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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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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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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

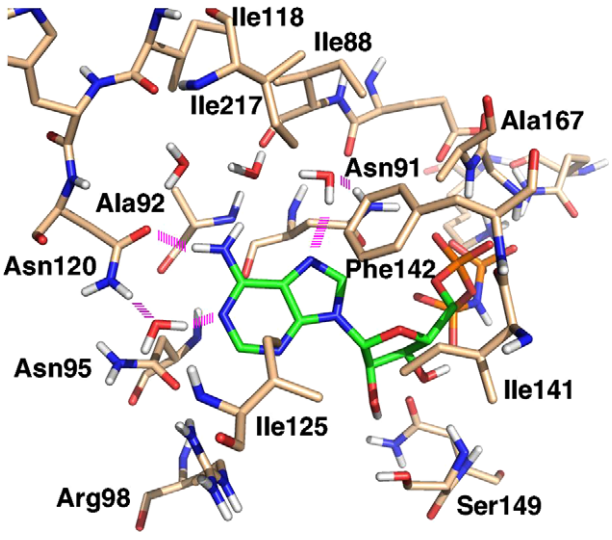


Figure 1. ATP-binding pocket of human topoisomerase IIα (ATPase domain) homology model. Key hydrogen bonds are indicated by dashed lines.

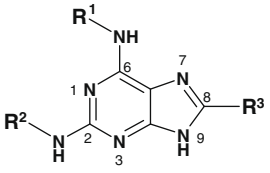


Figure 2. Designed purine scaffold.

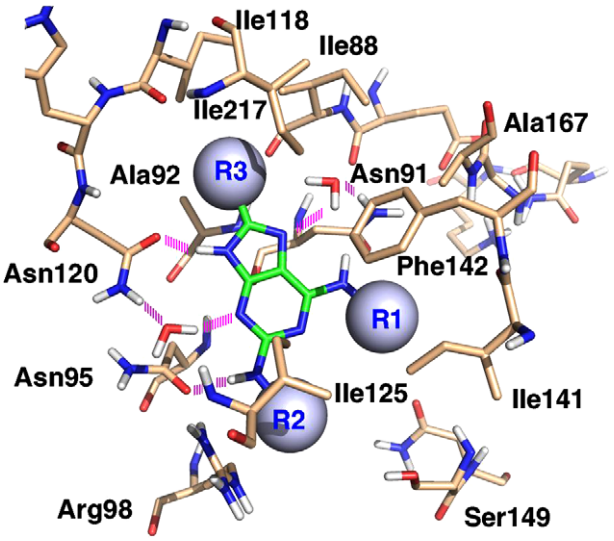


Figure 3. Model of designed purine scaffold docked in the ATP pocket. Key hydrogen bonds appear as dashed lines.

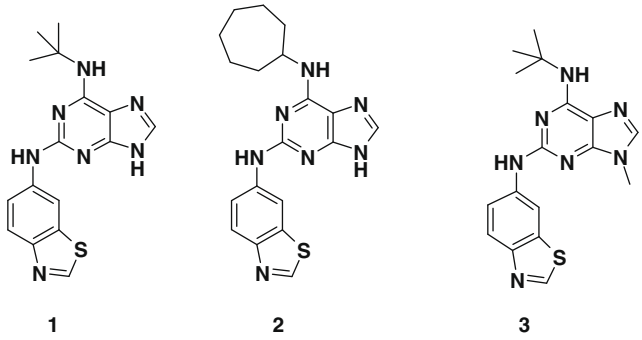


Figure 4. Chemical structures of the two micromolar hits and analog **3**.

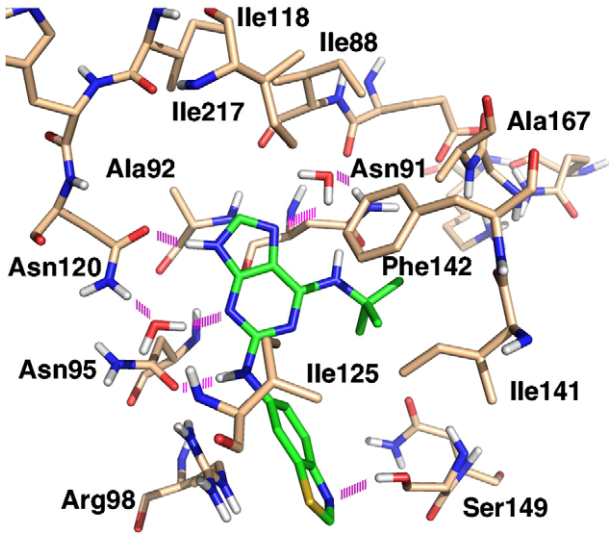
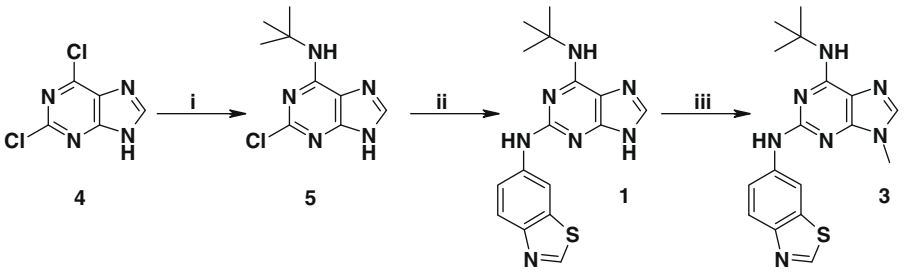


