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Orally bioavailable proteasome inhibitors: preclinical development of PR-047

Proteasome is a protease complex that is responsible for intracellular protein degradation via the ubiquitin-dependent pathway. Inhibition of the proteasome has been clinically validated as a promising strategy for the treatment of cancer. Although new agents continue to be developed, bortezomib is currently the only approved proteasome inhibitor and is used for the treatment of multiple myeloma and mantle cell lymphoma. While bortezomib represents a significant breakthrough in this area, it suffers from several drawbacks including high relapse rate, neuropathy and thrombocytopenia. Therefore, there is a need for new proteasome inhibitors. A recent report by Zhou *et al.* [1] details the preclinical development of PR-047 (**3**), an orally bioavailable follow up proteasome inhibitor to carfilzomib (**4**), a compound currently in phase II trials.

The epoxyketone peptide family of irreversible proteasome inhibitors is derived from the natural product epoxomicin (**5**). Efforts to develop an orally bioavailable peptide epoxyketone began with the previously identified carfilzomib (**4**), itself developed from epoxomicin. Because the tetrapeptidic structure was predicted to interfere with absorption, truncated versions of carfilzomib (**4**) were prepared and tested. It was discovered that while a tripeptide version preserved good activity the dipeptide did not. Thus, focusing on tripeptides, systematic variation of each of the amino acids moieties, examining solubility and metabolic stability as well as potency, led to the identification of PR-047 (**3**).

In vitro testing of PR-047 (**3**) demonstrated activity against the chymotyrpsin-like proteolytic activity of proteosome (IC_{50} of 82 nM in an ELISA-based LMP7 (low molecular mass polypeptide 7) active site assay). Orally administered PR-047 (once a day for two days, weekly) in either immunocompromised mice xenografted with the RL non-Hodgkin's lymphoma cell line or BALB/c mice bearing the colorectal tumor cell line CT-26, yielded a response equivalent to iv administered carfilzomib. PR-047 demonstrated moderate absolute oral bioavailability in several species: 17% (mouse), 21% (rat) and 39% (dog). On the basis of its favorable efficacy, bioavailability and pharmacological profile, PR-047 has been recommended for clinical development (Fig. 1).

Anticancer agents with CNS penetration: discovery of avixa

The discovery and development of new anticancer agents that are efficacious for the treatment of neurological malignancies is very difficult. This is due, at least in part, to the poor penetration of the blood–brain barrier (BBB),

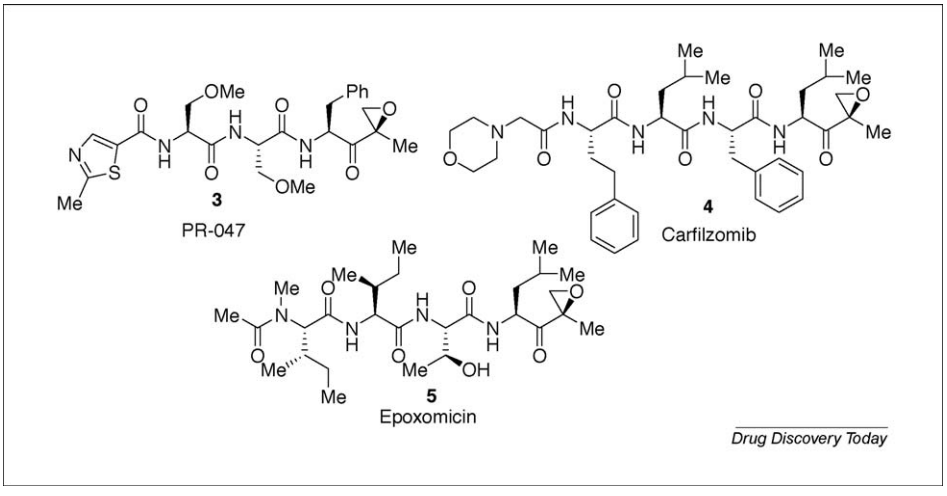

FIGURE 1

Structures of PR-047, carfilzomib and epoxomicin.

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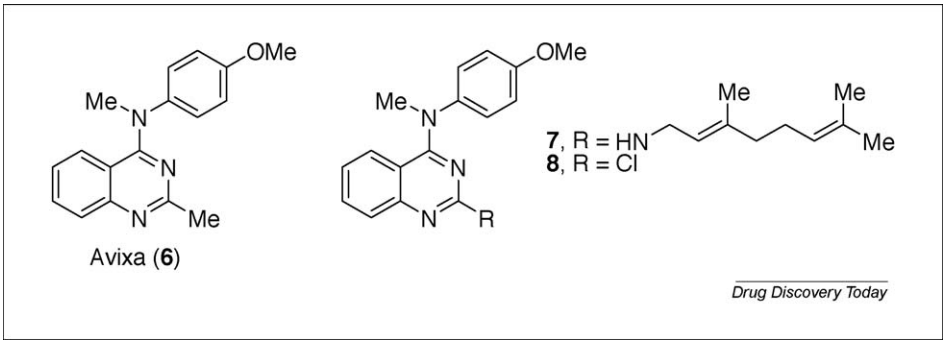

FIGURE 2

Quinazoline tubulin polymerization inhibitors 7, 8 and avixa.

a property that is common to most existing chemotherapeutics. Despite this challenge, efforts to develop new agents for the treatment of neurological cancers have lead to the identification of avixa (**6**, MPC-6827), a compound currently in phase II trials for the treatment of primary brain cancer [2].

A cell-based HTS (high throughput screening) campaign, using a caspase activation assay to identify potential anticancer agents, identified **7** and **8**, among others, as potent apoptosis inducers. Quinazoline **8**, in particular, displayed very potent cell culture activity (EC_{50} = 2 nM) against T47D (breast), HCT116 (colorectal carcinoma) and SNU-398 (hepatocellular carcinoma) cell lines and also demonstrated activity *in vivo* and high BBB penetration. Despite this encouraging activity profile, the chloride was seen as a liability due to its chemical reactivity. Consequently, SAR studies focused primarily on the replacement of the chloride functionality. This effort eventually led to the identification of avixa (**6**), wherein a methyl group serves as a suitable replacement for chloride yielding equipotent apoptosis activity. Further SAR indicated that 4-methoxy and *N*-methyl groups were the optimal substituents for the aniline portion of the molecule (Fig. 2).

Evaluation of the mechanism of avixa revealed that it inhibits tubulin polymerization with potency similar to that of vinblastine. Avixa is highly active against multidrug resistant cancer cell lines including P388/ADR, MCF-7/MX and MCF-7/VP. *In vivo* studies demonstrated good activity in a MX-1 breast tumor model, showing inhibition of tumor growth at 5 mg/kg. In addition, studies showed excellent blood–brain barrier penetration after intravenous dosing. Taking into consideration its ability to penetrate the CNS, avixa has been advanced into clinical development for primary brain cancer.

1 Zhou, H.-J. *et al.* (2009) Design and synthesis of an orally bioavailable and selective peptide epoxyketone proteasome inhibitor (PR-047). *J. Med. Chem.* 52, 3028–3038

2 Willardsen, J.A. *et al.* (2009) Discovery of *N*-(4-methoxyphenyl)-*N*,2-dimethylquinazolin-4-amine, a potent apoptosis inducer and efficacious anticancer agent with high blood brain barrier penetration. *J. Med. Chem.* 52, 2341–2351

Kevin Peese