Bioorganic & Medicinal Chemistry Letters 19 (2009) 4014–4017

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of a new class of catalytic topoisomerase II inhibitors targeting the ATP-binding site by structure based design. Part I

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ARTICLE INFO

Article history:
Received 18 May 2009
Revised 5 June 2009
Accepted 8 June 2009
Available online 13 June 2009

Keywords:
Anticancer agents
Structure based design
ATPase inhibitors
Topoisomerase II inhibitors

ABSTRACT

Topoisomerase II is a validated target in oncology. Among the different ways of blocking the function of this enzyme, inhibiting its ATPase activity has been relatively less investigated. In an effort to identify topoisomerase II inhibitors of a novel type, exerting their action by this mechanism, we have designed a purine inhibitor scaffold targeting the ATP-binding site of the enzyme. Searching the Novartis compound collection for molecules containing this purine motif has allowed the identification of two micromolar hits providing access to a new class of catalytic topoisomerase II inhibitors.

Topoisomerase II is a nuclear enzyme whose function, to allow the passage of a double strand of DNA through another one, is essential to dividing cells for disentangling the intertwined sister chromatids after replication.[1] In this process, the enzyme creates a break in one of the DNA double strands resulting in the formation of a transient covalent DNA-enzyme complex. Compounds called topoisomerase II poisons exert a potent cytotoxic effect by their ability to stabilize this complex, thereby causing DNA damage.[2] They constitute a well established class of antitumor agents used in cancer therapy since decades.[3] However, because they are also very toxic for normal dividing cells, topoisomerase II poisons present a narrow therapeutic window. Alternative ways, in principle not DNA damaging, to inhibit the activity of this crucial enzyme are provided by the catalytic inhibitors.[4,5] These compounds block the enzyme before DNA cleavage or in the last steps of the catalytic cycle after resealing of the DNA break. Although various catalytic inhibitors of topoisomerase II have been described, preclinical concepts for exploiting their antiproliferative activity based on molecular characteristics of the tumor cell have only recently emerged. These new concepts are based on studies suggesting that tumor cells bearing defects in certain checkpoint control mechanisms of the cell cycle may be particularly sensitive to topoisomerase II catalytic inhibitors.[6–8] The prospect of selectively inhibiting the proliferation of such cancer cells aroused our interest in topoisomerase II catalytic inhibitors, triggering our engagement in drug discovery efforts in this area of anticancer research. As part of these efforts, we report here the discovery by a structure-based approach of the first representatives of a new chemical class of topoisomerase II catalytic inhibitors. The optimization of the new class towards potent and selective inhibitors and the full biochemical and biological characterization of the resulting compounds are reported elsewhere.[9,10]

Topoisomerase II is an ATPase belonging to the GHKL (Gyrase, Hsp90, histidine Kinase, mutL) family.[11,12] It uses the energy derived from ATP hydrolysis to induce the different motions of the DNA double strands necessary for the completion of the catalytic cycle.[13] In our search for new catalytic inhibitors of this enzyme, we decided to focus on its ATPase activity. In particular, we wanted to identify compounds that inhibit the ATPase activity of the enzyme by occupying its ATP pocket, thereby preventing binding of the nucleotide. This approach has enjoyed considerable success in medicinal chemistry not only for the inhibition of protein kinases,[14] which is the most prominent example, but also for inhibiting two other members of the GHKL family: the bacterial DNA gyrase in the antibiotics area[15] and more recently Hsp90 in oncology.[16] At the initiation of the reported work, only the antibacterial drug Novobiocin was characterized as an ATP site directed inhibitor of topoisomerase II.[15] In the mean time, two other natural product derivatives have been described as catalytic inhibitors possibly having this mode of action.[17,18]

In current drug discovery, most projects start with a massive screening effort to identify initial active molecules. Recently, high throughput virtual screening has begun to play a role in this process.[19] Structure-based design offers an attractive alternative to these high throughput methods. In this highly focused approach, information on the three dimensional atomic structure of the targeted binding site is exploited to design by molecular modeling

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doi:10.1016/j.bmcl.2009.06.034

prototype ligand molecules, presenting favorable intermolecular contacts with the binding site. These are subsequently either synthesized or cherry-picked from available compound collections. This is the approach we followed to identify initial hits targeting the ATP-binding site of topoisomerase II.

At the time this project was conceived, the three dimensional structure of the ATPase domain of human topoisomerase II had not yet been determined.[20] Thus, we had to resort to protein homology modeling to obtain a structural model of the ATP-binding pocket of topoisomerase II on which to base the design of ligands. An initial model of the ATPase domain of human topoisomerase IIα was constructed using the coordinates of the crystal structure of domain B of the *Escherichia coli* DNA gyrase,[21] the only enzyme of the family whose structure had been determined at the time and presenting high sequence homology to our target in the region of the ATP site. This model was further refined by using, as a template, the crystal structure of the more homologous ATPase domain of yeast topoisomerase II[22] that became available soon after. The ATP-binding pocket of the resulting model is shown in Figure 1. As represented in this figure, the adenine moiety of ATP is engaged in three hydrogen bond interactions that define a mode of nucleotide binding conserved in the GHKL family of ATPases. Precisely, the adenine N6 and N1 atoms form hydrogen bonds in a bidentate manner with the side chain of residue Asn120,[23] one of the hydrogen bonds being mediated by a water molecule, while the N7 atom makes another water mediated hydrogen bond with the side chain of Asn91, a residue absolutely conserved in the GHKL ATPase family. In contrast to the adenine and triphosphate moieties, whose essential interactions with the enzyme are of the hydrogen bond type, the ribose moiety of ATP sits in the hydrophobic environment provided by the side chains of residues Ile141, Phe142 and Ala167. Other hydrophobic residues, Ile217, Ile118, Ile88 and Ala92, form a small subpocket at the bottom of the binding site filled with water molecules that establish a hydrogen bond network connecting the side chain of

