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MONOGRAPH

VERUBULIN HYDROCHLORIDE

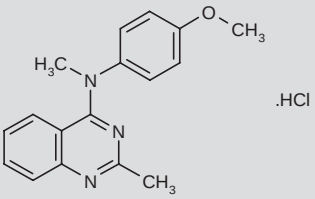
Prop INNM; USAN

Apoptosis Inducer
Vascular Disrupting Agent
Oncolytic

EP-128495
MPC-6827
MX-128495
Varbulin (former name)
Azixa™

N-(4-Methoxyphenyl)-*N*,2-dimethylquinazolin-4-amine hydrochloride

InChI: 1S/C17H17N3O.ClH/c1-12-18-16-7-5-4-6-15(16)17(19-12)20(2)13-8-10-14(21-3)11-9-13;/h4-11H,1-3H3;1H



$C_{17}H_{18}ClN_3O$
Mol wt: 315.797
CAS: 917369-31-4
CAS: 827031-83-4 (free base)
EN: 354069

SUMMARY

Despite the current focus on innovative drug delivery regimens and promising molecular therapies targeting growth factor signaling and angiogenesis, chemotherapy and radiation therapy remain the cornerstones of malignant glioma treatment. Therefore, it is critically important to evaluate new chemotherapeutic agents for their likelihood of success in the tightly protected and sensitive milieu of the central nervous system (CNS). Verubulin hydrochloride (MPC-6827) is a 4-arylaminoquinazoline with two distinct mechanisms of action. First, it causes apoptosis through the inhibition of tubulin polymerization, resulting in cell cycle arrest and cell death in a manner similar to other microtubule-interfering, proapoptotic chemotherapeutic agents, including the taxanes and the vinca alkaloids. Second, verubulin also has a vascular disrupting action through disrupting the microtubule cytoarchitecture in newly forming vessels. In preclinical testing, the agent was found to have excellent blood–brain barrier penetration, efficacy in multidrug-resistant cell lines and low CNS toxicity, making it a particularly attractive candidate as a brain tumor chemotherapeutic.

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*Synthesis prepared by J. Bolòs, R. Castañer. Thomson Reuters, Provença 388, 08025 Barcelona, Spain.

SYNTHESIS*

Verubulin can be prepared by several related ways:

a) Amination and cyclization of methyl 2-aminobenzoate (I) with acetonitrile in the presence of HCl at reflux gives 2-methyl-4-quinazolinone (II), which by chlorination by means of $POCl_3$ in the presence of DIEA in refluxing toluene or in the absence at 120 °C yields 4-chloro-2-methylquinazoline (III). Finally, intermediate (III) is condensed with *N*-(4-methoxyphenyl)-*N*-methylamine (IV) in the presence of HCl in isopropanol (1-4). Scheme 1.

