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Click chemistry to probe Hsp90: Synthesis and evaluation of a series of triazole-containing novobiocin analogues

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ABSTRACT

A series of triazole-containing novobiocin analogues has been designed, synthesized and their inhibitory activity determined. These compounds contain a triazole ring in lieu of the amide moiety present in the natural product. The anti-proliferative effects of these compounds were evaluated against two breast cancer cell lines (SKBr-3 and MCF-7), and manifested activities similar to their amide-containing counterparts. In addition, Hsp90-dependent client protein degradation was observed via Western blot analyses, supporting a common mode of Hsp90 inhibition for both structural classes.

Current cancer therapy strategies utilize the administration of multiple drug regimens that aim to halt multiple malignant processes simultaneously. Heat shock protein 90 (Hsp90) represents an exciting target for the treatment of cancer, as inhibition of this chaperone can affect multiple proteins that are directly associated with all six hallmarks of cancer.[1–4] Hsp90 is a 90 kDa molecular chaperone and is intimately involved in the post-translational conformational maturation of nascent polypeptides as well as the re-folding of denatured proteins and the re-solubilization of protein aggregates.[5] Pharmacological inhibition of Hsp90 effectively inhibits protein substrates dependent upon Hsp90 for conformational maturation, resulting in destabilization of the Hsp90-client protein heteroprotein complex, which leads to degradation of substrates via the ubiquitin-proteasome pathway.[4,6,7] Many proteins associated with malignant progression; including steroid hormone receptors, transcription factors and protein kinases, rely upon Hsp90 to reach their biologically active, three-dimensional conformation. As such, Hsp90 has emerged as a promising anti-cancer target, with more than 20 clinical trials currently in progress with small molecules that bind the N-terminal ATP binding site.[8]

The Hsp90 protein folding machinery requires co-chaperones and partner proteins to aid in the topological reorientation of polypeptide substrates.[7] This protein folding process is ATP-dependent, with hydrolysis occurring at the N-terminal nucleotide binding site of the Hsp90 homodimer.[9] Although promising data has emerged from these trials, many of these compounds exhibit undesired toxicity and/or complicated dosing schedules. In contrast, the development of Hsp90 inhibitors that target other small molecule binding regions, such as that contained in the C-terminus remains minimally investigated.[10] For example, novobiocin was shown to bind the C-terminus of Hsp90 in 2000 and provided the first example of a small molecule binding site outside of the N-terminus (Fig. 1).[11,12] However, novobiocin manifests only modest inhibitory activity (~500 μM). Since 2000, other inhibitors of the C-terminus have also been identified, such as epigallocatechin gallate (EGCG) (Fig. 1), but the development of such compounds has not been thoroughly sought after.[10]

Since the discovery of the Hsp90 C-terminal binding site, analogues of novobiocin have been synthesized and evaluated, with many of the compounds manifesting micromolar anti-proliferative activities.[13–17] Modifications to both the coumarin core and benzamide side chain have been pursued, resulting in the production of preliminary structure–activity relationships (SARs). The hydrogen bonding capabilities and the geometry of the amide bond appear important for novobiocin binding, however, modifications to this moiety have not been fully realized. It was proposed that inclusion of 1,2,3-triazoles as a bioisosteric replacement for the amide moiety could facilitate SAR analysis for the aryl side chain by utilizing 'click' chemistry. The triazole serves as a bioisostere due to similarities in both electronic and spacial characteristics to the amide bond. In addition, it is metabolically stable to hydrolysis and easily incorporated into small molecules.[18,19] In contrast, triazoles exhibit different hydrogen bonding capabilities and an altered geometry as compared to their amide counterparts, which aids in further elucidation of SAR. For these reasons, a series of 1,2,3-triazole-containing novobiocin analogues was prepared. The

