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Synthesis and in vitro antiproliferative activity of C5-benzyl substituted 2-amino-pyrrolo[2,3-*d*]pyrimidines as potent Hsp90 inhibitors

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ABSTRACT

A novel series of heat shock protein 90 (Hsp90) inhibitors was identified by X-ray crystal analysis of complex structures at solvent-exposed exit pocket C. The 2-amino-pyrrolo[2,3-*d*]pyrimidine derivatives, 7-deazapurines substituted with a benzyl moiety at C5, showed potent Hsp90 inhibition and broad-spectrum antiproliferative activity against NCI-60 cancer cell lines. The most potent compound, **6a**, inhibited Hsp90 with an IC_{50} of 36 nM and showed a submicromolar mean GI_{50} value against NCI-60 cell lines. The interaction of **6a** at the ATP-binding pocket of Hsp90 was confirmed by X-ray crystallography and Western blot analysis.

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Heat shock protein 90 (Hsp90) is expressed abundantly in the cytoplasm of normal cells to maintain protein homeostasis. It is an adenosine triphosphate (ATP)-dependent molecular chaperone that plays a key role in folding, stabilization, and maturation of the substrate client proteins involved in cell growth, differentiation, and survival.[1,2] Hsp90 is highly expressed in most tumor cells and plays a significant role in the proliferation, malignant transformation, and progression of cancer cells. Hsp90 client proteins include many oncoproteins necessary for tumor development, such as receptor tyrosine kinases, signal transducers, cell-cycle regulators, growth factors, and transcription factors.[3,4]

Inhibition of Hsp90 chaperone activity causes proteosomal degradation of various client oncoproteins that are involved in commonly deregulated pathways in cancer cells, including growth factor, transcription factor, angiogenesis, apoptosis, cell cycle, tissue invasion, metathesis, and oncogenic fusion protein.[5,6]

Inhibition of Hsp90 can simultaneously regulate numerous targets or signal pathways essential for cancer cell survival and proliferation. Cancer cells are highly reliant on this chaperoning activity (Hsp90-addiction). Hsp90 is an attractive target for cancer therapy, and many Hsp90 inhibitors have been evaluated in clinical trials for the treatment of cancers driven by Hsp90 client proteins.[7–10]

Although Hsp90 inhibitors have shown promising antitumor activity in clinical trials,[11–16] they have also shown limited single-agent activity due to low exposure, low solubility, and adverse events.[3,7] Combination therapy using Hsp90 inhibitors with other targeted anticancer drugs, including drugs targeting Hsp90 clients, showed synergistic effects by sensitizing cells or overcoming resistance.[17,18] The liver toxicity of first-generation geldanamycin analogs, tanespimycin (17-AAG), and alvespimycin (17-DMAG), and visual disorders of various second-generation Hsp90 inhibitors, were the most common adverse events and major limitations in the clinical studies.[3,19] Also, neurological toxicities of BIIB021, such as syncope and dizziness, were reported in a phase-I clinical trial.[20] Among Hsp90 inhibitors discovered by structure-based design using the X-ray crystal structures of Hsp90, radicicols (AT13387 and STA-9090), purine (PU-H71), and benzamide analogs (SNX-5422, XL888 and TAS-116) are currently being investigated in clinical trials (Fig. 1).[19]

Taldone and coworkers categorized the Hsp90 inhibitors using computational analysis of the cocrystal structure/docking studies based on their interactions with three *N*-terminal binding regions: the ATP-binding pocket A, lipophilic pocket B, and a

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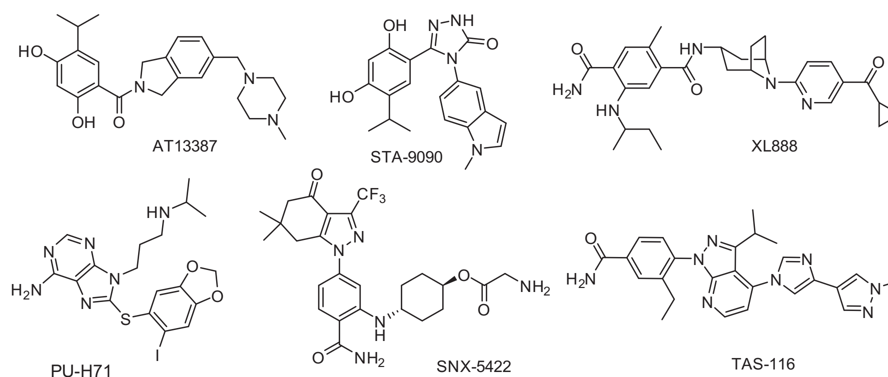


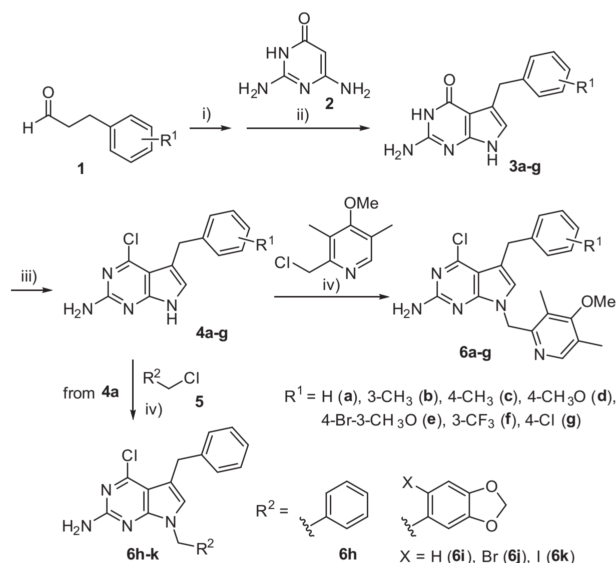
Fig. 1. Current Hsp90 inhibitors in active clinical trials.