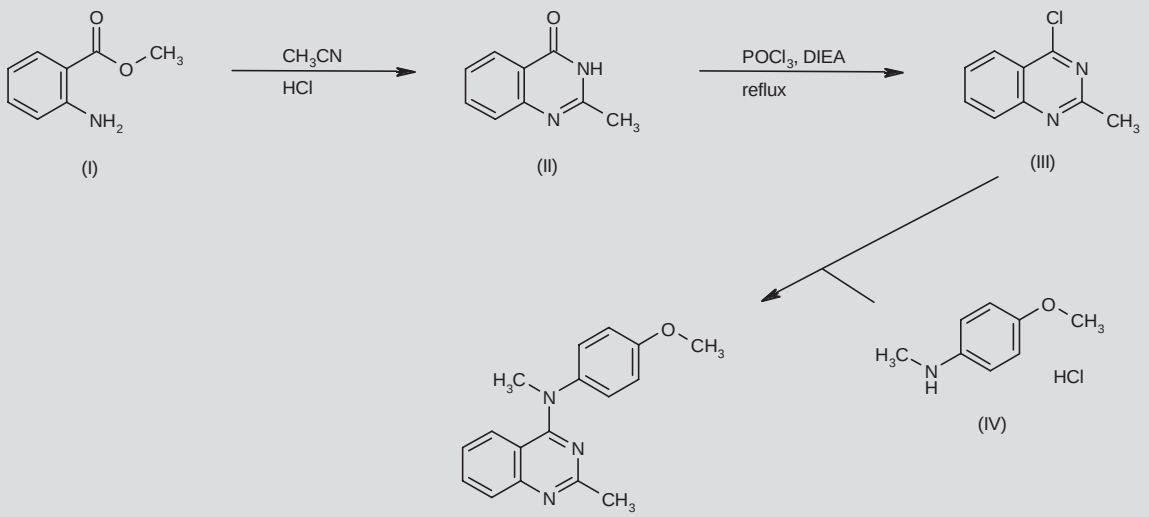
b) Treatment of 2-aminobenzonitrile (V) with *N,N*-dimethylacetamide dimethylacetal (VI) at 115 °C yields *N'*-(2-cyanophenyl)-*N,N*-dimethylacetamidine (VII), which by cyclization with 4-methoxyaniline (VIII) in AcOH/acetonitrile at 118 °C gives *N*-(4-methoxyphenyl)- 2-methylquinazolin-4-amine (IX). Finally, the secondary amine group in compound (IX) is methylated by means of NaH in DMF (5). Scheme 2.

c) Intramolecular cyclization of the acetamidine (II) with 4-methoxy-*N*-methylaniline (IV) in the presence of $AlCl_3$ in NMP at 200 °C (5). Scheme 2.