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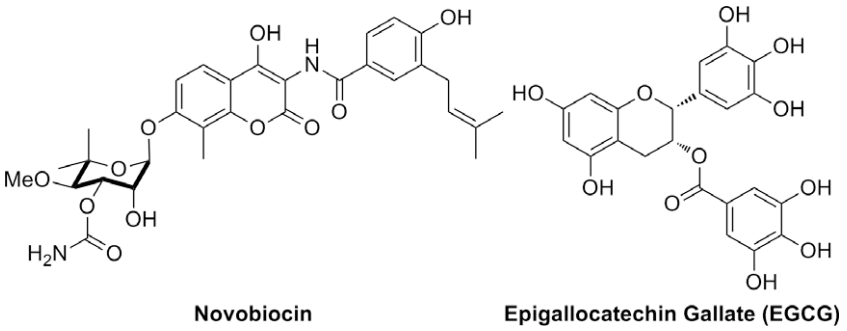
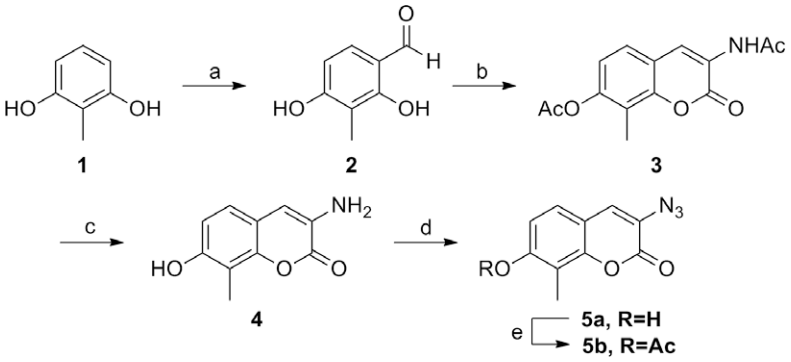


Figure 1. Hsp90 C-terminal inhibitors.

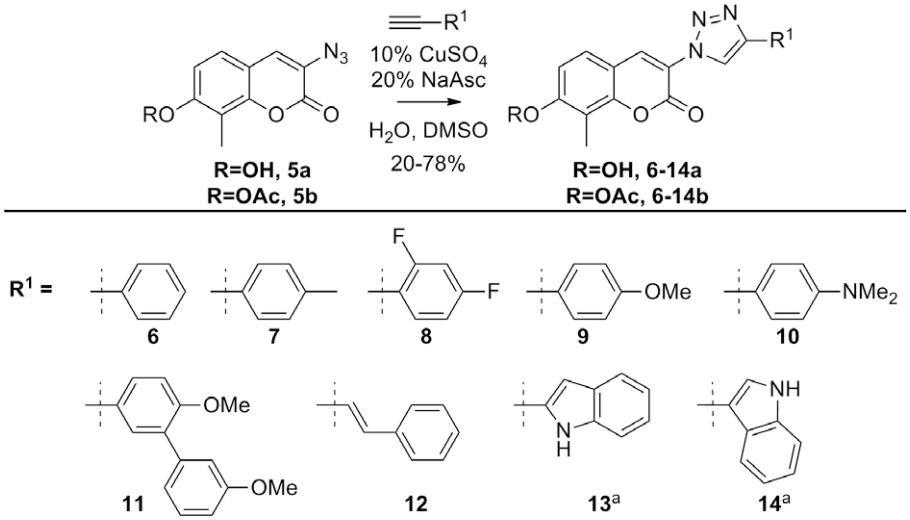


Scheme 1. Reagents: (a) (i) $POCl_3$, DMF, MeCN; (ii) H_2O (55%); (b) *N*-acetyl glycine, NaOAc, Ac_2O; (c) HCl, EtOH; (d) $NaNO_2$, HCl, EtOH, H_2O, then NaN_3 (52% three steps); (e) Ac_2O, pyridine, CH_2Cl_2 (>95%).

design, syntheses, and biological evaluation of these compounds are described herein.

Synthesis of the 8-methyl coumarin core, as found in novobiocin, was commenced with commercially available 2-methyl resorcinol, **1** (Scheme 1). Compound **1** was formylated under Vilsmeier–Haack conditions enlisting $POCl_3$ and DMF, followed by hydrolysis to afford formyl-resorcinol **2**. Similar to the procedure of Sivakumar and co-workers, condensation of **2** with *N*-acetyl glycine in the presence of acetic anhydride, produced the bis-acylated coumarin, **3**.[20] Deacetylation of both the phenol and amine was accomplished upon heating with HCl and EtOH to afford 3-amino-7-hydroxy-8-methyl-coumarin, **4**. Conversion of amino-coumarin **4** to the azide, which was required for the copper-catalyzed Huisgen 1,3-dipolar cycloaddition, was accomplished by in situ generation of the 3-diazonium salt upon treatment with sodium nitrite in aqueous acid, followed by the addition of sodium azide to afford 3-azido-coumarin, **5a**.[20] Acetylation of coumarin **5a** was accomplished with acetic anhydride in pyridine to afford **5b**.

Upon the generation of compounds **5a** and **5b**, the copper-catalyzed Huisgen 1,3-dipolar cycloaddition with the corresponding alkynes was set to generate compounds **6–14a** and **6–14b** (Scheme 2). Standard conditions were used to effect this transformation, and a combination of DMSO and H_2O were found most suitable for optimal product formation. Alkynes **6–10** were chosen



Scheme 2. 'Click' procedure with various alkenes used. (a) The TMS protected alkynes were used in these reactions with the addition of 1 equiv TBAF.