or ABL. Enzastaurin was discovered to target the PI3K/Akt pathway and inhibit glycogen synthase kinase-3 β (GSK-3 β) phosphorylation. Enzastaurin exerted potent anticancer activity in several preclinical cancer models and was chosen for further development as an anticancer agent (2, 28).

Preclinical Pharmacology

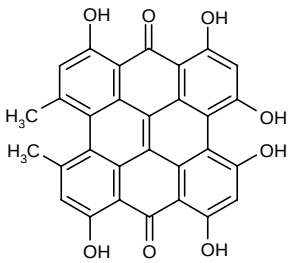
Results from kinase inhibition assays using human recombinant PKC isoforms showed that enzastaurin potently inhibited PKCβ with an IC_{50} value of 0.006 μM; IC_{50} values obtained for PKCα, PKCγ and PKCε were 0.039, 0.083 and 0.110 μM, respectively. The agent (0.1-10 μM) also directly suppressed proliferation of U-87 MG glioblastoma cells, PC-3 prostate carcinoma cells and HCT 116 colon carcinoma cells *in vitro* at low micromolar concentrations. Similar antiproliferative effects were seen against several other cancer cell lines, including leukemia (K-562, MOLT-4), NSCLC (A549, EKVX, HOP-92), colon cancer (COLO 205, SW620), central nervous system (CNS) cancer (SF-2956, SF-539, U-87 MG, U-251), melanoma (LOXIMVI, M14, SK-MEL-5, SK-MEL-28, UACC-257), ovarian (OVCAR-3, OVCAR-4, OVCAR-8), renal (Caki-1), prostate (PC-3) and breast cancer (MCF7, NCI/ADR-RES, MDA-MB-435). The most sensitive cell lines were K-562, MOLT-4, HOP-92 and PC-3. Several cell lines were unresponsive to the agent. These included prostate cancer DU 145, breast cancer Hs 578T, BT-549 and T-47D, melanoma Malme-3M, lung cancer HOP-62, NCI-H23, NCI-H322M and NCI-H460, ovarian cancer OVCAR-5 and SK-OV-1, and renal cancer 786-O, A-498, ACHN, RXF393 and TK10. Enzastaurin was also shown to induce concentration-dependent apoptosis in HCT 116 and U-87 MG cells, where treatment at concentrations of 1-4 μM resulted in oligonucleosomal fragmentation. Further examination of the mechanism of action of enzastaurin revealed that the agent suppressed GSK-3 β^{Ser9}, ribosomal protein $S6^{Ser240/244}$ and Akt^{Thr308} phosphorylation in human tumor cells, including U-87 MG, HT-29, PC-3 and Raji lymphoma cells. Enzastaurin also inhibited proliferation, induced apoptosis and altered cell cycle distribution in cancer cells engineered to be resistant to the EGFR inhibitors gefitinib and cetuximab. Increased expression of VEGFR-1 (Flt-1) was detected in those cells that were more sensitive to enzastaurin as compared to cells less sensitive to the agent. Results suggest that enzastaurin may also act via a VEGFR-1-mediated mechanism (28, 29).

Table I: Protein kinase C (PKC) inhibitors under active development for the treatment of cancer (from Prous Science Integrity®).

Drug	Source	Phase
1. Enzastaurin hydrochloride	Lilly	III
2. Hypericin	Yeda/National Cancer Centre Singapore	II
3. UCN-01 (NSC-638850, KRX-0601)	Keryx Biopharmaceuticals/National Cancer Institute	II
4. PhGα1*	PharmaGap	Preclinical



1



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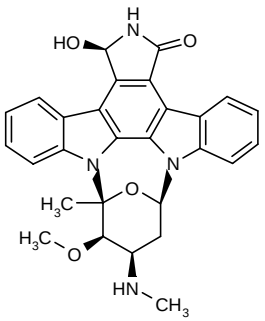

3

*Structure not available

The antiangiogenic effects of enzastaurin were examined *in vitro*. The agent more potently inhibited the growth of human umbilical vein endothelial cells (HUVECs) as compared to several human tumor cell lines, including human renal cell Caki-1 and small cell lung cancer (SCLC) SW2 cells. The agent was also shown to effectively inhibit both VEGF- and basic fibroblast growth factor (bFGF)-induced neovascularization in rat cornea (30, 31).

