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Discovery of novel aminoquinazolin-7-yl 6,7-dihydro-indol-4-ones as potent, selective inhibitors of heat shock protein 90

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ABSTRACT

A novel class of Hsp90 inhibitors, structurally distinct from previously reported scaffolds, was developed from rational design and optimization of a compound library screen hit. These aminoquinazoline derivatives, represented by compound **15** (SNX-6833) or 1-(2-amino-4-methylquinazolin-7-yl)-3,6,6-trimethyl-6,7-dihydro-1*H*-indol-4(5*H*)-one, selectively bind to Hsp90 and inhibit its cellular activities at concentrations as low as single digit nanomolar.

Heat shock protein 90 (Hsp90) has received significant attention as a therapeutic target for cancer treatment.[1] Hsp90 acts as a molecular chaperone that aids the folding,[2] maturation, transport, and maintenance of conformational stability of its client proteins.[3] Many of these client proteins are involved in critical cellular functions that promote cell growth, proliferation and survival, and are also themselves being pursued as anti-cancer targets, for example, Her2, c-Met, and Cdk-4 as well as a wide range of mutated proteins.[4] Hsp90 is also over-expressed in malignant cells thus these cells may be more dependent on the Hsp90 chaperoning function.[5] Targeting Hsp90 blocks multiple oncogenic signaling pathways,[3] thus diminish the potential for resistance to Hsp90 inhibitors.[6] Inhibition of Hsp90 N-terminal domain ATPase activity[7] disrupts an ongoing 'folding' cycle, leads to destabilization, ubiquitination, and ultimately proteasomal degradation of client proteins.[6] The natural products geldanamycin[8] and radicicol[9] are inhibitors of Hsp90. More recently, additional classes of Hsp90 inhibitors have been developed.[10–14] Here we report our synthetic and medicinal chemistry efforts in the discovery of a novel aminoquinazoline scaffold that inhibits Hsp90.[15]

Through screen of a focused compound library against sets of ATP binding proteins, we identified 4-(2,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydro-1*H*-indol-1-yl)benzamide (**1**, Fig. 1) as a moderate hit for Hsp90 binding (K_d = 3.7 μM), although it was inactive in multiple cellular assays (IC_{50} >50 μM).[13] Structure-based design led us to synthesize 1-(4-aminoquinazoline-7-yl)-2,6,6-trimethyl-6,7-dihydro-1*H*-indol-4(5*H*)-one (**2**), which showed improved activity in an Her2 degradation assay (SKBR3 cells; IC_{50} = 10 μM). This scaffold appears not only small but also drug-like: both its

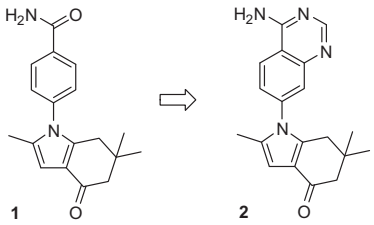

Figure 1. Two related Hsp90 inhibitors: an initial screen hit benzamide **1**, and a 4-aminoquinazoline **2** with a variant cyclic binding head.

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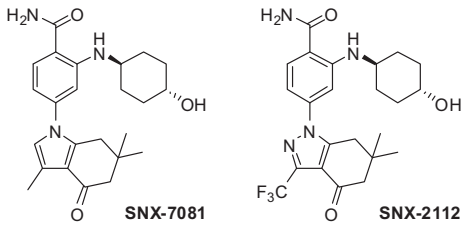
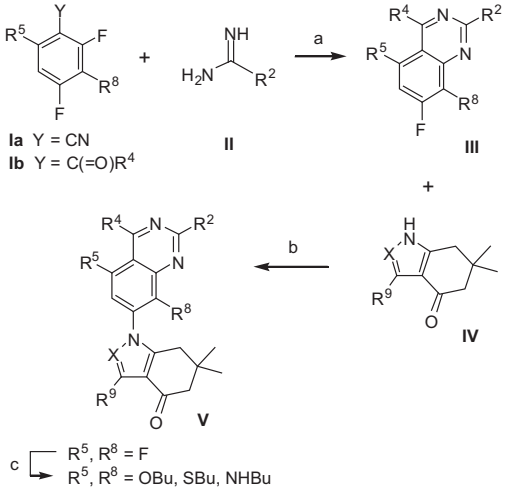


Figure 2. Previous development of screen hit **1** has led to potent Hsp90 inhibitors: benzamide SNX-7081 and SNX-2112.