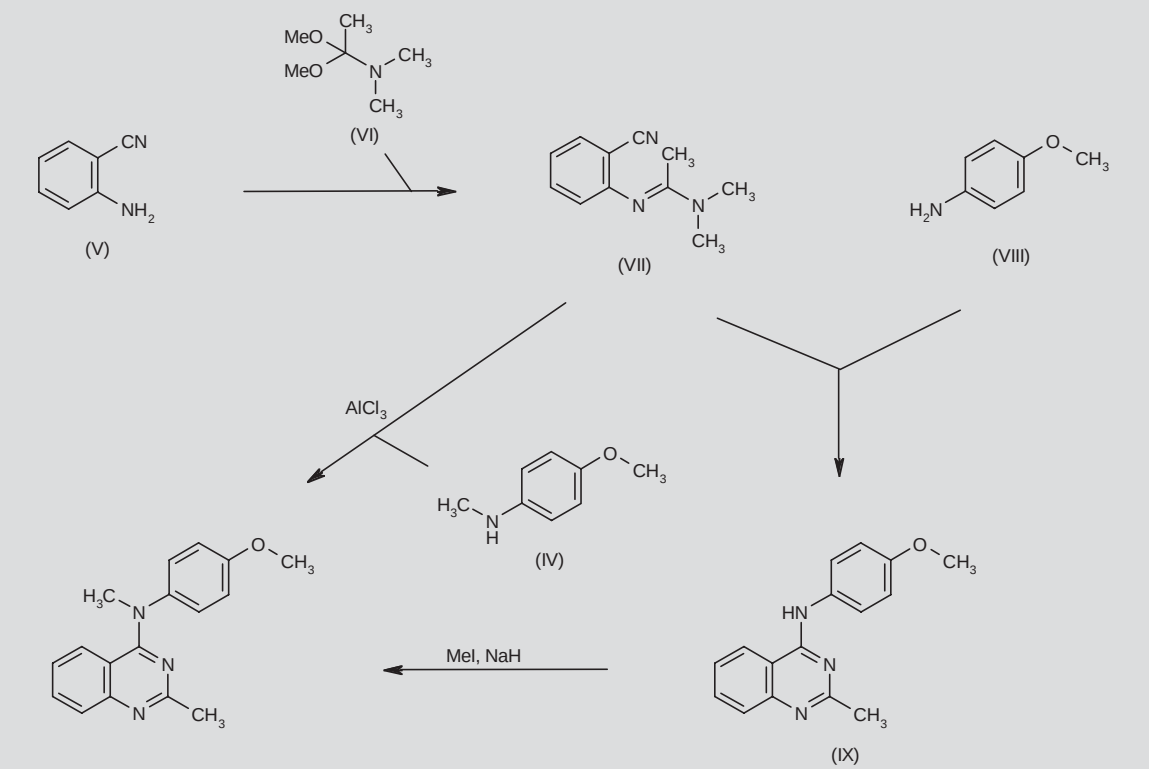
BACKGROUND

According to the Central Brain Tumor registry of the United States, an estimated 62,930 new cases of primary nonmalignant and malignant brain and central nervous system (CNS) tumors are

Scheme 1. Synthesis of Verubulin



Scheme 2. Synthesis of Verubulin



expected to be diagnosed in the U.S. in 2010 (6). Glioblastoma, the most common and devastating of these tumors, has approximately a 15-month median survival for patients below 70 years of age at diagnosis (7).

Despite numerous phase II and III studies examining dozens of agents, almost 30 years elapsed between the seminal publication in 1976 demonstrating the superiority of irradiation over supportive care (8) and the first convincing evidence demonstrating the impact

of adding chemotherapy in the form of temozolomide over irradiation alone (9). Such a dismal performance for chemotherapy results not only from the relative chemoresistance of these cancers, but also from a number of additional unique obstacles that impede delivery of effective amounts of agent into brain tumors.

In addition to its activity versus the malignant glioma cell itself, the effectiveness of a chemotherapeutic agent is highly dependent on several factors relating to its ability to attain high enough concentrations in the CNS, including the ability to penetrate the blood–brain barrier (BBB), the extent to which it is actively transported out of the brain and its volume of distribution in the brain parenchyma (10). Thus, because agents must first dissolve in the lipid membranes of the BBB, unidirectional transfer is highly correlated with lipophilicity. Additionally, the transfer coefficient of many agents falls well below what would be predicted by their lipophilicity due to other important factors, such as plasma protein binding, solute molecular weight and active efflux transport (11). For instance, agents such as vincristine and etoposide bind more than 90% to plasma proteins, reducing the free fraction of drug in plasma available to cross the BBB (12). Furthermore, passage can be impeded due to size; the BBB blocks transvascular leakage of most molecules larger than 180 kD, a weight exceeded by many chemotherapeutic agents (13). Finally, the BBB expresses high levels of drug efflux pumps such as P-glycoprotein and other multidrug resistance transporters that actively remove chemotherapeutic drugs such as vincristine, etoposide and paclitaxel (14). This effect can be profound; inhibiting these pumps can increase brain uptake by as much as 50-fold (15). Finally, in addition to the difficulty in delivering chemotherapy to the tumor, chemotherapeutic effectiveness is hampered by the exquisite sensitivity of the CNS to drug toxicity, resulting in a therapeutic index for most agents that is essentially zero.

Almost all conventional chemotherapeutic agents have some degree of CNS toxicity (16). Toxicity is influenced by the dose, frequency of exposure and route of administration. Common CNS toxicities include acute and chronic encephalopathy, seizures, headache, vasculopathy and stroke, myelopathy, posterior reversible encephalopathy syndrome, aseptic meningitis, dementia and cranial nerve palsies. These toxicities are often the dose-limiting factor. Not surprisingly, therefore, a great deal of effort has been directed towards the development of methods that would increase drug concentration in tumors. One approach that has been intensively studied is to deliver therapy via intra-arterial catheters; for example, the etoposide concentration was increased fourfold after intra-arterial infusion compared with i.v. infusion (17). This approach has been studied extensively with a variety of agents, including nitrosoureas and platinum-based agents, in a number of studies dating back to the 1980s; unfortunately, a survival advantage has not been demonstrated.