Enzastaurin exerted inhibitory activity against various types of lymphoma. Concentration-dependent antiproliferative effects were observed against five mantle cell lymphoma cell lines (Granta 519, HBL-2, JeKo-1, NCEB-1, REC-1) and two hematological control cell lines (Jurkat, Karpas 422). Cells exhibited differential susceptibility to the agent, as reflected by the IC_{50} values at 24 h for each (IC_{50} = 8 μM for JeKo-1 to > 50 μM for Granta 519, REC-1 and Karpas 422). The agent also induced apoptosis, moderate accumulation of cells in the G2/M phase of the cell cycle and alteration of Bcl-2, cyclin D1 and p14 RNA and protein expression. Sequence-dependent synergistic effects were observed when enzastaurin was combined with cytarabine or mitoxantrone, with superadditive effects observed when cells were first treated with either agent followed by enzastaurin; no synergistic effects were detected with combination treatment including fludarabine. Enzastaurin was cytotoxic against a panel of diffuse large cell lymphoma cell lines (OCI-Ly3, OCI-Ly7, OCI-Ly10, OCI-Ly19, SUDHL-4, SUDHL-6, Farage, Toledo), with an average IC_{50} value of 2 μM obtained at 48 h of incubation (IC_{50} = 0.5-4 μM). Enzastaurin was also effective in PKCβ-positive diffuse large B-cell lymphoma cell lines, where treatment decreased PKC enzymatic activity to undetectable levels and markedly inhibited proliferation (> 80%) and increased apoptosis (22-70%); PKCβ-negative diffuse large B-cell lymphoma cell lines were generally resistant to enzastaurin. Enzastaurin exerted proapoptotic effects on cutaneous T-cell lymphoma cell lines (HUT78 and HH). Treatment with the agent reduced cell viability and increased annexin V binding and the proportion of sub-G1 populations. Apoptosis appeared to involve caspase-3-activated cleavage of PARP and downregulation of AKT activity and its downstream effectors GSK-3 β and ribosomal S6. Significant concentration- and time-dependent antiproliferative activity for enzastaurin (2.5-20 μM) was demonstrated against the lymphoplasmacytic lymphoma (or Waldenström's macroglobulinemia) BCWM1 and WM-WSU cell lines. Enzastaurin induced concentration-dependent inhibition at 24 and 48 h, with induction of caspase-3, -8 and -9 and PARP cleavage noted, as well as a reduction in Bcl-x_L. Treatment inhibited the migration of these cells and Akt phosphorylation, Akt kinase activity, p-MARCKS and ribosomal p-S6. Synergistic activity was observed when enzastaurin was combined with bortezomib (2.5-10 nM) (32-36).

Several studies have demonstrated the *in vitro* efficacy of enzastaurin against multiple myeloma cell lines and multiple myeloma cells from patients. Experiments using dexamethasone-sensitive MM.1S, dexamethasone-resistant MM.1R, RPMI 8226, doxorubicin-resistant RPMI-Dox40, melphalan-resistant RPMI-LR5, IL-6-dependent INA6, U266, NCI-H929, OPM-1 and OPM-2 cells indicated that the agent inhibited phorbol ester (TPA)-triggered phosphorylation of PKC isoforms and phosphorylation of downstream PKCμ/PKD, MARCKS, GSK-3 β, JNK1/2, ERK1/2 and c-myc. In addition, proliferation, survival and migration of the cell lines and multiple myeloma cells isolated from multidrug-resistant patients were inhibited, as well as PKC activation stimulated by growth factors and

