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N-Aryl-benzimidazolones as novel small molecule HSP90 inhibitors

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ABSTRACT

We describe the development of a novel series of *N*-aryl-benzimidazolone HSP90 inhibitors (**9**) targeting the N-terminal ATP-ase site. SAR development was influenced by structure-based design based around X-ray structures of ligand bound HSP90 complexes. Lead compounds exhibited high binding affinities, ATP-ase inhibition and cellular client protein degradation.

Heat shock protein 90 (HSP90) is a highly conserved chaperone protein that is ubiquitously expressed, comprising approximately one to two percent of total protein in normal cells.[1] HSP90, as well as other members of the chaperone family, are essential for normal cellular housekeeping functions and mediate numerous aspects of post-translational protein processing. These functions include the facilitation of proper folding of newly synthesized proteins, prevention of client protein aggregation, translocation of client proteins across membranes, and the modulation of active client protein conformation and signaling for normal protein turnover.[2] Chaperone proteins are upregulated as an adaptive response to a variety of toxic stresses and promote cell survival.[3,4]

HSP90 is 2–10-fold more abundant in tumor cells compared to their normal counterparts.[5] There is an additional dependence of cancer cells on HSP90 because many of its client proteins are oncogenes. HSP90 client proteins are known to be essential to tumorigenesis and tumor maintenance and to the development of the six characteristic hallmarks of cancer.[6–11] Inhibition of HSP90 leads to the degradation of client proteins and is a promising therapeutic approach by which multiple oncogenic pathways can be targeted simultaneously. Given that cancer is a heterogeneous disease, often involving alterations in multiple proliferative and survival pathways, a multi-targeted approach is likely essential for effective therapeutic strategy. Like most chaperone proteins, HSP90 functions as a homodimer that is an integral part of a larger super-chaperone complex made up of other chaperones, co-chaperones, accessory and adaptor proteins (e.g., HSP70, HSP40, HIP, HOP, CDC37, p50, p23, AHA1, and immunophilin).[12–14] HSP90 contains a Bergerat ATP binding fold in the N-terminus and a second, less characterized nucleotide binding-site in the C-terminus.[15] The chaperone properties of the complex are energetically driven by the binding and hydrolysis of ATP in an iterative cycle. Unlike other ATP binding protein targets, such as kinases, HSP90 does not covalently modify its substrates. Client proteins first bind HSP90 as part of an intermediate complex. ATP binding and hydrolysis act as a molecular switch that drives conformational changes in HSP90 and results in altered co-chaperone recruitment.[16] The transition to the 'mature' complex results in an active client protein state and propagates the chaperone catalytic cycle. Inhibition of the ATP-ase activity effectively turns off the switch and prevents transition. The complex is locked in the immature state, and ultimately results in client protein degradation.

Geldanamycin analogs[17] (including 17-AAG, **1**)[18] and radicicol (**2**)[19] are very efficient HSP90 inhibitors (Fig. 1). Both compounds are very potent in in vitro assays but have potential limitations in an in vivo setting (formulation difficulties, off-target toxicities, and/or instability in plasma).[20,21] Pharmaceutical companies and academic institutions have exerted significant efforts to discover small molecule inhibitors of HSP90 that would overcome problems associated with natural product based analogs.[22–24] One such example is the diarylpyrazole, VER-49009 (**3**). Here we describe our efforts to identify small molecule inhibitors of HSP90 that resulted in the discovery of a novel series of *N*-arylbenzimidazolones.

To identify compounds that bind the N-terminal ATPase domain of human HSP90α, we employed several high-throughput screen-

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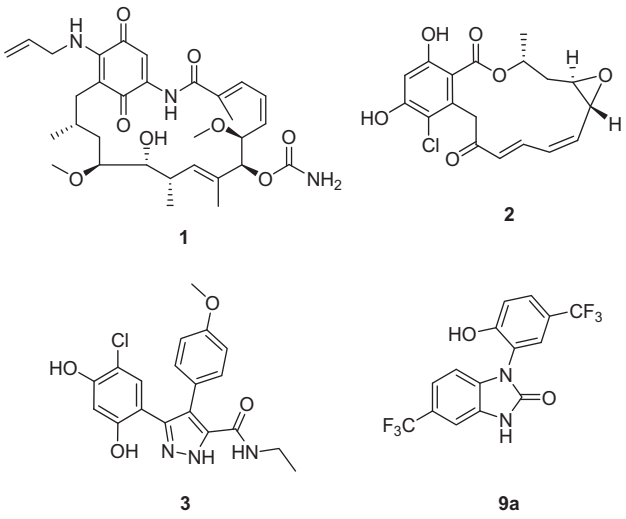
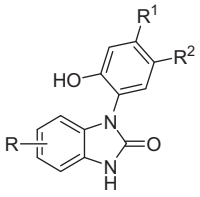