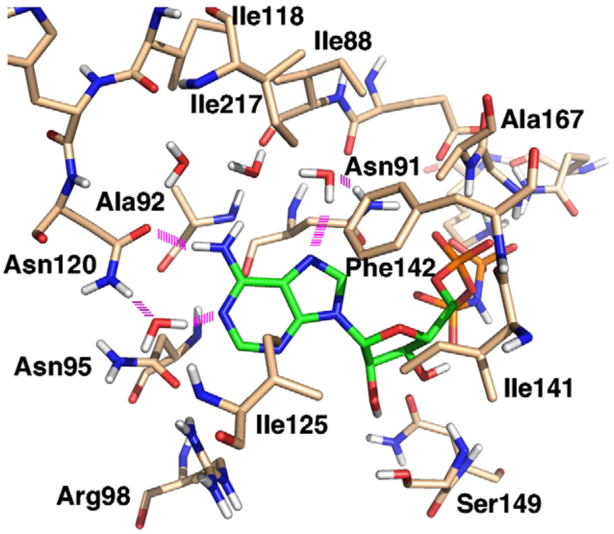


Figure 1. ATP-binding pocket of human topoisomerase IIα (ATPase domain) homology model. Key hydrogen bonds are indicated by dashed lines.

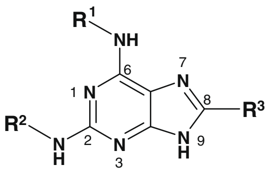


Figure 2. Designed purine scaffold.

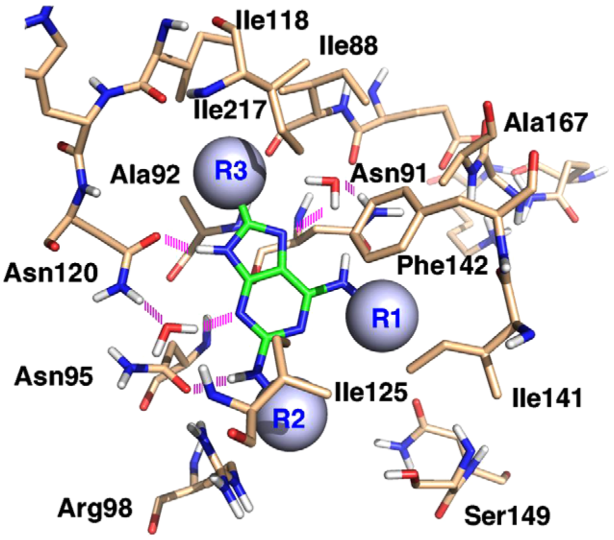
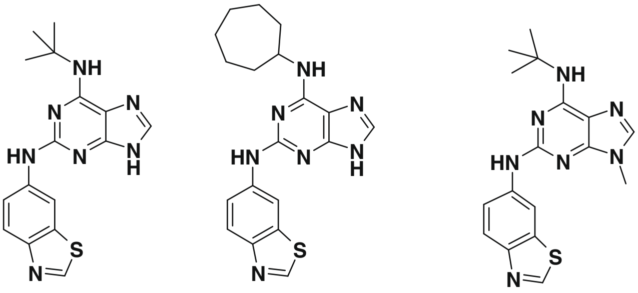


Figure 3. Model of designed purine scaffold docked in the ATP pocket. Key hydrogen bonds appear as dashed lines.



1 2 3

Figure 4. Chemical structures of the two micromolar hits and analog **3**.

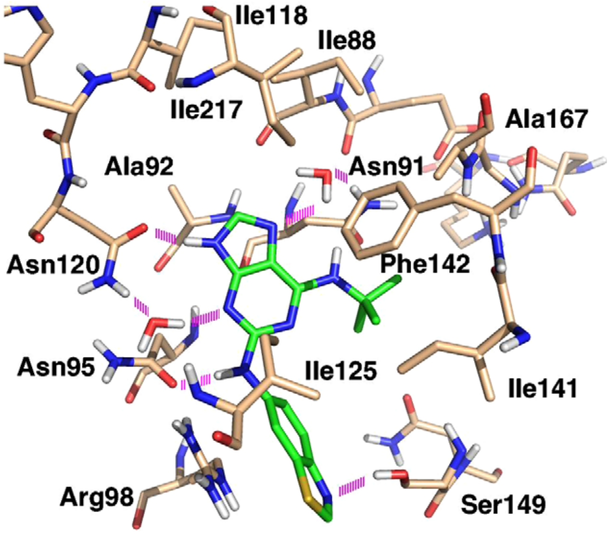


Figure 5. Model of compound **1** docked in the ATP pocket. Hydrogen bonds are represented as dashed lines.