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Discovery of benzamide tetrahydro-4*H*-carbazol-4-ones as novel small molecule inhibitors of Hsp90

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ABSTRACT

Hsp90 maintains the conformational stability of multiple proteins implicated in oncogenesis and has emerged as a target for chemotherapy. We report here the discovery of a novel small molecule scaffold that inhibits Hsp90. X-ray data show that the scaffold binds competitively at the ATP site on Hsp90. Cellular proliferation and client assays demonstrate that members of the series are able to inhibit Hsp90 at nanomolar concentrations.

Heat shock protein 90 (Hsp90) has emerged as a promising target for the treatment of cancer and other diseases.[1] The cellular function of Hsp90 is to chaperone the folding and then maintain the conformational integrity of multiple proteins. Hsp90's preferred clients encompass a range of signaling proteins involved in oncogenesis, including Her2, c-Kit, Met, Hif-1α, and androgen receptor. Additionally, mutated proteins that are implicated in cancer are often sensitive clients and are generally more dependent on Hsp90 chaperone function than their wild-type counterparts.[2] Hsp90 is an ATPase and this function is essential to its chaperone capability.[3,4] Inhibition of the ATPase function results in client proteins being ubiquinated and then degraded via proteosome pathways. The discoveries that the natural products geldanamycin and radicicol could competitively inhibit ATP binding to Hsp90[5,6] have prompted significant research into the discovery of small molecule inhibitors of Hsp90.[7,8] We report here the discovery of a novel class of small molecule carbazol-4-one benzamides that are unrelated to published ansamycins, resorcinols, or purine-based inhibitors. They potently inhibit Hsp90 and demonstrate nanomolar cellular inhibition of the target.

Screening of a focused library, designed to inhibit proteins with purine binding sites, yielded a novel benzamide hit for Hsp90 (Fig. 1). Synthetic and modeling analyses of this chemical scaffold

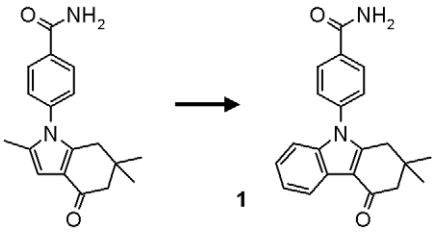

Figure 1. Hsp90 benzamide screening hit and the 1,2,3,9-tetrahydro-4*H*-carbazol-4-one analog **1**.

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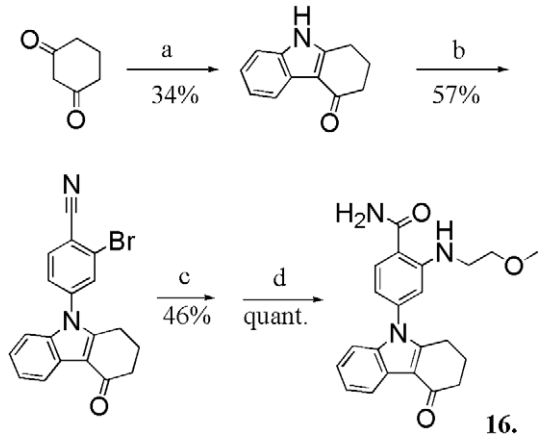
prompted effort to combine the benzamide with a 1,2,3,9-tetrahydro-4*H*-carbazol-4-one moiety. This carbazol-4-one functionality has demonstrated favorable pharmacokinetic properties as it is incorporated into ondansetron, a well tolerated anti-emetic medicine.