cytokines released from bone marrow stromal cells, fibronectin, VEGF, IL-6 or serum from patients with multiple myeloma. Enzastaurin in combination with bortezomib resulted in synergistic activity, while moderate synergism or additive effects were observed when it was combined with melphalan or lenalidomide. Further examination of the mechanism of action of enzastaurin was performed using the MM.1S cell line, with results showing that the presence of IL-6 or the pan-caspase inhibitor ZVAD-fmk did not influence cell death induced by the agent (1-3 μM for 72 h); insulin-like growth factor-I (IGF-I), although ineffective at the higher enzastaurin dose of 3 μM, abrogated cell death when the agent was added at 1 μM. The agent decreased both GSK-3 β and Akt phosphorylation and combination treatment including dexamethasone caused additive effects (37-39).

Enzastaurin significantly downregulated GSK-3 β and inhibited the proliferation (IC_{50} = 10 μM) of glioma cell lines. The agent decreased viability and induced cell cycle arrest in the G2/M phase and significantly upregulated the Wnt pathway, with concentration- and time-dependent inhibition of β-catenin phosphorylation observed (40).

Enzastaurin (1400 nM) inhibited the proliferation and decreased the survival of ML-1, CGTH-W1 and BHT-101 human thyroid cancer cell lines. Synergistic antiproliferative and cytotoxic effects were observed when enzastaurin was combined with the antifolate pemetrexed against all cell lines when continuously exposed to the agents; synergistic activity was also observed in the ML-1 and CGTH-W1 cell lines, but not BHT-101, after 1-h exposure to combination treatment (41).

The antiangiogenic activity of enzastaurin was demonstrated in the rat corneal micropocket assay and in nude mice bearing human SCLC xenografts (SW2). Oral administration of the agent (b.i.d. on days 1-10 postimplantation) reduced VEGF- or bFGF-induced neovascularization by about 50% (at 10 mg/kg) and 26% (at 30 mg/kg), respectively, in the rat corneal micropocket assay. Antitumor efficacy was observed, including a dose-dependent reduction in intratumoral vessels and tumor growth delay in mice treated with the agent (3, 10 or 30 mg/kg b.i.d. p.o. on days 14-30 postimplantation). Synergistic antitumor effects were noted when animals were first treated with paclitaxel (24 mg/kg i.v.) or carboplatin (50 mg/kg i.p.) followed by enzastaurin (42).

Enzastaurin showed considerable activity *in vivo* in several other tumor xenograft models. The agent (30 mg/kg b.i.d. p.o. for 8 weeks) significantly delayed tumor growth, prolonged survival and reduced angiogenesis in beige-nude Xid mice bearing s.c. MM.1S xenografts; body weight was not affected by treatment. Enzastaurin (80 mg/kg b.i.d. p.o.) also significantly inhibited tumor growth in SCID mice bearing WM xenografts (36, 37).

Treatment of athymic nude mice bearing s.c. human colon cancer cell (HCT 116) or human glioblastoma (U-87 MG) xenografts with enzastaurin (75 mg/kg b.i.d. p.o. starting when tumors reached about 100 mm^3 and continuing for 21 days) significantly inhibited tumor growth and GSK-3 β phosphorylation in tumor tissue and peripheral blood mononuclear cells (PBMCs). Moreover, mice bearing human SW2, Caki-1 or HCT 116 carcinoma xenografts and treated with the agent (100 mg/kg b.i.d. p.o. on days 14-30 postimplantation for SW2 and HCT 116 and days 21-39 for Caki-1) exhibited reductions in intratumoral vessel density of almost 40%. Significant reductions in plasma VEGF levels were also observed in enzastaurin-treated mice bearing SW2 and Caki-1 but not HCT 116 xenografts. Treatment had no effect on plasma bFGF or TGF-β levels (28, 43).

Although enzastaurin did not exert potent cytotoxic activity *in vitro* against a number of human cancer cell lines, it displayed potent antitumor effects in xenograft models using the same cell lines and enhanced the activity of other chemotherapeutics.