Figure 5. Model of compound **1** docked in the ATP pocket. Hydrogen bonds are represented as dashed lines.



Scheme 1. Synthesis of compound **3**. Reagents and conditions: (i) 2–4 equiv amine, *n*-butanol 50–80 °C; (ii) arylamine, HCl cat., NMP 130 °C; (iii) CH_3I, K_2CO_3,DMF, rt.

Asn120 to that of Asn91. On the opposite side, three residues, Ile125 together with Arg98 and Ser149, constitute the entrance of the cavity.

Using the above model, we started the interactive design[24] of molecular scaffolds mimicking ATP in its main interactions with the enzyme. A key consideration in the design was the objective of identifying molecules having higher binding affinity for the ATP pocket than the nucleotide to obtain potent inhibition of the enzyme ATPase activity. This is why attention was also paid to the possibility of creating additional favorable interactions with the binding site, not exploited by ATP. This strategy led to the design of the purine scaffold shown in Figure 2. As illustrated in Figure 3, the designed scaffold is able to establish the same hydrogen bonds as the adenine moiety of ATP with residues Asn120 and Asn91 but employing a different orientation of the purine ring system in the cavity. Thus, the purine N3 and N9 atoms are used for creating the bidentate hydrogen bonds with Asn120 while the N7 atom can form the water mediated hydrogen bond with Asn91. From position 6 of the ring, it is possible to access the ribose subpocket using a NH linkage. Modeling suggested that aromatic, alkyl and cycloalkyl substituents were appropriate to fill this part of the ATP pocket by making favorable hydrophobic contacts with residues Ile141, Phe142 and Ala167. In addition, positions 2 and 8 of the purine scaffold enable access to parts of the cavity not exploited by ATP in its binding interactions. Thus, from the model we speculated that it should be possible to displace the water molecules filling the small subpocket formed by residues Ile217, Ile118, Ile88 and Ala92 by adding a small alkyl group in position 8. On the other hand, a secondary amine group placed in position 2 is able to donate a hydrogen bond to the side chain of Asn95 while interacting with the residues of the entrance of the pocket Ile125, Arg98 and Ser149. Aromatic groups looked particularly interesting considering the shape of the ATP pocket in this region.

Before engaging in any synthesis work, we checked if compounds conforming to the above design idea were available in the Novartis compound collection. To this end, we carried out a substructure search of this database using the designed purine scaffold as query. Approximately 50 molecules were retrieved and were tested in a biochemical assay measuring the inhibition of topoisomerase IIα ATPase activity.[25]

Encouragingly, from the selected set of archive compounds, we obtained two micromolar hits, compounds **1** and **2**, inhibiting the ATPase activity with IC_{50} values of 1.7 and 8.4 μM, respectively (Fig. 4). A representation of compound **1** docked in the enzyme ATP pocket according to our design principle is shown in Figure 5. Orientating the purine moiety of the molecule such that it forms the aforementioned hydrogen bond interactions with Asn120, Asn95 and Asn91, positions its *tert*-butyl group in the hydrophobic ribose subpocket while placing its benzothiazole ring at the entrance of the cavity. For the latter chemical group, the model suggests the formation of a π–π stacking interaction with the guanidinium group of Arg98[26] on the one hand and that of a hydrogen bond between the thiazole nitrogen atom and the side chain of Ser149 on the other hand. Thus, the ATPase inhibitory activity of the identified hits can be explained by a plausible set of productive interactions with the enzyme. As a further validation of the design concept, we prepared (scheme 1) and tested compound **3**, the analog of **1** methylated at position N9. In our binding mode hypothesis, N9 is engaged in a direct hydrogen bond with the side chain of Asn 120. Alkylation of this nitrogen was therefore expected to disrupt this key interaction and result in a significant loss of inhibitory activity. In full agreement with this notion, **3** turned out to be essentially inactive in the ATPase assay, retaining only marginal inhibitory activity (7%) at a concentration of 10 μM.