Figure 1. Compounds **1** 17-AAG, **2** Radicicol, **3** VER-49009, and **9a** NS1619.

ing methods. Techniques included NMR screening, affinity selection/mass spectrometry (ASMS) and Time Resolved-Fluorescence Resonance Energy Transfer (TR-FRET) assays. ASMS screening provided a variety of HSP90 inhibitors including the *N*-arylbenzimidazolone NS1619 (**9a**)[25] that exhibited low micromolar affinity. Compound binding affinity for the N-terminal ATPase domain of HSP90α was determined by competition binding using TR-FRET. Functional activity was assessed via the Malachite Green ATP-ase assay.

Table 1 summarizes the binding affinity data for substituted *N*-aryl-benzoimidazolones. Removal of the CF_3 substituent from the benzimidazolone ring of NS1619 (**9a**) had negligible effect on the binding activities of **9b** (Table 1), and we thus performed the first round of SAR studies on a benzimidazolone core containing an unsubstituted phenyl ring. Removal of the meta-CF_3 group and introduction of the hydroxy group at the 4-position of *N*-phenyl ring (**9c**) afforded a nearly 10-fold increase in activity. The overlay of X-ray crystal structures of radicicol and **9c** (Fig. 2) clarifies this significant boost in binding affinity. Similar to radicicol, **9c** gathers the water-mediated hydrogen bonding interaction with the protein back bone. Based on this X-ray data, we incorporated a 5-chloro group to afford analog **9e**, which showed a heightened binding affinity relative to **9c**. Other positional combinations of hydroxyl and halogen substituents at the 4- and 5-positions conferred significant decreases in activities, thus the 5-chloro-2,4-dihydroxy substitution pattern was used for all following analogs.

Table 1
Binding data for substituted 1-aryl-benzimidazolones



Compound	R^1	R^2	R	IC_{50}, FRET[a] (μM)	IC_{50}, MG ATPase[a] (μM)
9a (NS1619)	H	CF_3	5-CF_3	3.32	81
9b	H	CF_3	H	2.32	127
9c	OH	H	H	0.22	85
9d	H	OH	H	34.9	200
9e	OH	Cl	H	0.054	15.2
9f	Cl	OH	H	2.53	200

[a] All determinations are triplicate values.