Enzastaurin was not very effective against the human T98G glioblastoma multiforme cell line *in vitro* (IC_{50} > 250 μM), but it was shown to enhance the antitumor activity of the nitrosurea BCNU in experiments using nude mice bearing s.c. or intracranial xenografts. Sequential treatment with BCNU (15 mg/kg i.p. on days 7-11 postimplantation) followed by enzastaurin (3, 10 or 30 mg/kg b.i.d. p.o. on days 14-30 postimplantation) was shown to be more effective in inhibiting tumor growth as compared to simultaneous treatment. Treatment with enzastaurin (30 mg/kg) alone increased survival of nude mice bearing intracranial T98G xenografts to 72 days as compared to 41 and 37 days for BCNU-treated animals and untreated controls, respectively. Moreover, while sequential treatment only increased survival to 74 days, simultaneous treatment further increased survival to 102 days (44).

Minimal cytotoxic activity was observed for enzastaurin against monolayer cultures of human renal carcinoma Caki-1 and colon carcinoma HT-29 cells (IC_{50} about 10 and 23 μM, respectively). However, antiangiogenic and antitumor activity for the agent (10 or 30 mg/kg b.i.d. p.o. on days 14-30 postimplantation) was demonstrated in nude mice bearing these xenografts. Treatment reduced the number of intratumoral vessels by 20% and 40-50%, respectively. Enzastaurin (10 or 30 mg/kg b.i.d. p.o. on days 7-11 postimplantation) also enhanced HT-29 tumor growth delay when simultaneously combined with 5-fluorouracil (5-FU; 30 mg/kg i.p.) or cisplatin (10 mg/kg i.p.). Caki-1 tumor growth delay was enhanced when enzastaurin (3, 10 or 30 mg/kg b.i.d. p.o. on days 4-18 or 14-30 postimplantation) was combined with fractionated radiation therapy (3 Gy on days 7-11 and 14-18 postimplantation) and/or gemcitabine (60 mg/kg i.p. on days 8, 11, 14 and 17 postimplantation) in a simultaneous, overlapping or sequential regimen (45).

Enzastaurin was not highly cytotoxic against human Hep 3B hepatocellular and Hs 746T gastric cancer cell lines *in vitro* (IC_{50} > 250 and 100 μM, respectively), but it exhibited antitumor efficacy when administered orally (3, 10 or 30 mg/kg b.i.d. on days 4-18 or 14-30 postimplantation) to nude mice bearing Hep 3B or Hs 746T xenografts. While only small reductions were observed in CD31-positive Hs 746T intratumoral vessels, a 60%

decrease was seen in CD31-positive Hep 3B intratumoral vessels; the agent caused larger decreases in CD105-positive intratumoral vessels in both models. Tumor growth delays with treatment were 6.5-15 days and 5-25 days for the Hs 746T and Hep 3B models, respectively. Enzastaurin enhanced Hep 3B tumor growth delays observed with 5-FU (30 mg/kg i.p. on days 7-11 postimplantation) when administered simultaneously or sequentially (31 and 43 days, respectively, *vs.* 9 days with 5-FU alone) and enhanced gemcitabine (60 mg/kg i.p. on days 8, 11, 14 and 17 postimplantation)-induced Hs 746T tumor growth delay when the agents were given simultaneously or sequentially (40 and 30 days, respectively, *vs.* 15 days with gemcitabine alone) (46).

While it exerted little activity *in vitro* against Calu-6 cells (IC_{50} = 26 μM), effective antiangiogenic and antitumor effects were observed for enzastaurin (30 mg/kg b.i.d. p.o. on days 4-18 or 14-30 postimplantation) against s.c. Calu-6 xenografts in nude mice. Treatment decreased CD31- and CD105-positive intratumoral vessels by 50%. In this model and a Lewis lung carcinoma xenograft model, enzastaurin enhanced tumor growth delays seen with paclitaxel (24 mg/kg i.v. on days 7, 9, 11 and 13 postimplantation), carboplatin (50 mg/kg i.p. on day 7, 10 or 13 postimplantation) and gemcitabine (60 mg/kg i.p. on days 7, 10 and 13 postimplantation) by 5-, 1.7- and 2-fold, respectively, as well as those observed with fractionated radiation therapy. Moreover, the number of lung metastases was reduced with each combination treatment (47).

