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Extended-spectrum antiprotozoal bumped kinase inhibitors: A review

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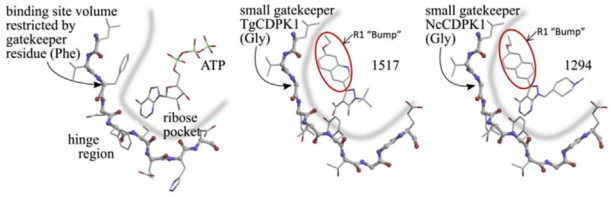
HIGHLIGHTS

- Many apicomplexan calcium-dependent protein kinases (CDPKs) are validated drug targets.
- These CDPKs have atypically small gatekeeper residues and are inhibited by bumped kinase inhibitors (BKIs).
- Highly selective ATP-competitive BKIs inhibit apicomplexan parasites growth at concentrations non-toxic to mammalian cells.
- A proof-of-concept therapeutic potential have been demonstrated in animal model of infections for some BKIs.
- BKIs have potential as therapeutics for diseases caused by apicomplexan pathogens.

GRAPHICAL ABSTRACT



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ABSTRACT

Many life-cycle processes in parasites are regulated by protein phosphorylation. Hence, disruption of essential protein kinase function has been explored for therapy of parasitic diseases. However, the difficulty of inhibiting parasite protein kinases to the exclusion of host orthologues poses a practical challenge. A possible path around this difficulty is the use of bumped kinase inhibitors for targeting

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calcium-dependent protein kinases that contain atypically small gatekeeper residues and are crucial for pathogenic apicomplexan parasites' survival and proliferation. In this article, we review efficacy against the kinase target, parasite growth *in vitro*, and in animal infection models, as well as the relevant pharmacokinetic and safety parameters of bumped kinase inhibitors.

1. Introduction

Protein kinase inhibitors have been sought for therapy of parasitic diseases, but the challenge of specific inhibition of parasite kinases versus mammalian kinases has limited their practical application. Bumped kinase inhibitors (BKIs) were originally generated in Prof. Kevan Shokat's laboratory to build specificity into protein kinase inhibitors that compete with ATP for the kinase active site (Bishop et al., 1998; Bishop and Shokat, 1999). The "bump" on BKIs precludes their binding to almost all mammalian kinases, since these have a bulky gatekeeper residue in the ATP-binding pocket (see Fig. 1, below). Dr. Shokat's group pioneered the use of BKIs in genetically-altered mammalian kinases, in which the bulky gatekeeper residue was mutated to glycine or alanine, rendering the mutant kinase susceptible to BKIs (Bishop et al., 2000; Liu et al., 1999). If the mutant protein kinase was active, the mutation could be introduced into the corresponding gene in mice, allowing the function of that kinase to be probed anytime during the life of the mice by inhibiting that kinase specifically with BKIs (Au-Yeung et al., 2010). BKIs had no discernable effects on

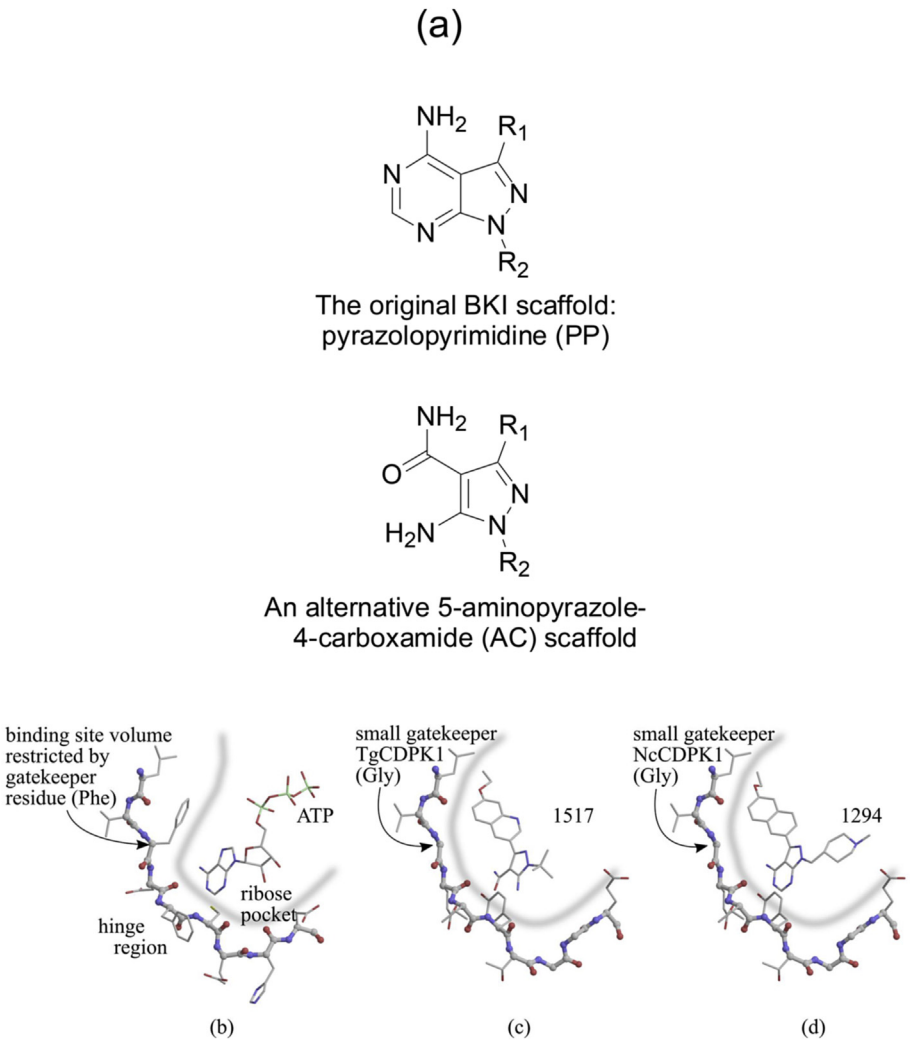

Fig. 1. BKIs core scaffold and orientation with enzymes ATP binding sites: (a) Pyrazolopyrimidines (PP) and alternative 5-aminopyrazole-4-carboxamide (AC) scaffold chemical backbone for CDPK inhibitors. **(b)** Typical protein kinase active site. The volume accessible for ATP binding, indicated by the thick blue line, is limited by the presence of a large gatekeeper residue, in this case phenylalanine 103 of human CDK9. **(c)** Active site of *T. gondii* CDPK1 with AC scaffold BKI-1517. The large R1 substituent occupies a hydrophobic region made accessible by the absence of sidechain atoms in the glycine gatekeeper residue. **(d)** Active site of *N. caninum* CDPK1 with PP scaffold BKI-1294. In addition to the large R1 group, this inhibitor contains a large R2 group that extends deeper into the ribose pocket. The three crystal structures shown are 3BLQ, 4ONA, and 4MX9.