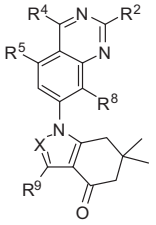
Scheme 1. Preparations for the aminoquinazoline analogs. Reagents and conditions: (a) NaH, DMF, 100 °C; (b) NaH, DMF, 50–150 °C; (c) NaH, DMF, BuOH or BuSH or $BuNH_2$, 120 °C microwave.

top 4-aminoquinazoline and lower dihydro indolone moieties have been utilized in marketed drugs, for example, the anti-cancer medicine Erlotinib and anti-schizophrenia agent Molindone, respectively. Such favorable trend and features, combined with our previous success on structurally related SNX-7081 and SNX-2112, a clinical candidate (Fig. 2),[13] have encouraged us to further explore and optimize this novel scaffold also carrying an amino [6,6] fused pyrimidine core that is shared less commonly by reported Hsp90 inhibitors.[16]

The syntheses of 4-aminoquinazoline analogs started with an available 2,4-difluorobenzonitrile (**Ia**, Scheme 1). Treating that with formamidine, acetamidine, or guanidine (**II**) and sodium hydride in DMF[15,17] at 100 °C afforded 4-amino-7-fluoroquinazoline (**III**). This was then coupled with either a derivative of 6,6-dimethyl-6,7-dihydro-1*H*-indol-4(5*H*)-one or 6,6-dimethyl-6,7-dihydro-1*H*-indazol-4(5*H*)-one (**IV**)[13] in heated mixture of NaH and DMF to afford a 4-amino regioisomer (**V**). Additional substituents could be optionally introduced later, for example, at the C5 (R^5) or C8 (R^8) of the quinazoline ring, by displacement of existing extra fluorine(s) carried on the benzonitrile with nucleophiles such as alkoxide, thio or amine in heated DMF. The corresponding regioisomeric 2-aminoquinazolines were prepared similarly, from guanidine and either a 2,4-difluoro benzaldehyde or acetophenone (**Ib**).[15,18]

The biological data of these analogs are summarized (Table 1). A binding assay, as previously described,[21] was used to determine the compound affinity to monomeric Hsp90. Hsp90 specific cellular effects on client proteins were also evaluated, including Her2 degradation as measured in either SKBR3 or AU565 cell lines using an imaging assay. In addition, the anti-proliferative activities of these analogs were evaluated in multiple cancer cell lines.

Table 1
Structures and biological data for aminoquinazoline analogs



Compd	R^2	R^8	R^5	R^4	R^9	X	Hsp90[a]	Her2[b]
2	H	H	H	NH_2	H	CMe	2.941	9.974[d]
3	H	H	H	NH_2	Me	CH	0.245	0.568[c]
4	H	H	H	NH_2	Me	N	0.628	5.813[d]
5	Me	H	H	NH_2	Me	CH	0.628	0.731[c]
6	NH_2	H	H	NH_2	Me	CH	2.549	1.898[c]
7	H	H	F	NH_2	Me	CH	>10	>20[c]
8	H	F	H	NH_2	Me	CH	1.078	0.665[c]
9	H	$-O^nBu$	H	NH_2	Me	CH	9.804	7.369[c]
10	H	$-NH^nBu$	H	NH_2	Me	CH	> 10	7.752[c]
11	H	$-S^nBu$	H	NH_2	Me	CH	0.980	0.401[c]
12	H	–S-allyl	H	NH_2	Me	CH	2.157	3.655[c]
13	H	$-SC_2H_4NH_2$	H	NH_2	Me	CH	>10	>20[c]
14	NH_2	H	H	H	Me	CH	0.157	0.705[d]
15	NH_2	H	H	Me	Me	CH	0.098	0.004[d]
16	H	H	H	NH_2	Me	CMe	ND[e]	0.498[d]
17	H	H	H	NH_2	Et	N	ND[e]	4.510[d]
18	H	H	H	NH_2	CF_3	N	>10	16.96[d]
19	H	F	H	NH_2	Me	N	ND[e]	3.783[d]
20	NH_2	H	–SEt	NH_2	Me	CH	>10	16.84[d]
21	H	H	–OMe	NH_2	Me	CH	>10	>20[d]