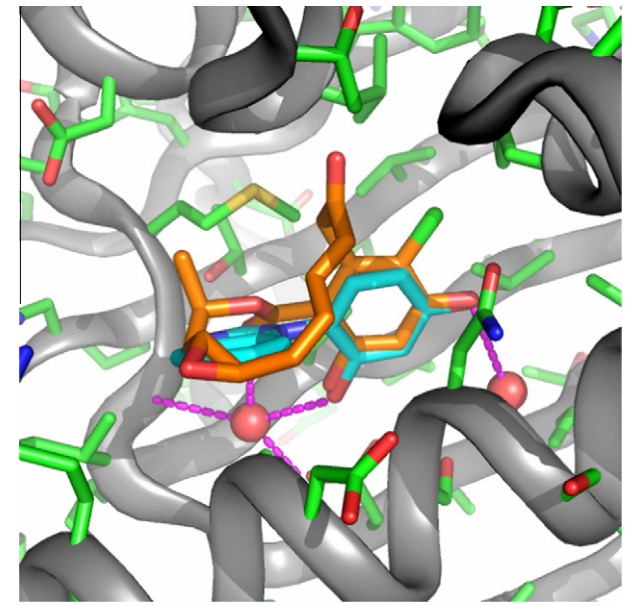
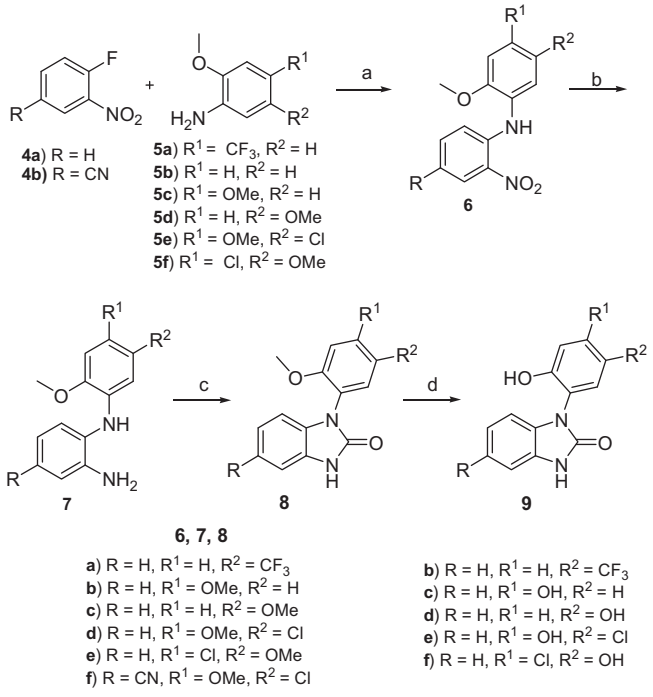


Figure 2. X-ray crystal structure of benzoimidazolone **9c** (cyan) (3OW6) overlaid with radicicol **2** (orange) (1BGQ) bound to the N-terminal ATPase domain of human HSP90.

Benzimidazolones **9** were conveniently prepared in four steps from commercially available 2-fluoro-nitrobenzenes **4** and substituted anilines **5** (Scheme 1). Coupling of **4** and **5** under basic conditions and elevated temperatures provided substituted nitroanilines **6** in moderate yields. Hydrogenation of nitroanilines



Scheme 1. Reagents and conditions: (a) K_2CO_3, DMA, 120 °C, (26–46%); (b) H_2, Pd/C; (c) phosgene; (31–66%, two steps); (d) BBr_3 (44–74%).

6 followed by treatment with phosgene afforded methylated benzimidazolone intermediates **8**. Subsequent demethylation with BBr_3 afforded the final benzimidazolones **9**.

A comparison of the X-ray crystal structures of **9c** and VER-49009 (**3**),[26] (Fig. 3) indicated that the 5-chloro-2,4-dihydroxyphenyl ring occupies the same site when bound to the N-terminal ATPase domain of human HSP90. VER-49009 (**3**) takes advantage of additional interactions of the carboxamide group with Gly97 and Lys58 residues via hydrogen bonding.[27] We next examined substitution at the 5-position in order to mimic these interactions.

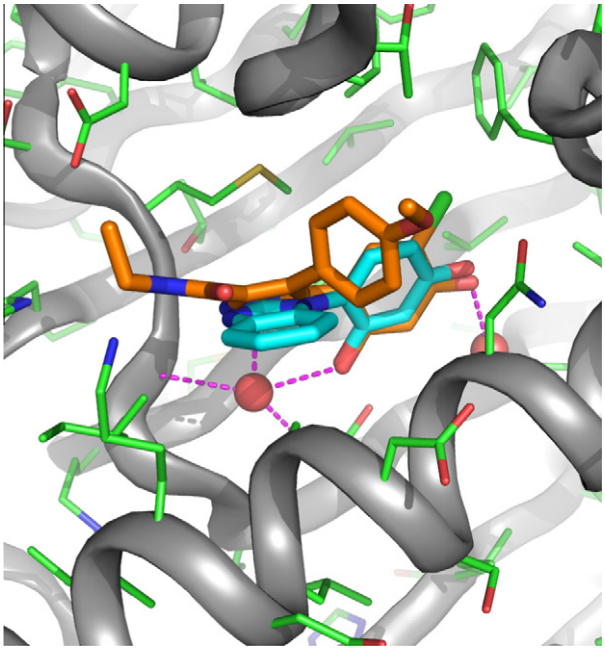
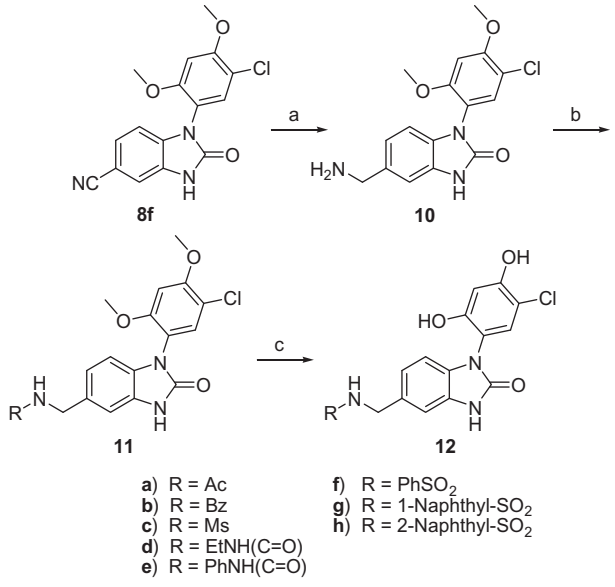