The benzamides in this study were obtained in a straightforward manner and a representative synthesis is shown in Scheme 1. The 1,2,3,9-tetrahydro-4*H*-carbazol-4-one ring system was established by means of combining 1,3-cyclohexanedione and phenyl hydrazine via the Fischer indole synthesis in a Personal Chemistry microwave apparatus.[9] Use of dimedone or the mono-methyl reagent instead of 1,3-cyclohexanedione yielded the related analogs described in the SAR. The purified tetrahydro-4*H*-carbazol-4-one was then reacted with the desired 4-fluorobenzonitrile in the presence of sodium hydride. For most analogs, the next step was to incorporate the amine side chain of interest via a palladium catalyzed aromatic amination utilizing $Pd(OAc)_2$ and DPPF with microwave irradiation.[10] Hydrolysis of the nitrile to the carboxamide gave the desired product.

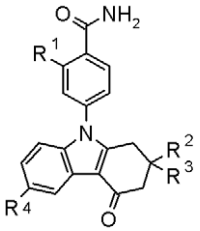
The SAR for this series of compounds is reported in Table 1. Shown are data for a non-enzymatic binding assay, previously described,[8] that allowed determination of binding affinity to monomeric Hsp90. An assay measuring effects on Her2 stability was also established to evaluate cellular effects of the compound.[11] It is important to note that Hsp90 function is complex, involving structural rearrangements as well as co-chaperone protein binding, and so inhibition of monomeric Hsp90 is necessary but not sufficient for cellular activity.[4] Compound **1** showed promising submicromolar Hsp90 binding although it lacked measurable cellular activity. Investigation focused initially on the importance of the gem-dimethyl group present in the hit. Removal of the methyl groups was viewed as potentially desirable for solubility and molecular weight reasons. Although compound **2** showed reduced activity, it still retained moderate affinity. Addition of either a bromo (**3**, **4**) or cyano (**5**) group at the benzamide 2 position effectively killed Hsp90 binding. The methoxy analog **6** was also inactive.

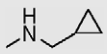
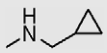
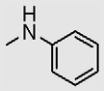
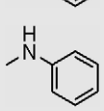
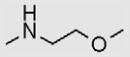
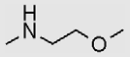
Compound **7** with an amino group showed reduced but still measurable affinity, and binding models suggested room for additional substitution at this position. The affinities of cyclopropyl analogs, **8**, **9**, and **10** were consistent with this observation, and moreover, these compounds showed the first cellular activity observed for the series. Compounds **9** and **10** demonstrated improved binding relative to compound **1**, and the gem-dimethyl analog **8** showed nanomolar cellular activity. Cylcopropyl methyl analogs



Scheme 1. Preparation of **16**: (a) phenylhydrazine (1.2 equiv), TFA, microwave/140 °C/600 s; (b) NaH (2 equiv), DMF, 2-bromo-4-fluorobenzonitrile (1.3 equiv); (c) methoxyethylamine (5 equiv), $Pd(OAc)_2$ (0.01 equiv), DPPF (0.01 equiv), NaO*t*-Bu (2 equiv), toluene, microwave/110 °C/1200 s; (d) DMSO (cat.), EtOH, KOH (ca. 10 equiv), H_2O_2 (32%, XS), 60 °C, 2.5 h.

Table 1
Structures and activities for benzamide tetrahydro-4*H*-carbazol-4-one analogs



Compound	R^1	R^2	R^3	R^4	Hsp90[a]	Her2[b]
1	H	Me	Me	H	0.75	>50
2	H	H	H	H	1.8	>50
3	Br	Me	H	H	>10	ND
4	Br	H	H	H	>10	>10
5		H	H	H	>10	>50
6	–OMe	H	H	H	>10	>50
7	$–NH_2$	H	H	H	2.9	>10
8		Me	Me	H	0.35	0.61
9		Me	H	H	1.7	7.0
10		H	H	H	0.35	5.4
11		H	H	F	0.25	1.3
12		Me	H	H	2.9	6.9
13		H	H	H	0.27	1.3
14		Me	Me	H	>10	ND
15		H	H	H	1.0	2.1
16		H	H	H	0.29	8.9
17		H	H	F	0.78	4.1
18		H	H	H	0.35	5.3

(*continued on next page*)