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Design and synthesis of novel macrocyclic 2-amino-6-arylpyrimidine Hsp90 inhibitors

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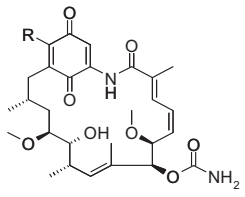
ABSTRACT

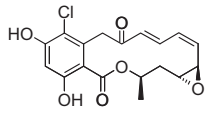
Macrocyclic compounds bearing a 2-amino-6-arylpyrimidine moiety were identified as potent heat shock protein 90 (Hsp90) inhibitors by modification of 2-amino-6-aryltriazine derivative (CH5015765). We employed a macrocyclic structure as a skeleton of new inhibitors to mimic the geldanamycin-Hsp90 interactions. Among the identified inhibitors, CH5164840 showed high binding affinity for N-terminal Hsp90α (K_d = 0.52 nM) and strong anti-proliferative activity against human cancer cell lines (HCT116 IC_{50} = 0.15 μM, NCI-N87 IC_{50} = 0.066 μM). CH5164840 displayed high oral bioavailability in mice (F = 70.8%) and potent antitumor efficacy in a HCT116 human colorectal cancer xenograft model (tumor growth inhibition = 83%).

Heat shock protein 90 (Hsp90) is an ATP-dependent molecular chaperon which is constitutively and ubiquitously expressed in mammalian cells. It governs the conformational maturation, stability and function of these substrate proteins, called client proteins.[1] Because client proteins are known to play an important role in controlling proliferation, survival, invasion, metastasis, and angiogenesis, Hsp90 inhibitors could cause potent inhibition of tumor growth and progression by simultaneous degradation of client proteins.[2–4] Indeed, it has been reported that the ansamycin antibiotic geldanamycin (GM, **1a**)[5] and the macrocyclic lactone antibiotic radicicol (RD, **2**)[6] demonstrated anti-proliferative activity against tumor cells by inhibiting Hsp90 (Fig. 1). The selective effect of these inhibitors to tumor cells and not to normal cells might stem from the fact that Hsp90 exists in an activated state in tumor cells by forming a complex with a series of co-chaperones.[7–10] Consequently, Hsp90 is believed to be an attractive molecular target for anticancer agents.[2–4] Actually, several Hsp90 inhibitors such as semi-synthetic analogs of GM, 17-allylamino-17-demethoxygeldanamycin (17-AAG, **1b**)[11] and 17-(2-dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG, **1c**)[12] along with synthetic small molecules such as CNF-2024 (**3**),[13] NVP-AUY922 (**4**)[14] and AT13387 (**5**)[15] are being evaluated in clinical trials (Fig. 1).

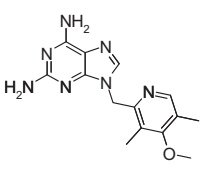
We recently reported the identification of a new class of orally available Hsp90 inhibitor bound to the N-terminal ATP binding site, CH5015765 (**6**), by a combination of fragment screening, virtual screening, and structure-based drug design with the assistance of X-ray cocrystal structures of ligand-Hsp90 complexes.[16]



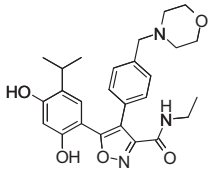
1a, Geldanamycin; R = OMe
1b, 17-AAG; R = $NHCH_2CH{=}CH_2$
1c, 17-DMAG; R = $NHCH_2CH_2NMe_2$



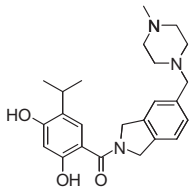
2, Radicicol



3, CNF-2024



4, NVP-AUY922



5, AT13387

Figure 1. Known Hsp90 inhibitors.

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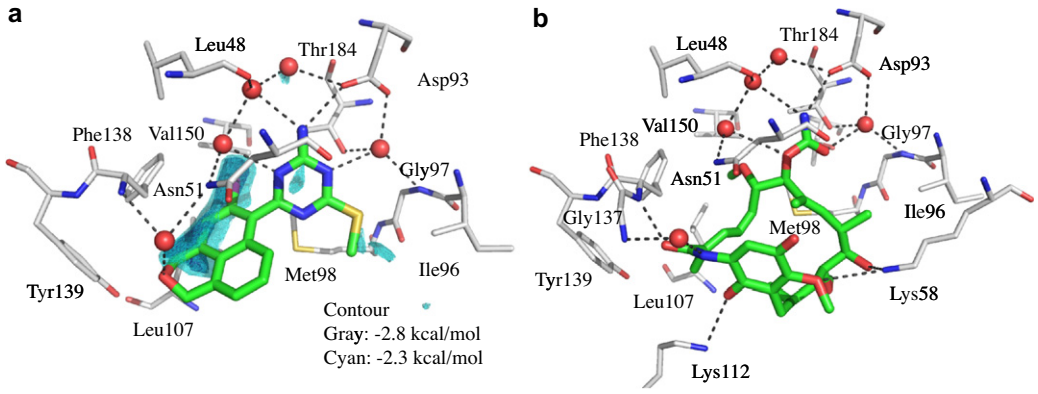
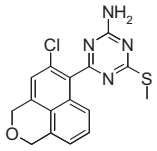