[a] Values (μM) are estimated K_d's derived from an 8 point binding assay as described in 'Text' and in 'References and Notes'.[19]
[b] Values (μM) are IC_{50} determinations from a Her2 imaging assay in either SKBR3 or AU565 cells as described in 'Text' and 'References and Notes'.[20]
[c] SKBR3 cell line.
[d] AU565 cell line.
[e] Not determined.

One key initial improvement we made on analog **2** was to relocate the 2-methyl of the lower indolone moiety to the nearby 3-position (**3**), based on our knowledge of SAR on a related SNX-7081 scaffold.[13] This increased both the affinity to Hsp90 and cellular potency for Her2 degradation by more than 10-fold. The parallel 3-methyl indazol-4-one analog (**4**), designed to improve solubility and metabolic stability, lost affinity and potency significantly. Additional modifications to the lower subunit did not provide more improvement, evidenced by the 2,3-dimethyl indolone analog (**16**), larger 3-ethyl (**17**) or 3-trifluoromethyl (**18**) indazolone analog. Likewise, reduction of the indolone carbonyl to a hydroxyl or removal of one to all three methyl(s) led to partial or total loss of activities (data not shown). Thus the 3,6,6-trimethylindol-4-one moiety appears optimized, and the steric hindrance imposed by the methyl groups could enhance the metabolic stability of the indolone and its analogs.[22]

Addition of a methyl group at the quinazoline C2 position (**5**) caused a minor decrease in compound activities whereas an amino group (**6**) was less favored. Introduction of a fluorine at C5 (**7**) was not tolerated, but was somewhat tolerated at C8 of (**8**) and (**19**) and we do not have a good explanation to these. Displacement of the C8 fluoride with *n*-butanol (**9**) or *n*-butylamine (**10**) was disfavored, whereas the *n*-butylthio (**11**) gave only slight improvement.