Another approach to increase drug delivery involves direct intratumoral placement of chemotherapy. The Gliadel wafer, a biodegradable polymer infused with carmustine (BCNU) that is implanted into the tumor resection cavity, is an FDA-approved polymeric system for the local delivery of drugs to brain tumors that circumvents the BBB through direct installation of chemotherapy. Two phase III studies in newly diagnosed and recurrent glioblastoma multiforme (GBM) demonstrated a modest survival benefit of approximately 8 weeks with the Gliadel wafers compared with placebo (18, 19). Since treatment was compared with placebo wafers, it is unclear, however, whether this represented a superior effect over i.v. BCNU. Another drug delivery method currently undergoing investigation involves implanting a catheter in the brain and diffusing macromolecules into the brain at a set rate via a pump under a set pressure. This has been shown to distribute molecules further into the brain parenchyma, potentially providing a larger therapeutic target, but evidence that this clearly results in benefit is still lacking (20).

An alternative strategy to increasing the delivery of an agent is BBB disruption. RMP-7 is a bradykinin analogue receptor-mediated permeabilizer that increases tight junction permeability by activating bradykinin B_2 receptors on endothelial cells. Although it showed promise in experimental animal studies, a phase II study revealed no benefit for this agent in improving the outcome for patients receiving carboplatin for recurrent glioma (21).

Based on the above, one could posit a number of features that would characterize an agent with particular effectiveness versus malignant brain tumors, including high activity versus brain cancer cells, lipophilicity, resistance to multidrug resistance transporters, low protein binding and the absence of significant CNS toxicity at effective therapeutic doses. Verubulin appears to favorably address many of these major challenges facing any agent to be used against CNS malignancies (Table I).

PRECLINICAL PHARMACOLOGY

The effectiveness of tubulin targets in the treatment of glioblastoma has been well recognized in the success of the taxanes and vinca alkaloids, at least in vitro (22). Verubulin causes apoptosis through the inhibition of tubulin polymerization, resulting in cell cycle arrest and cell death in the G_2/M phase of the cell cycle, similar to the taxanes and vinca alkaloids, an effect attributable to it being a potent activator of caspases, leading to cell death in various tumor lines (1). Competition studies revealed that verubulin was found to compete with colchicines and paclitaxel but not vincristine in binding a targeted protein, suggesting that the target of verubulin is likely tubulin (23). In addition, verubulin has a second mechanism of action as a vascular disrupting agent, perturbing proper microtubule function in developing blood vessels. Glioblastomas are known to be highly vascular tumors and recent experience has demonstrated a survival benefit for patients with recurrent GBM using the antiangiogenic agent bevacizumab (24). Therefore, the prospect of an agent with dual actions in being toxic to both tumor-associated blood vessels and glioma cells is a very attractive aspect of this agent.

Table I. *Important determinants of efficacy for an active agent versus central nervous system malignancies.*

Verubulin	
Activity vs. glioma cells	Yes
Lipophilic compound	Yes
Low molecular weight	Yes
Minimal to no binding to plasma proteins	No
Not actively exported by efflux pumps	Yes
High CNS/plasma concentrations	Yes

In preclinical studies verubulin was shown to be effective in cell lines expressing high levels of multidrug resistance (MDR). Compared with vinblastine and docetaxel, verubulin was much more potent versus human breast carcinoma and leukemia cell lines overexpressing multidrug resistance protein 1 pumps and was demonstrated not to be a substrate for the MDR transporters (23).

PHARMACOKINETICS AND METABOLISM

The pharmacokinetics of verubulin have been evaluated in mice, rats and dogs in preclinical studies. Following i.v. administration of a single dose, the half-life ($t_{1/2}$) of verubulin was in the range of 2.75 to 4.4 h. Furthermore, analysis of plasma from blood samples and homogenized brain samples obtained from mice after an i.v. dose of 2.5 mg/kg revealed the brain/plasma ratio for exposure to be 16, indicating rapid and extensive distribution into brain tissue (1).