Although little efficacy was observed for enzastaurin against MX-1 breast cancer cells and SK-OV-3 ovarian carcinoma cells *in vitro* (IC_{50} = 8.1 and 9.5 μM, respectively), significant antiangiogenic and growth-inhibitory activity and improved survival were observed in nude mice bearing s.c. xenografts and treated with the agent (30 mg/kg b.i.d. p.o. on days 4-25 or 14-30 postimplantation). Reductions in CD31- and CD105-positive MX-1 (35% and 43%, respectively) and SK-OV-3 (60% and 75%, respectively) intratumoral vessels, significant growth delays (20 days for MX-1) and significant increases in survival (73 days *vs.* 38 days for SK-OV-3) were reported with treatment. Enzastaurin also enhanced paclitaxel- and carboplatin-induced MX-1 tumor growth delays by 1.7- and 3.8-fold, respectively, and potentiated growth delays seen with fractionated radiation therapy. Moreover, enzastaurin enhanced survival rates in mice bearing SK-OV-3 xenografts treated with carboplatin (by 1.8-fold) or paclitaxel (100% survival at 120 days) (48).

Preclinical data suggest that enzastaurin may potentiate the radiosensitivity of murine 4T1 breast cancer cell bone tumors, suppressing tumor growth, bone osteolysis and cancer-induced bone pain. Experiments performed in C3H/SCID mice bearing intrafemoral 4T1 tumors showed that treatment with enzastaurin (75 mg/kg b.i.d. p.o.) or local irradiation (30 Gy) alone had no significant effect on bone destruction scores. However, their combination significantly decreased these indices. Moreover, combination therapy also significantly decreased bone pain (49).

Safety

Enzastaurin had no tetratogenic or detrimental effects on embryofetal development in experiments conducted in rats (5, 20 and 75 mg/kg) and New Zealand white rabbits (100 and 300 mg/kg). Rabbits receiving 1000 mg/kg enzastaurin exhibited severe adverse events, including prolonged anorexia, reductions in body weight and increases in abortion rate. Reductions in maternal body weight and food consumption were also observed in rabbits given the two lower enzastaurin doses. The no-observed-adverse-effect level (NOAEL) for developmental toxicity in the rabbit was concluded to be 300 mg/kg; the maternal NOAEL for rabbits could not be determined in this study. Dose-related reductions in rat maternal body weight and food consumption were seen with 20 and 75 mg/kg and the maternal and developmental NOAELs for rats were concluded to be 5 and 75 mg/kg, respectively (50).

Clinical Studies

Ex vivo determination of intracellular phosphoprotein signaling, including PKC activity in PBMCs from 5 patients with advanced nonhematological malignancies participating in a phase I dose-escalation trial and receiving enzastaurin (525 mg/day p.o. for up to 28 days), revealed inhibition of PMA-induced PKC activity in all but 1 patient sample. Similarly, analysis of PBMC samples from 9 cancer patients involved in another phase I trial and who had received enzastaurin in combination with capecitabine demonstrated significant reductions in PKC activity after enzastaurin (350 or 525 mg/day p.o. for 14 days) (51).

A phase I dose-escalation study was conducted in 47 patients with advanced cancer (mostly lung and head and neck cancers) to determine the recommended phase II dose of enzastaurin (5-, 25- or 100-mg capsule formulation starting at 20 mg once daily for 28 days followed by a 4-day washout period) in this patient population. All patients received at least one dose of the agent (median = 2 cycles). The maximum tolerated dose (MTD) was not reached even at doses up to 700 mg/day. The recommended dose was concluded to be 525 mg/day and 12 additional patients were accrued at this dose. Dose-limiting toxicity (DLT) of Q-T_c alteration was seen in 1 patient at 700 mg/day, leading the patient to discontinue treatment, and in 2 patients receiving 525 mg/day. Plasma levels of enzastaurin and its metabolites increased with doses up to 240 mg, with peaks detected at 525 and 700 mg. No significant grade 3 or 4 adverse events were reported and 2 deaths occurred that were unrelated to treatment. The most frequent adverse events were grade 1 chromaturia, fatigue and other gastrointestinal toxicities. Stable disease was seen in 21 patients (45%) for 2-16 cycles (52).