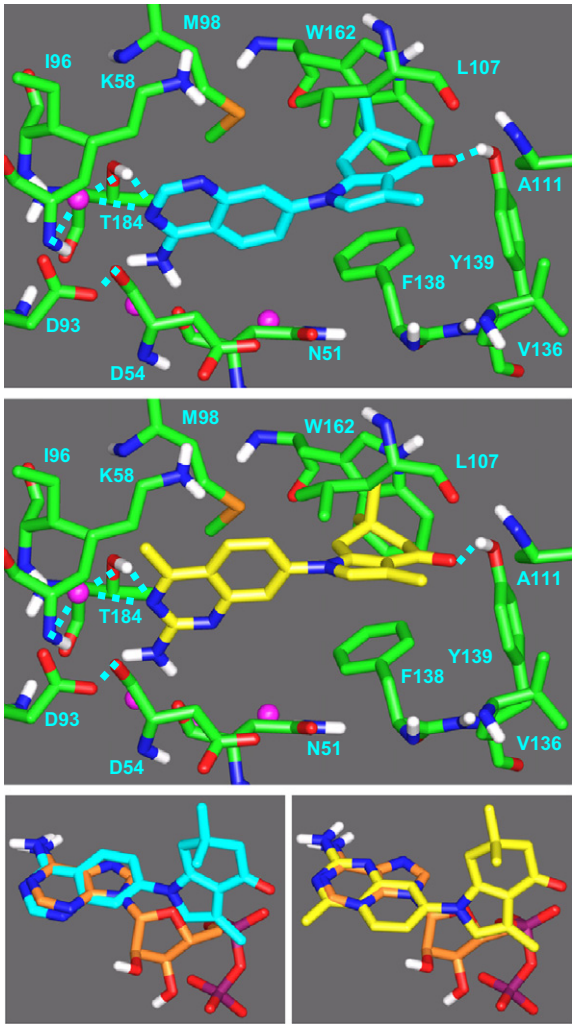


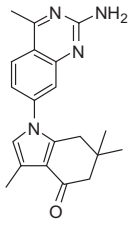
Figure 3. Binding models for compounds **3** (top) and **15** (middle). Protein structure and active site water molecules are derived from protein databank entry 3D0B.[24] Expected hydrogen bonds are indicated with dashed lines. Lower panel shows protein based overlaps to ADP as taken from Hsp90—ADP structure.[25]

Other C8 attachments carrying additional functional groups such as an alkene (**12**) or amine (**13**) did not improve potencies, nor for a small ethylthio (**20**) or methoxyl (**21**) group placed at C5.

Another intriguing SAR feature of the scaffold was discovered, when the 4-amino group of analog **6** was deleted, or the 4-amino of **3** was relocated to C2. The resulted 2-amino analog **14** was equally potent compared to its 4-amino regioisomer **3**. In vitro studies, however, indicated **14** to be not selective for Hsp90. Unlike most analogs in this series, it also appeared to inhibit tubulin polymerization and active in cellular assays that measure progression through M-phase[23] (IC_{50} = 0.11 μM). Since C4 position of quinazoline is also known prone to metabolic oxidation, a blocking methyl group was introduced there to lead to compound **15**. Serendipitously and unexpectedly, this modification greatly boosted both Hsp90 affinity and cellular client activities to nanomolar range; and surprisingly, this also eliminated the off-target activity against tubulin (IC_{50} >10 μM, M block).

Computational models were used to assist in design and understanding of the observed SAR for this quinazoline scaffold. Development of initial models for the 4-aminoquinazoline series was straightforward, based on X-ray data for binding of either a related benzamide analog[24] or ADP[25] to the N-terminal domain

Table 2
Cellular activities for compound **15**, SNX-6833



Assay	IC_{50}, μM[a]
A375 proliferation	0.026 ± 0.016
HT29 proliferation	0.005 ± 0.005
LNCAP proliferation	0.015 ± 0.008
MCF-7 proliferation	0.0007 ± 0.0009
MDAMB231 proliferation	0.020 ± 0.011
NCI-H460 proliferation	0.064 ± 0.028
PC-3 proliferation	0.094 ± 0.035
SKMEL5 proliferation	0.104 ± 0.031
SW620 proliferation	0.014 ± 0.008
Her2 degradation, AU565[b]	0.004 ± 0.001
HSP70 induction, A375[b]	0.005 ± 0.001
pErk inhibition, AU565[b]	0.036 ± 0.020
pS6 inhibition, A375[b]	0.011 ± 0.004

[a] Values are means of four experiments with standard deviation shown.
[b] Both cellular readout and cell type are indicated.

of Hsp90. In particular the quinazoline ring was modeled to closely overlay the purine of ADP with the indolone moiety binding into a hydrophobic pocket induced by displacement of Leu 107 (Fig. 3). The key relocation of the 2-methyl group for the 3-methyl (comparison of **2** to **3**) results in the 3-methyl occupying a small hydrophobic pocket formed by residues Ala 111, Val 136, and Tyr 139. It is interesting to note that this region is in part composed by residues of the 'lid' segment of Hsp90 which plays a central role in the conformational rearrangements that occur as part of the chaperoning mechanism. Modulation of the lid conformation may offer a plausible rationalization for the significantly improved cellular activity of compound **3**. The 2-aminoquinazoline analog **15** was modeled to maintain overlap of the N3 aromatic nitrogen and 2-amino group with N1 and N6 of ADP, thereby allowing the 2-amino group to engage in a well-formed hydrogen bond with Asp 93. This overlap was accomplished through rotation about the biaryl torsion, which in turn allowed the indolone to maintain a similar positioning relative to compound **3**. The quinazoline ring is also positioned to strongly interact with Met 98.