solvent-exposed exit pocket C.[21] The 2-amino and 9-pyridyl groups of purine BIIB021 (PDB code: 3QDD) bind to the essential pocket A and the hydrophobic pocket B, respectively.[22] The purine PU-H71 (PDB code: 2FWZ) has an additional hydrophilic substituent at *N*9 for binding to exit pocket C.[23] Unlike most Hsp90 inhibitor series, both purine compounds have a conserved α-helix 4 outside of pocket B. EC144, a 7-deazapurine compound with the *N*7 of purine replaced with a carbon atom, also binds to pocket C with a C5 substituent (PDB code: 3NMQ), and the outer helix is conserved in the cocrystal structure.[24]

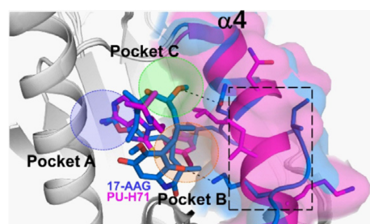
We determined that the hydrophilic substituent at C5 of 7*H*-2-amino-pyrrolo[2,3-*d*]pyrimidine, 7-deazapurine, allowed binding at the hydrophobic channel of exit pocket C (Fig. 2). This hydrophobic binding in the purine series at exit pocket C enables additional interaction by maintaining the hydrogen bonding in pocket A and π–π stacking in pocket B with a conserved outer α-helix 4, based on comparison with the cocrystal structures of other purine inhibitors. In this report, we describe the synthesis of 7*H*-2-amino-pyrrolo[2,3-*d*]pyrimidine derivatives having a benzyl moiety at C5 as novel potent Hsp90 inhibitors and discuss their antiproliferative activities.

All the title compounds reported herein were prepared as illustrated in Scheme 1.[25] First, α-bromination of 3-arylpropyl aldehydes with bromine in the presence of TMSBr, and subsequent cyclization with 2,6-diaminopyrimidin-4(3*H*)-one **2**, afforded 3-benzyl-substituted pyrrolo[2,3-*d*]pyrimidin-4-ones **3a–g** in moderate yields. The *para*-position in the phenyl ring of 3-(3′-methoxyphenyl)propanaldehyde was also brominated, and the resulting dibrominated product was used in the next step to give **3e**. Chlorination of **3a–g** using $POCl_3$ in the presence of *N*,*N*-dimethylaniline as the base, with prior *N*-acetyl protection and final deprotection with methanolic hydrochloric acid, provided the 4-chloro-2-amino-pyrrolo[2,3-*d*]pyrimidines **4a–g** in good yields via three steps. The chlorination of benzyl pyrimidin-4-one compounds substituted with methyl and methoxy groups at the *ortho*-position of the benzyl group was unsuccessful, likely due to steric hindrance. The desired 7-deazapurine compounds **6a–k** were prepared via *N*-alkylation with various alkyl halides **5** or 2-chloromethyl-4-methoxy-3,5-dimethyl-pyridine, in moderate to good yields.

The binding affinities of 5-benzyl-2-amino-7*H*-pyrrolo[2,3-*d*] pyrimidines derivatives **6a–k** to Hsp90α were tested using a fluorescence polarization (FP) competitive binding assay with fluorescently labeled geldanamycin (FITC-GM) at Reaction Biology Corporation (RBC, Malvern, PA, USA).[26] BIIB021, PU-H71, and 17-AAG were used as positive controls (Table 1).



Scheme 1. Reagents and reaction conditions: (i) TMSBr, Br_2 (1 equiv), DCM/1,4-dioxane (10/1), rt; (ii) **2**, NaOAc (2 equiv), CH_3CN/H_2O (1/1), rt, 27–75%; (iii) Ac_2O, reflux, 3 h; $POCl_3$, $PhNMe_2$, $BnEt_3N^+Cl^-$, CH_3CN, 100 °C, 1 h; 3 N HCl in MeOH, 50 °C, 2 h, 21–92% in three steps; (iv) **5** or 2-chloromethyl-4-methoxy-3,5-dimethyl-pyridine, K_2CO_3, DMF, 40 °C, 31–63%.



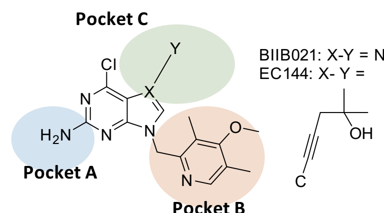


Fig. 2. Structural superimposition of complex structures in the ATP-binding pocket of *m*Hsp90ND shows that the outer α-helix 4 over pocket B is conserved at PU-H71 (PDB code: 2FWZ, purple) and collapsed at 17-AAG (PDB code: 1A4H, blue). Schematic representation of purine analogs and their interactions in the binding pockets of Hsp90α (right). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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