Bioorganic & Medicinal Chemistry Letters 25 (2015) 1338–1342

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of novel oxazepine and diazepine carboxamides as two new classes of heat shock protein 90 inhibitors

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ARTICLE INFO

Article history:
Received 4 December 2014
Revised 8 January 2015
Accepted 12 January 2015
Available online 20 January 2015

Keywords:
HSP90 inhibitor
Oaxazepine
Diazepine
Macrocycle
Structure based design

ABSTRACT

Two novel series of oxazepine and diazepine based HSP90 inhibitors are reported. This effort relied on structure based design and isothermal calorimetry to identify small drug like macrocycles. Computational modelling was used to build into a solvent exposed pocket near the opening of the ATP binding site, which led to potent inhibitors of HSP90 (**25**–**30**).

Heat shock protein 90 (HSP90) is an ATP-dependent 90 kDa molecular chaperone that plays a critical role in the regulation of a wide variety of client proteins.[1,2] HSP90 has been an attractive target for both cancer therapy[3,4] and neurodegenerative indications,[5,6] and various inhibitor chemotypes have been developed as shown in Figure 1.[7–12]

Many classes of HSP90 inhibitors have been discovered using structure based drug design.[7–12] Multiple examples of macrocyclic inhibitors of HSP90, as illustrated by 17-AAG (**1**) and macrocycle **4**,[13–15] which have ring sizes of 19 and 12 atoms respectively, have been disclosed in the literature. Our goal was to build on this previous work using X-ray guided structural analysis to design smaller, more efficient macrocylic inhibitors with improved CNS drug-like properties, that is, lower molecular weight (MW <450), reduced number of hydrogen bond donors (HBD <3), acceptable Clog*P* (1–5), and reduced polar surface area (PSA <90).[16] To this end, we used benzamide **5**, which is a simplified version of SNX-0723 (**2**), as our starting point. We proposed that benzamide **5** could be cyclized into a smaller more efficient macrocycle compared to compound **4**.

We determined from X-ray analysis, that the aryl-aryl dihedral angle of benzamide **5** was 47.4 degrees when bound to the HSP90 protein. We hypothesized that cyclization from the meta-position of the carboxamide phenyl ring to the 2-position of the tetrahydroindolone ring of benzamide **5** would lock the biaryl ring system into a preferred conformation as illustrated in Figure 2. Computational analysis predicted that a seven-membered macrocycle was the smallest ring system that could produce an aryl-aryl dihedral angle close to the experimentally observed 47.4 degree angle of benzamide **5**. Macrocycle **6** (Fig. 2) had a calculated dihedral angle of 44.1 degrees and closely matched the configuration of benzamide **5**. We proposed that rigidifying this conformation should lead to a more favorable entropy of binding and translate to an increase in binding affinity.

We then synthesized oxazepine **6** (Scheme 1) for direct comparison of the dihedral angles and binding affinities with benzamide **5**. X-ray analysis of oxazepine **6** bound to HSP90 showed a dihedral angle of 46.8 degrees, which compared very well with the 47.4 degree dihedral angle of benzamide **5**. Oxazepine **6** also showed a 3-fold increase in binding affinity compared to benzamide **5** (Fig. 3). These data are consistent with a favorable entropic contribution to binding from cyclization.

Isothermal titration calorimetry (ITC) studies were implemented to study the binding thermodynamics of the oxazepine system. These studies showed a favorable $\Delta\Delta G^{\circ}$ of -1.0 ± 0.1 kcal mol^{-1} for oxazepine **6** versus benzamide **5**. However, the key parameter driving increased binding affinity

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http://dx.doi.org/10.1016/j.bmcl.2015.01.023
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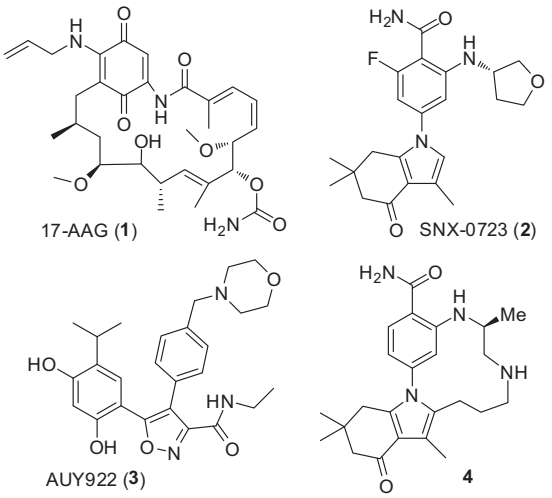


Figure 1. Clinical HSP90 candidates 17-AAG (**1**), SNX-0723 (**2**), AUY922 (**3**), and macrocyclic HSP90 inhibitor **4**.