Compound **15**, or SNX-6833, potently inhibited cancer cell proliferation across multiple cell lines at nanomolar concentrations (Table 2). Importantly, it also promoted degradation of direct client proteins such as Her2 and pERK, as well as induced Hsp70 synthesis, at comparable concentrations. In addition to examining effects on direct Hsp90 clients, markers for various signaling cascades were studied. As expected for a compound that inhibited AKT and MAPK pathways, compound **15** potently reduced levels of pS6 (Fig. 4).

In conclusion, we have discovered a novel, selective, and drug-like 2-amino-4-methylquinazolin-7-yl 6,7-dihydro-indol-4-one scaffold that potently inhibits Hsp90 across multiple cellular assays. The highly favorable features of this series of inhibitors revealed here invite further investigations.

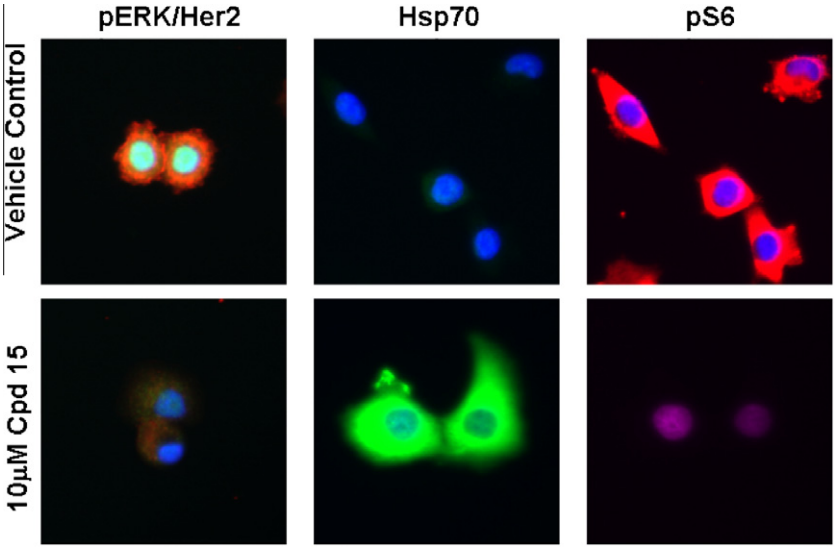


Figure 4. Merged images showing levels of pERK (green) and Her2 (red) in AU565 cells. Hsp70 (green, FITC) and pS6 (red, TRITC) were measured in A375 cells. Hsp70/pS6 images are of identical cells for each treatment condition (vehicle control or test compound), but were captured at different wavelengths. For all images, DNA was stained with Hoescht (blue).

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19. Test compound affinity for Hsp90 was determined as follows: Hsp90 from porcine spleen extract was isolated by affinity capture on a purine-affinity media. The Hsp90 loaded media was then challenged with test compound at a given concentration, ranging from 0.8 to 500 μM, and the amount of Hsp90 liberated at each concentration was determined by Bradford protein assay. The resulting IC_{50} values were corrected for the ATP ligand concentration and presented as K_d values.
20. All cell lines were purchased from ATCC. Proliferation rates were measured by seeding cells into 96-well plates, followed by compound addition 24 h later. After addition of compound, cells were allowed to grow for either an additional 72 or 144 h, depending on rate of growth. At harvest, media was removed and DNA content for individual wells was determined using CyQuant® DNA dye (Invitrogen). Her2 degradation in SKBR3 cells was measured by cellular ELISA using a mouse anti-Her2 primary antibody (Millipore) and anti-mouse peroxidase (Invitrogen) conjugated secondary antibody on cells treated for 24 h with compound followed by methanol fixation. High content analysis (HCA) was done using an ArrayScan 4.5 (Pittsburg, PA) or Becton Dickenson 435 (Rockville, MD) imager on cells treated 24 h with compound. After fixation in 4% PBS-buffered formalin, cells were probed with anti-Her2 (Millipore), antiphospho-S6 (pS6), anti-pERK (cell signaling) and anti-Hsp70 (assay design) primary antibodies, followed by TRITC or FITC conjugated secondary antibodies.

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