Verubulin is 98% protein-bound in rat and 98.2% protein-bound in human plasma. Its major metabolites are *O*-demethyl and *N*-demethyl metabolites and their glucuronide and sulfate conjugates. Verubulin and its major metabolites are metabolized mainly by the liver and excreted renally. The major cytochrome P450 enzymes involved in the metabolism of verubulin are CYP2C19, followed by CYP1A2 and CYP3A4. Verubulin has the greatest in vitro inhibition with CYP2C19, the enzyme responsible for the metabolism of clopidogrel (Plavix®). It is speculated that the concurrent use of verubulin with clopidogrel could result in elevated levels of clopidogrel and subsequent toxicity. For this reason, the use of clopidogrel in conjunction with verubulin has been avoided (Beelen, personal communication).

The pharmacokinetics of verubulin have also been studied in clinical trials. In a dose-escalation study verubulin demonstrated a $t_{1/2}$ of 5.46 ± 2.35 h and 7.4 ± 3.76 h, respectively, on days 1 and 15 following i.v. administration of 3.3 mg/m^2. Exposure as measured by AUC and C_{max} was variable, an effect likely due to variations in infusion duration and sampling times. A consistent increase in exposure paralleling an increase in dose was found, suggesting linear kinetics and lack of saturation. Pharmacokinetic analysis demonstrated a rapid redistribution of verubulin. The $t_{1/2}$ of the *O*-demethyl metabolite appeared to mirror that of the parent compound. In a combination study of verubulin and temozolomide, increasing doses of verubulin did not affect the pharmacokinetics of temozolomide (Beelen, personal communication).

Dose-escalating studies demonstrated that the maximum tolerated dose of verubulin is 3.3 mg/m^2 administered over 2 h. The pharmacokinetic parameters of verubulin were found to be similar as monotherapy and in combination with temozolomide or carboplatin and required no dose adjustments (Beelen, personal communication).

SAFETY

Verubulin has been generally well tolerated in clinical studies to date. In monotherapy studies, observed toxicities in patients receiving verubulin at doses at or below 3.3 mg/m^2 were generally mild and included fatigue, nausea, constipation and headache. Unlike the experience with other vinca alkaloids, neurological complications such as neuropathy have not been observed as yet. As recently as March 2010 there had been nine serious adverse events reported that were considered possibly, probably or definitely related to verubulin. These include two hypersensitivity events in one patient, two nonfatal myocardial infarctions, one cerebral ischemia, one elevated troponin, three events of cerebral hemorrhage and CNS hemorrhage, and one event of grade IV hypertension.

CLINICAL STUDIES

The clinical experience with verubulin is restricted to phase I and II studies in which it has been used both as monotherapy and in combination with other chemotherapeutics. When used in combination with carboplatin (23) in patients with relapsed glioblastoma multiforme who had failed temozolomide, verubulin was found to be well tolerated and no dose reduction of carboplatin was necessary. Two patients achieved a partial response and six remained stable, with an overall response rate (i.e., partial response and stable disease) of 42%. Response duration extending to 7 months was noted, although the median progression-free survival (PFS) was only 1.8 months (25).

The combination of multiple doses of verubulin and temozolomide has also been studied in 22 patients with stage IV metastatic melanoma, 10 of whom had brain metastasis (26). Twenty patients had had prior systemic chemotherapy with either temozolomide or dacarbazine. Verubulin was dosed at 2.1, 2.7 or 3.3 mg/m^2 in combination with temozolomide 75 or 85 mg/m^2. Dose reduction of temozolomide was not necessary and verubulin was safe and well tolerated at all dose levels. Two patients had partial responses of 4 and 8 months and another 10 achieved stable disease. Median PFS was 2.8 months, which compared favorably with a prior phase III study of temozolomide and dacarbazine in which PFS was < 2 months (27).

Currently, plans are under way for the next clinical study with verubulin, which will address its efficacy as an upfront therapy for newly diagnosed GBM patients in a randomized, two-arm phase IIb study in combination with standard-of-care radiation therapy and temozolomide compared to standard of care alone.

SOURCES

EpiCept Corp. (US); licensed to Myrexis, Inc. (US).

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