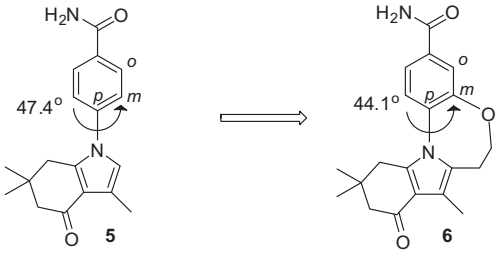
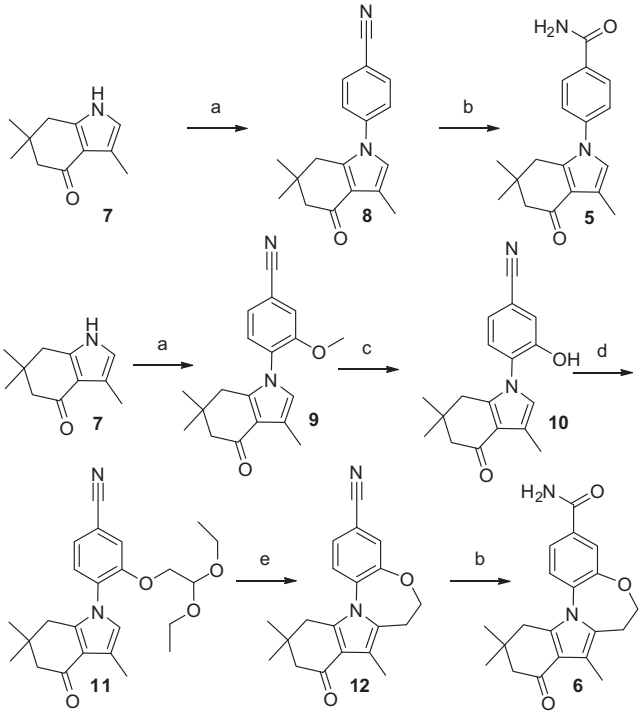


Figure 2. Benzamide **5** with X-ray derived aryl-aryl dihedral angle and macrocycle **6** with calculated aryl-aryl dihedral angle.

was a favorable $\Delta\Delta H^{\circ}$ of -2.6 ± 0.2 kcal mol^{-1} and not $\Delta\Delta S^{\circ}$, which had a value of $+1.6 \pm 0.2$ kcal mol^{-1}. These data suggested the enhanced binding affinity of compound **6** was mainly driven by enthalpic contributions. Closer inspection of the structural information showed the favorable enthalpic contribution was potentially coming from a hydrogen bond between the water network in the HSP90 protein and the macrocyclic chain oxygen atom of oxazepine **6**, which is not present in benzamide **5** (Fig. 4). The water molecules associated with this hydrogen bonding water network are well-ordered. Maintaining this ordered water network during ligand binding, is most likely the cause of the unfavorable entropic component.

The insights gained through ITC experiments illustrate the usefulness of this tool to help test and elucidate the thermodynamic component in structure based design. In our particular case, it helped to elucidate the increased binding affinity from an enthalpically favored hydrogen bonding water network versus an entropically favored ring cyclization.

We next explored increasing the binding affinity of oxazepine **6** by designing in a new ligand–protein interaction. The benzamide structural class, represented by SNX-0723 (**2**), achieves a substantial potency gain by filling a solvent exposed pocket near the opening of the ATP binding site with various alkyl, aryl, and ether groups from the *ortho*-position of the carboxamide phenyl ring.[8] The alkyl and aryl groups of the benzamide class provided the benefit of a favorable hydrophobic interaction with Met-98 residue and the ether substituents potentially benefited from an additional hydrogen bond with Lys-58.[8] We hypothesized that building off the ether chain of oxazepine **6** could effectively access the same pocket as the THF ring of SNX-0723 (**2**). This was illustrated by the computational docking pose of oxazepine **13** into the X-ray



Scheme 1. Synthetic scheme for the synthesis of compounds **5** and **6**. Reagents and conditions: (a) 4-Fluoroaromatic nitrile, NaH, DMF, 150 °C, 69–83%; (b) NaOH, H_2O_2, EtOH, DMSO, 98%; (c) BBr_3, DCM, 25 °C, 58%; (d) 2,2-diethoxyethanol, DIAD, PPh_3, THF, 98%; (e) (1) formic acid, 75 °C, 91%, (2) 10% Pd/C, EtOAc, 1 atm H_2, 99%.

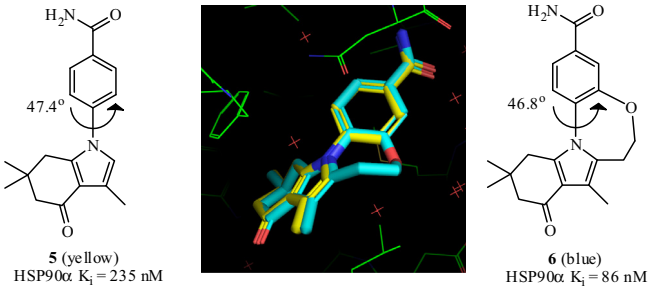


Figure 3. HSP90α X-ray overlay and binding affinity of compounds **5** and **6** with experimental aryl-aryl dihedral angles.

structure of SNX-0723 (**2**) bound with HSP90 (Fig. 5). This docking showed reasonable overlay of the propyl group of oxazepine **13** and the THF ring of SNX-0723 (**2**).

To this end, we synthesized (Scheme 2) analogs to explore substitution on the ether chain of the oxazepine structural class as shown in Table 1.

We initially explored the effect of a chlorine atom at R^1 (Table 1) and found that chlorine and hydrogen substituents at this position were essentially equipotent, as illustrated by compounds **6** and **15**. We carried a chlorine atom through much of the exploration at R^1 due to ease of synthesis. We next explored substitution of the ether chain with alkyl groups as shown in compounds **13** and **14**. Alkyl substituents at R^2 did not result in dramatically increased binding affinity. We concluded that substitution at this position was not effective at accessing the pocket near the opening of the ATP binding site. X-ray analysis of compound **13** bound with HSP90 protein showed disorder for the propyl group and supported this hypothesis.

Another way to access this pocket would be to replace the macrocyclic chain oxygen with a nitrogen atom. This nitrogen atom