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Structure–activity relationship study of beta-carboline derivatives as haspin kinase inhibitors

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ABSTRACT

Haspin is a serine/threonine kinase that phosphorylates Thr-3 of histone H3 in mitosis that has emerged as a possible cancer therapeutic target. High throughput screening of approximately 140,000 compounds identified the beta-carbolines harmine and harmol as moderately potent haspin kinase inhibitors. Based on information obtained from a structure–activity relationship study previously conducted for an acridine series of haspin inhibitors in conjunction with in silico docking using a recently disclosed crystal structure of the kinase, harmine analogs were designed that resulted in significantly increased haspin kinase inhibitory potency. The harmine derivatives also demonstrated less activity towards DYRK2 compared to the acridine series. In vitro mouse liver microsome stability and kinase profiling of a representative member of the harmine series (**42**, LDN-211898) are also presented.

The serine/threonine kinase haspin (*Ha*ploid Germ Cell-*S*pecific Nuclear *P*rote*i*n Kinase, also known as *G*erm Cell-*S*pecific *G*ene-2; Gsg2)[1] functions in mitosis, where it phosphorylates histone H3 at Thr-3 (H3T3ph).[2] During mitosis, this phosphorylation generates a binding site on H3 for Survivin and thereby positions the Chromosome Passenger Complex at centromeres to regulate chromosome segregation,[3,4] and it also displaces proteins such as TFIID that normally bind to H3 through methylated Lys-4.[5] Depletion of haspin by RNA interference, or microinjection of H3T3ph antibodies, causes chromosome alignment defects and failure of normal mitosis.[2,3,6]

Human haspin has ATP-binding and catalytic sites structurally similar to other members of the eukaryotic protein kinase (ePK) superfamily with several notable exceptions. For example, the highly conserved DFG motif involved in ATP-binding and the APE motif involved in stabilizing the C-terminal lobe among ePKs are altered or absent and the activation loop region is substantially rearranged in haspin compared to other ePKs.[7,8]

Haspin kinase inhibitors are expected to be useful probes for elucidating the cellular roles of this protein and may have therapeutic utility in treating cancer. A recently described small molecule, CHR-6494 (**1**), that inhibits haspin displayed anti-tumor activity in a mouse xenograft model.[9] Also, 5-iodotubercidin (**2**) has been reported as an effective haspin kinase inhibitor.[7,10]

We previously utilized a time-resolved fluorescence resonance energy transfer (TR-FRET) high throughput screening (HTS) assay to identify the acridine derivative **3** (LDN-192960) as another potent haspin inhibitor (Fig. 1; IC_{50} = 0.010 μM).[11,12] This assay has now also been used to discover the beta-carbolines harmine, **4**, and harmol, **5**, as moderately potent haspin inhibitors with IC_{50} values of 0.59 and 0.77 μM, respectively. Harmine has previously been identified as an inhibitor of DYRK family kinases, with IC_{50} values between 0.03 and 0.35 μM reported for DYRK1A, and approximately 50-fold lower potency toward DYRK2.[13] Herein, we describe the design, synthesis and improved potency of the beta-carboline series for haspin inhibition utilizing the structure–activity relationships previously determined for the acridine series[12] combined with in silico docking using a recently disclosed crystal structure of the kinase.[7] In addition, in vitro mouse liver microsome stability and kinase profiling of a representative beta-carboline analog are presented.

A crystal structure of haspin bound to AMP was used for docking calculation.[7,14] Analysis of this structure revealed key hydrogen bonds between nitrogen atoms of the adenine ring of AMP and protein backbone atoms of residues E606 and G608 (Fig. S1).[15] A 12 Å docking grid was generated using the AMP center of mass as the

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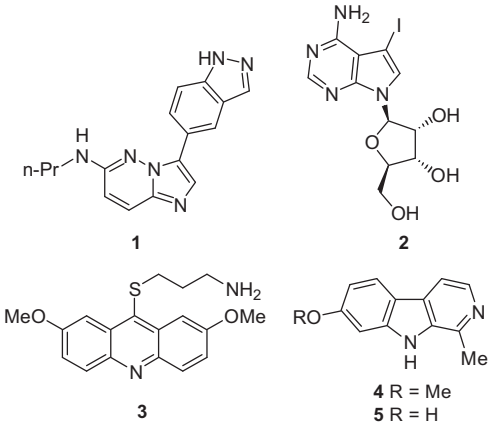

Figure 1. Haspin inhibitors identified by radiometric, thermal stability shift and TR-FRET HTS assays.

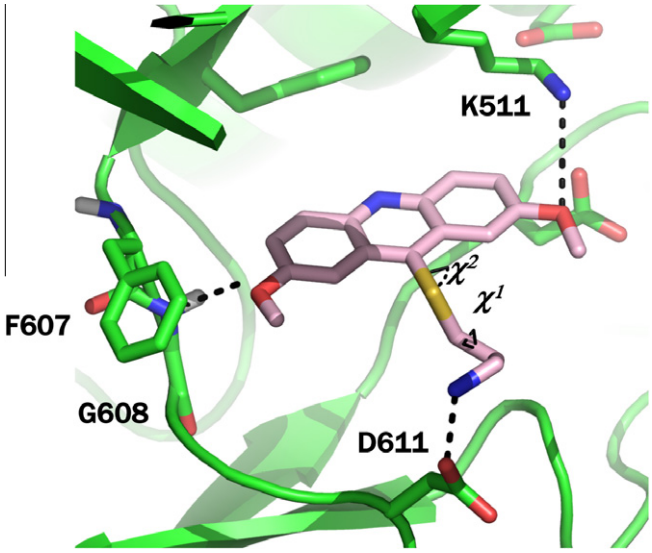

Figure 2. Molecular docking of **3** at the ATP-binding site of haspin. X^1 and X^2 are the torsion angles that were sampled during the metadynamic calculation.