Figure 2. X-ray structure analysis of small molecular Hsp90 inhibitors in complex with human N-terminal Hsp90α. The numbering of the residues corresponds to those in the X-ray cocrystal structure (gray $C_{Hsp90\alpha}$, green C_{ligand}, red O, blue N, yellow S, purple Cl). Hydrogen bonds are indicated by dashes. (a) Detailed binding mode of compound **6** (PDB code: 3B28). Hydrophobic space was predicted by the Site Finder application in MOE and its location is shown in cyan (−2.3 kcal/mol) and gray (−2.8 kcal/mol). (b) Detailed binding mode of GM (PDB code: 1YET).

Table 1
Chemical structure of **6** and its biological,[16] physicochemical, and pharmacokinetic profiles



6, CH5015765

(a) Binding affinity, in vitro anti-proliferative activity, and physicochemical properties of **6**

	IC_{50} (μM)				LM CL (μL/min/mg protein)	
K_d (nM)[a]	HCT116	NCI-N87	PSA (Å^2)[b]	Solubility (μM)[c]	Human	Mouse
3.4	0.46	0.57	59.1	29	43	60

(b) Pharmacokinetic profiles of **6**

Administration	Dose (mg/kg)	$t_{1/2}$ (h)	t_{max} (h)	C_{max} (μg/mL)	AUC_{inf} (μg·h/mL)	CL or CL/F (mL/h/kg)	F[d] (%)
iv	10	0.25	—	—	8.55	1170	—
po	200	2.62	0.5	3.92	7.65	26100	4.5

[a] Values were measured by surface plasmon resonance (SPR) using human N-terminal Hsp90α.
[b] Molecular polar surface area (PSA) was calculated by TPSA.
[c] Solubility in FaSSIF. The use of FaSSIF may over-predict the solubility in physiological in vivo conditions.
[d] Oral bioavailability.

Compound **6** showed high affinity for N-terminal Hsp90 (K_d = 3.4 nM) and in vitro anti-proliferative activity against cell lines such as KRAS mutant HCT116 (IC_{50} = 0.46 μM) and HER2-overexpressing NCI-N87 (IC_{50} = 0.57 μM). In mice, however, it showed several drawbacks such as limited oral bioavailability (F = 4.5%) and moderate tumor growth inhibition (TGI = 54%) against the xenografted NCI-N87 (po, 400 mg/kg). These drawbacks probably came from the low liver microsomal (LM) stability (mouse LM clearance (CL) = 60 μL/min/mg protein) and low water solubility (solubility in fasted state simulated intestinal fluid (FaSSIF) = 29 μM). In this work, compound **6** was chosen as a lead compound for further optimization, and biological,[16] physicochemical, and pharmacokinetic profiles of **6** are summarized in Table 1.

In an X-ray cocrystal structure of **6** with human Hsp90 (PDB code: 3B28), we observed the following effective interactions, as depicted in Figure 2a: (1) an amino group on the 2-position of triazine forms multiple hydrogen bonds with the carboxylic side chain of Asp93 and a water molecule which is conserved in the cocrystal structures of all Hsp90 ATP site binders; (2) a methylthio group on the 4-position of triazine forms a hydrophobic interaction with hydrophobic side chains of Ile96 and Met98; (3) a hydrophobic part from the chloro group to benzylic methylene carbon of a 5-chloro-1*H*,3*H*-benzo[*de*]isochromene moiety fills the proximal hydrophobic space formed by hydrophobic side chains of Val150, Leu107, and Phe138 (explicitly visualized by the Site Finder application in Molecular Operating Environment[17]); (4) an etheric oxygen of a 5-chloro-1*H*,3*H*-benzo[*de*]isochromene moiety forms a hydrogen bond with a water molecule which is hydrogen-bound to the backbone of Phe138 and the side chain of Asn51. On the other hand, we analyzed the binding mode of GM (PDB code: 1YET) and identified important interactions such as one with the side chain of Lys58 (Fig. 2b).[16] In our molecular design of new inhibitors, we maintained the interactions found between compound **6**-Hsp90 and tried to incorporate GM-Hsp90 interactions.

We considered that a macrocyclic structure like GM would offer more chances to make effective interactions. In general, macrocyclic compounds are more conformationally restricted than their acyclic analogs, which potentially give higher target bindings and selectivities and improve oral bioavailabilities.[18,19] We chose pyrimidine instead of triazine as a core structure since a nitrogen