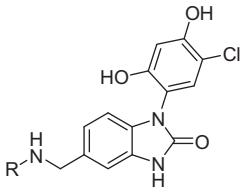
Figure 3. X-ray crystal structure of benzimidazolone **9c** (cyan) (3OW6) overlaid with VER-49009 **3** (orange) (3OWB) bound to the N-terminal ATPase domain of human HSP90.



Scheme 2. Reagents and conditions: (a) BH_3–THF (91%); (b) R-Cl or R-N=C=O, Et_3N, (56–76%); (c) BBr_3 (37–81%).

To probe the effects of substitution at the 5-position, we utilized easily accessible 5-aminomethyl-benzimidazolone as a synthetic precursor for variety of amides **12a**,**b**, ureas **12d**,**e** and sulfonamides **12c**,**f**,**g**,**h**. Substituted benzimidazolones **12** were prepared from the 5-cyano analog **8f**. Borane–THF reduction of **8f** yielded 5-aminomethyl-benzimidazolones **10** which was subsequently coupled with isocyanates, acyl- and sulfonyl-chlorides to yield

Table 2
Binding data for 5-substituted 1-(5-chloro-2,4-dihydroxyaryl)-benzimidazolones



Compound	R	IC_{50}, FRET[a] (μM)	IC_{50}, MG ATPase[a] (μM)
1 (17-AAG)	—	0.02	9.8 (2)
2 (radicicol)	—	0.016 ± 0.001 (33)	0.53 ± 0.03 (34)
3 (VER-49009)	—	0.019 ± 0.002 (3)	0.88 ± 0.15 (3)
12a	Ac	0.040	24.8
12b	Bz	0.072	22.9
12c	CH_3SO_2	0.050	25.2
12d	EtNH(C=O)	0.033	14.2
12e	PhNH(C=O)	0.060	15.4
12f	$PhSO_2$	0.027	3.42
12g	1-Naphthyl-SO_2	0.030	0.55
12h	2-Naphthyl-SO_2	0.047	1.14

[a] ±Standard error (number of determinations). All determinations are triplicate values.

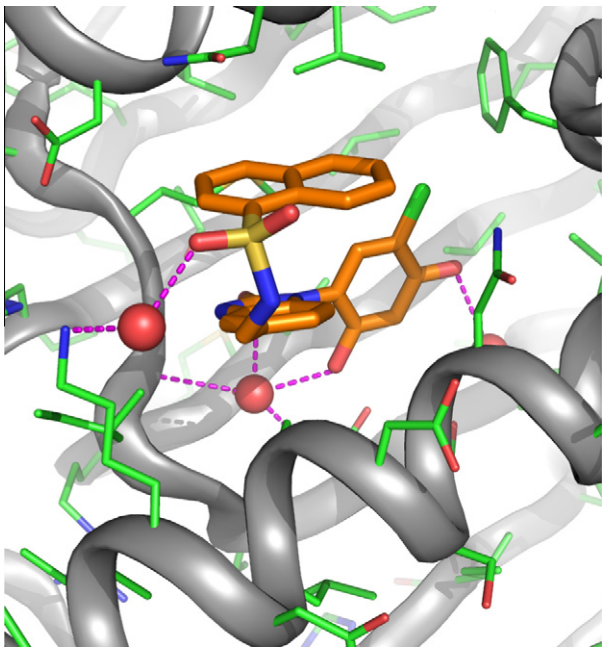


Figure 4. X-ray structure of benzimidazolone **12g** (3OWD)bound to the N-terminal ATPase domain of human HSP90.