The safety and antitumor efficacy of enzastaurin (400 mg t.i.d. as loading dose on day 4 of a 21-day cycle and 1500 mg p.o. once daily on days 5-21) in combination

with pemetrexed (500 mg/m^2 i.v. on day 1) were evaluated in a phase 1b study in 42 patients with advanced or metastatic cancer, the majority of whom had received prior chemotherapy. Patients received daily oral folic acid and vitamin B_{12} every 9 weeks and 5-7 days before cycle 1. A total of 36 patients received 2 or more cycles, with 8 patients receiving 6 or more. Reported drug-related grade 3 or higher hematological adverse events reported included anemia (n=2), leukopenia (n=1), thrombocytopenia (n=4) and neutropenia (n=3). One case each of grade 3 ulcer and gastrointestinal-liver dysfunction were reported. A partial response was observed in 2 patients with thyroid carcinoma and 11 patients had stable disease (median duration = 5.6 months) (53).

Another phase I dose-escalation study examined the safety and efficacy of oral enzastaurin (350-700 mg/day on days 1-21) combined with capecitabine (750-1000 mg/m^2/day b.i.d. p.o. on days 1-14). A total of 27 patients with advanced, refractory solid tumors were treated for a median of 3 cycles. There was no significant pharmacokinetic interaction between the agents and combination treatment was well tolerated. No grade 4 adverse events were reported. Grade 3 anemia (n=1), headache (n=1), intestinal perforation (n=1 with gastric cancer), fatigue (n=1), hand-foot syndrome (n=2) and cardiac ischemia (n=1) were seen. No objective tumor responses were achieved, although 5 patients with cancer of the lung, breast, pancreas, head and neck or hemangiopericytoma had stable disease for 6 months or more. The recommended phase II dose was concluded to be 500 mg once daily enzastaurin and 1000 mg/m^2 b.i.d. capecitabine on days 1-14 every 21 days (54, 55).

The safety and efficacy of enzastaurin (350 mg once daily to 500 mg b.i.d. p.o. alone in a 14-day lead-in period in cycle 1 to achieve steady state and on days 1-21 in subsequent cycles) combined with gemcitabine (1000 or 1250 mg/m^2 by 30-min i.v. infusion on days 1 and 8 every 21 days) and cisplatin (60 or 75 mg/m^2 by 3-h i.v. infusion on day 1 every 21 days) for up to 7 cycles were examined in a phase I trial conducted in 33 patients with advanced, refractory solid tumors. Combination therapy was well tolerated, with no significant pharmacokinetic interactions observed among the agents. The MTD was not reached. Two patients in the b.i.d. dosing groups experienced DLTs of grade 2 Q-T_c prolongation and grade 3 fatigue during cycle 1. Grade 3/4 neutropenia (n=3/6), grade 3/4 thrombocytopenia (n=1/6), grade 3 leukopenia (n=2), hyperglycemia (n=1) and elevated creatinine (n=1) were also reported. Twice-daily enzastaurin at doses above 250 mg resulted in more discontinuations in the early cycles, more dose adjustments and higher rates of serious adverse events possibly related to treatment and low-grade toxicities as compared to once-daily dosing. A total of 24 patients were evaluable for response. Partial responses were achieved in 3 patients with pancreatic, head and neck or ovarian cancers and stable disease for 2 cycles or more was obtained in 13 patients (56).