point of origin with a single hydrogen bond constraint on the backbone amide of G608. Docking calculations were performed on **3**, which demonstrated that this inhibitor was well accommodated within the binding site and satisfied the hydrogen bonding constraint on G608 (Fig. 2). In addition, the inhibitor also made a hydrogen bond with K511, which likely disrupts a key salt bridge between this residue and E535 that is necessary for closure of the ATP-binding cleft enabling kinase activity. A metadynamic simulation sampling the two torsion angles (X^1 and X^2) of the alkylamine as collective variables was also conducted. One low energy conformation was found (Fig. S2) that allowed a hydrogen bond between the amine and D611 (Fig. 2).

Next, docking calculations were performed on four harmine analogs (**7a**–**b** and **9a**–**b**) that incorporate at two different positions alkylamines similar to that present in **3**. The two derivatives with the alkylamine on the N^9-position (**9a** and **9b**) were well accommodated within the binding site making a hydrogen bond with D687 as well as the hydrogen bonding constraint on G608 (Fig. 3B and C). In contrast, compounds **7a** and **7b** resulted in steric hindrance with the region around F607 and G608 and were not well situated within the ATP-binding site, suggesting that they would unlikely be haspin

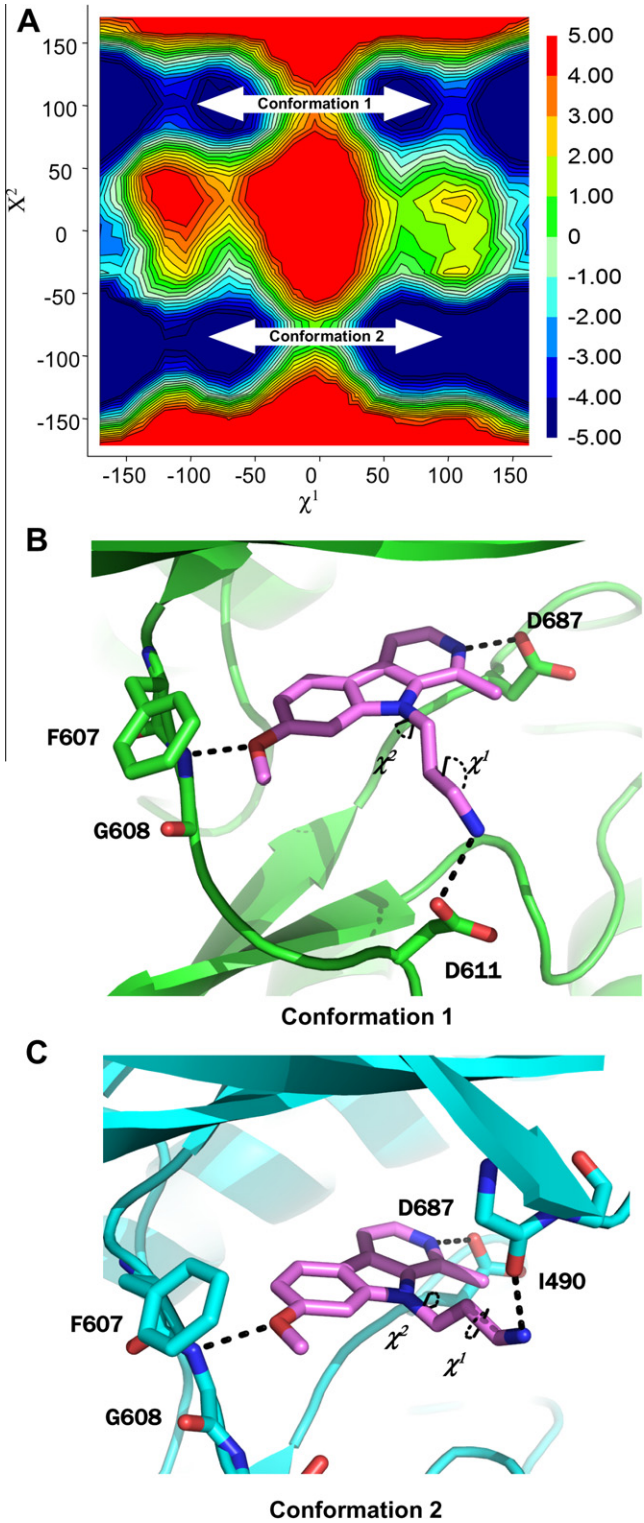

Figure 3. (A) Free energy surface map from metadynamic calculations sampling two torsion angles (X^1 and X^2) of **9a**. Blue and red represent low and high energy conformations, respectively. (B and C) Docking of **9a** at the ATP-binding site of haspin for the two conformations of the alkylamine side-chain based on metadynamic calculations.

inhibitors. A similar metadynamic simulation for **9a** sampling two torsion angles (X^1 and X^2) of the alkylamine as collective variables found two low energy conformations (Fig. 3A). One of these allowed a hydrogen bond between the amine and D611 (Fig. 3B), similar to **3**. However, the second conformation permitted a hydrogen bond