In conclusion, by testing a small number of compounds from our chemical archives comprising a purine motif designed to interact favorably with the ATP-binding site of topoisomerase II, we have discovered prototypes of a new class of catalytic inhibitors of this enzyme. Optimization of the potency and selectivity of these inhibitors[9] has allowed us to carry out cellular experiments[10] strongly suggesting that ATP-site directed inhibitors of topoisomerase II have the potential to be developed as new anticancer agents for treating tumors with certain molecular alterations.

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References and notes

1. Wang, J. C. *Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 430.
2. Wilstermann, A. M.; Osheroff, N. *Curr. Top. Med. Chem.* **2003**, *3*, 321.
3. Hande, K. R. *Biochim. Biophys. Acta* **1998**, *1400*, 173.
4. Larsen, A. K.; Escargueil, A. E.; Skladanowski, A. *Pharmacol. Ther.* **2003**, *99*, 167.
5. Nitiss, J. L. *Nat. Rev. Cancer* **2009**, *9*, 338.
6. Haggarty, S. J.; Koeller, K. M.; Kau, T. R.; Silver, P. A.; Roberge, M.; Schreiber, S. L. *Chem. Biol.* **2003**, *10*, 1267.
7. Nakagawa, T.; Hayashita, Y.; Maeno, K.; Masuda, A.; Sugito, N.; Osada, H.; Yanagisawa, K.; Ebi, H.; Shimokata, K.; Takahashi, T. *Cancer Res.* **2004**, *64*, 4826.
8. Damelin, M.; Bestor, T. H. *Br. J. Cancer* **2007**, *96*, 201.
9. Schoepfer, J.; Chène. P.; Radimerski, T.; Meier, P.; Furet, P., in preparation.
10. Chène, P.; Rudloff, J.; Schoepfer, J.; Furet, P.; Meier, P.; Zhiyan, Q.; Schlaeppi, J. M.; Schmitz, R.; Radimerski, T. *BMC Chem. Biol.* **2009**, *9*, 1.
11. Corbett, K. D.; Berger, J. M. *Annu. Rev. Biophys. Biomol. Struct.* **2004**, *33*, 95.
12. Chène, P. *Nat. Rev. Drug Disc.* **2002**, *1*, 665.
13. Bates, A. D.; Maxwell, A. *Biochemistry* **2007**, *46*, 7929.
14. Backes, A. C.; Zech, B.; Felber, B.; Klebl, B.; Mueller, G. *Expert Opin. Drug Disc.* **2008**, *3*, 1409.
15. Maxwell, A.; Lawson, D. M. *Curr. Top. Med. Chem.* **2003**, *3*, 283.
16. Drysdale, M. J.; Brough, P. A. *Curr. Top. Med. Chem.* **2008**, *8*, 859.
17. Hu, C. X.; Zuo, Z. L.; Xiong, B.; Ma, J. G.; Geng, M. Y.; Lin, L. P.; Jiang, H. L.; Ding, J. *Mol. Pharmacol.* **2006**, *70*, 1593.
18. Qin, Y.; Meng, L.; Hu, C.; Duan, W.; Zuo, Z.; Lin, L.; Zhang, X.; Ding, J. *Mol. Cancer Ther.* **2007**, *6*, 2429.
19. Rester, U. *Curr. Opin. Drug Discovery Dev.* **2008**, *11*, 559.
20. A X-ray crystal structure of this domain became later available: Wei, H.; Ruthenburg, A. J.; Bechis, S. K.; Verdine, G. L. *J. Biol. Chem.* **2005**, *280*, 37041. The reported modeling and design work was repeated using this crystal structure and led to the same results as those obtained with the homology model in line

with the fact that, besides minor differences in the conformation of a few residue side chains, the homology model is identical to the X-ray structure.
21. Homology modeling was performed with the program WHAT IF: Vriend, G. *J. Mol. Graph.* **1990**, *8*, 52, after sequence alignment in T-Coffe: Notredame, C.; Higgins, D. G.; Heringa, J. *J. Mol. Biol.* **2000**, *302*, 205. The DNA gyrase structure used is reported in : Brino, L.; Urzhumtsev, A.; Mousli, M.; Bronner, C.; Mitschler, A.; Oudet, P.; Moras, D. *J. Biol. Chem.* **2000**, *275*, 9468 (PDB code 1EI1). The sequence of human topoisomerase IIα was retrieved from the Swiss-Prot database (entry code P11388).
22. Classen, S.; Olland, S.; Berger, J. M. *Proc. Natl. Acad. Soc.* **2003**, *100*, 10629 (PDB code 1PVG).
23. This residue is either an asparagine or an aspartic acid in the GHKL ATPase family.
24. Modeling and docking was performed with a version of MacroModel enhanced for graphics by A. Dietrich. MacroModel: Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.
25. The ATPase assay is described in Ref. 10.
26. For a review on this type of interaction see: Flocco, M. M.; Mowbray, S. L. *J. Mol. Biol.* **1994**, *235*, 709.