Table 3
Cellular effect of HSP90 inhibitors on client protein knockdown and HSP70 up-regulation in human tumor cell lines

Compound	In-cell western		Luciferase reporter	Cell viability assay
	ErbB2 EC_{50}[a] (μM)	HSP70 EC_{50}[a] (μM)	Hif-1α EC_{50}[a] (μM)	EC_{50}, SKBR3[a] (μM)
1 (17-AAG)	<0.10	0.06	0.12	0.025
2 (VER-49009)	0.35	0.50	0.21	0.25
12g	<0.10	0.06	0.19	0.012

[a] All determinations are triplicate values.

amides, sulfonamides and ureas **11**. Demethylation with BBr_3 provided **12** in good yields (Scheme 2).

All compounds maintained good binding affinities and aryl sulfonamides displayed improved enzymatic inhibition in the Malachite green assay. 1-Naphthyl sulfonamide **12g** showed activity comparable to both radicicol (**2**) and VER-49009 (**3**) in this assay (Table 2).

The X-ray crystal structure of **12g** bound to HSP90 is shown in Figure 4. The 1-naphthylsulfonamido moiety of **12g** adopts a 'bent-back' conformation possibly due to hydrophobic collapse, allowing the sulfonamide group to engage in a water-mediated hydrogen bond with Lys58. The orientation and binding of the 5-chloro-2,4-dihydroxyphenyl ring is maintained as in previous structures.

The cellular effects of **12g**, 17-AAG (**1**), and VER-49009 (**3**) were first evaluated in viability assays in the SKBR3 human breast cancer cell line.[28] Compound **12g** displayed equal or greater cell killing behavior than 17-AAG (**1**) or VER-49009 (**3**) (Table 3).

Several assays were employed to directly examine the effects of **12g** on the degradation of known HSP90 client proteins in human tumor cell lines. Hypoxia induced factor 1α (Hif-1α) is an oncogenic protein regulated by HSP90.[29] Inhibition of HSP90 causes degradation of Hif-1α and abolishes Hif-1α regulated transcription. To examine the effect of HSP90 inhibitor **12g** on the degradation of Hif-1α, we developed a luciferase reporter assay in which the enolase promoter (Hif-1α regulated) was used to drive the expression of a reporter construct containing the luciferase gene. The Hif-1α regulated luciferase reporter construct was integrated into NSCLC cell line H1299. Inhibition of Hif-1α mediated transcription results in a decrease in fluorescent signal. As shown in Table 3 and 17-AAG (**1**), VER-49009 (**3**), and benzimidazolone **12g** efficiently inhibit luciferase activity with EC_{50} values of 120, 210, and 190 nM, respectively.

It has been shown that HSP90 inhibition also leads to ErbB2 degradation and HSP70 up-regulation.[30, 31] An in-cell western approach was developed for rapidly monitoring both degradation of ErbB2 and induction of HSP70 in the SKBR3 cell line known for its high overexpression of ErbB2.[28] The results summarized in Table 3 shows **12g** to be equipotent to 17-AAG in modulating Erb2 and HSP70 in a cellular context.

In summary, we describe here the discovery of a novel series of *N*-aryl-benzimidazolone HSP90 inhibitors targeting the N-terminal ATP-ase site. Interestingly, Novartis has developed a parallel series of benzimidazolone analogs[32] that appears to be based on the same commercially available lead compound, NS1619 (**9a**). Development of structure activity relationships was heavily influenced by structure-based design based on X-ray structures of ligand bound HSP90 complexes. Lead compounds exhibited binding affinities, ATP-ase inhibition and cellular client protein degradation activities comparable to or better than existing inhibitors. These compounds may serve as viable leads for further optimization.

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