A phase II trial in 53 patients with advanced stage IIIB or metastatic stage IV NSCLC who had failed at least one prior therapy examined the tolerability and efficacy of enzastaurin (500 mg once daily every 28 days) as a second- or third-line treatment. Thirty-four patients were alive at interim analysis. Ten patients received 6 or more cycles, 3 of whom continued for more than 9 cycles. The most frequent toxicity reported was fatigue (n=21), occurring within 1 week of onset of treatment but not seen in patients with stable disease. The mean progression-free survival (PFS) was 1.84 months and the PFS rate at 6 months was 10.4%. No partial or complete responses were observed, although stable disease was achieved in 18 patients (57).

The efficacy and safety of enzastaurin (525-mg capsules amended to 500-mg tablets once daily in a 28-day cycle) in 55 patients with relapsed diffuse large B-cell lymphoma were investigated in another phase II trial. All patients received at least one dose of the agent and were evaluable and 12 were alive and progression-free at the end of 2 cycles. One case each of grade 4 hypomagnesemia and grade 3 thrombocytopenia were reported; no grade 3 or 4 neutropenia was seen. Other grade 3 toxicities included fatigue (n=2), edema (n= 1), headache (n=1) and motor neuropathy (n=1). Two of the 12 surviving patients with elevated lactate dehydrogenase (LDH) experienced normalization of levels with treatment. Stable disease was seen in 9 patients (2 of whom had relapsed after prior stem cell therapy) for at least 4 cycles. One of these patients achieved a complete response and 5 achieved a minor response (lesion shrinkage of 25-< 50%). Three patients were progression-free for 12 cycles or more (58).

The efficacy and safety of enzastaurin (500 mg p.o. once daily) were examined in a single-arm, multicenter phase II trial conducted in 60 patients with relapsed or refractory CD20$^+$ mantle cell lymphoma and a maximum of four prior chemotherapies. Treatment was well tolerated. No drug-related deaths or grade 4 toxicities were seen. Three patients discontinued due to adverse events (diarrhea, renal impairment or syncope) possibly related to treatment. The most frequent adverse event was grade 1 or 2 fatigue (n=5). One case each of grade 3 anemia, diarrhea, dyspnea, vomiting, hypotension and syncope were also reported. Although no objective tumor responses were observed, 22 patients were progression-free for 3 cycles or more, 6 of whom had stable disease for 6-22 months (59).

The tolerability and antitumor efficacy of oral enzastaurin (500 mg/day in a 6-week cycle) were also demonstrated in a phase II trial in patients with recurrent high-grade gliomas. In group A (n=70, not on antiepileptic drugs) and group B (n=15, receiving antiepileptics), 31 and 5 patients, respectively, received more than 1 cycle. Treatment was well tolerated, with few cases of adverse events of > grade 1; these included grade 2, 3 and 4 hematological toxicities in 4, 2 and 1 patient, respectively, and grade 2 hepatotoxicity in 1 patient. Thirteen patients were stable for more than 3 months and 14 showed objective radiographic responses, including 1 complete response. The patient population was in-

creased to 120 (72% with glioblastoma multiforme) and further results from 104 patients were reported regarding the effects of treatment on health-related quality of life (HRQL). The rates of patients benefiting from at least 2 treatment cycles were 13%, 11% and 10%, respectively, for the brain tumor subscale, trial outcome index and total Functional Assessment of Cancer Therapy-Brain score. It was concluded that the majority of patients experienced stabilization or improvements in HRQL with enzastaurin treatment (60, 61).

Enzastaurin continues to undergo phase III development to determine its efficacy in preventing relapse of lymphoma and in combination with lomustine for the treatment of intracranial glioblastoma. Phase I-II testing is ongoing in patients with recurrent high-grade gliomas, as well as phase II development for relapsed or refractory diffuse large B-cell lymphoma, relapsed mantle cell lymphoma, breast, ovarian, pancreatic, metastatic colorectal, advanced NSCLC and other advanced or metastatic cancers (62-75). A phase II trial evaluating the efficacy of enzastaurin for preventing NSCLC in former smokers has been suspended (76).

Source

Eli Lilly and Company (US).

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