European Journal of Medicinal Chemistry 89 (2015) 442–466

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Design, synthesis and biological evaluation of biphenylamide derivatives as Hsp90 C-terminal inhibitors

Huiping Zhao [a], Gaurav Garg [a], Jinbo Zhao [a], Elisabetta Moroni [b], Antwan Girgis [a], Lucas S. Franco [a], Swapnil Singh [a], Giorgio Colombo [b], Brian S.J. Blagg [a, *]

[a] Department of Medicinal Chemistry, 1251 Wescoe Hall Drive, Malott 4070, The University of Kansas, Lawrence, KS 66045-7563, USA
[b] Istituto di chimica del riconoscimento molecolare, CNR, Via Mario Bianco 9, 20131 Milano, Italy

ARTICLE INFO

Article history:
Received 2 May 2014
Received in revised form
13 August 2014
Accepted 12 October 2014
Available online 14 October 2014

Keywords:
Heat shock protein 90
Hsp90 C-Terminal inhibitors
Biphenyl
Structure–activity relationship
Breast cancer

ABSTRACT

Modulation of Hsp90 C-terminal function represents a promising therapeutic approach for the treatment of cancer and neurodegenerative diseases. Current drug discovery efforts toward Hsp90 C-terminal inhibition focus on novobiocin, an antibiotic that was transformed into an Hsp90 inhibitor. Based on structural information obtained during the development of novobiocin derivatives and molecular docking studies, scaffolds containing a biphenyl moiety in lieu of the coumarin ring present in novobiocin were identified as new Hsp90 C-terminal inhibitors. Structure–activity relationship studies produced new derivatives that inhibit the proliferation of breast cancer cell lines at nanomolar concentrations, which corresponded directly with Hsp90 inhibition.

1. Introduction

The 90 kDa heat shock proteins (Hsp90) are highly conserved molecular chaperones responsible for the conformational stability of more than 200 client proteins, many of which are essential to cancer cell survival [1–3]. Abnormal expression of Hsp90 has been implicated in a variety of disease states: In cancer, over-expression of Hsp90 is critical for the maturation and biological activity of numerous oncogenic proteins (eg., Her2, Raf1, Akt, CDK4, Src, c-Met, etc.) that are distributed amongst all six hallmarks of cancer [4,5]. In neurodegenerative diseases, Hsp90 serves as the master regulator of the prosurvival heat shock response, and provides buffering capabilities for damaged proteins that accumulate beyond normal concentrations and can result in neuronal death [6]. Research has demonstrated that small molecule Hsp90 N-terminal inhibitors manifest two cellular activities, the first of which is induced degradation of proteins that are dependent upon the Hsp90 protein folding machinery. The second is concomitant induction of the pro-survival heat shock response (HSR). The HSR expands the chaperone buffering capacity to counter misfolded proteins that accumulate upon exposure to cellular stress, and thus, aids cell survival. These contradictory effects can provide unique therapeutic opportunities for the treatment of cancer and neurodegenerative diseases, if segregated [7,8]. 17 Small molecule Hsp90 N-terminal inhibitors have entered clinical trials for the treatment of various cancers, however, the heat shock response manifested by these compounds appears detrimental, as the concentration needed for client protein degradation also induces the pro-survival response [9]. Similarly, these two effects hinder their application as neuroprotective agents, as cytotoxic client protein degradation is observed at the same concentration that induces the prosurvival HSR.

Recent studies have identified small molecules that bind the Hsp90 C-terminus and allosterically modulate Hsp90 function [10,11]. In contrast to N-terminal inhibitors, C-terminal inhibitors can segregate the heat shock response from client protein degradation, thus providing a therapeutic opportunity for the treatment of neurodegenerative diseases or elimination of the pro-survival, heat shock response for cancer [12,13]. Although several scaffolds are now known to bind the C-terminus (Fig. 1) [13–16], medicinal

* Corresponding author.
E-mail address: bblagg@ku.edu (B.S.J. Blagg).

http://dx.doi.org/10.1016/j.ejmech.2014.10.034
0223-5234/

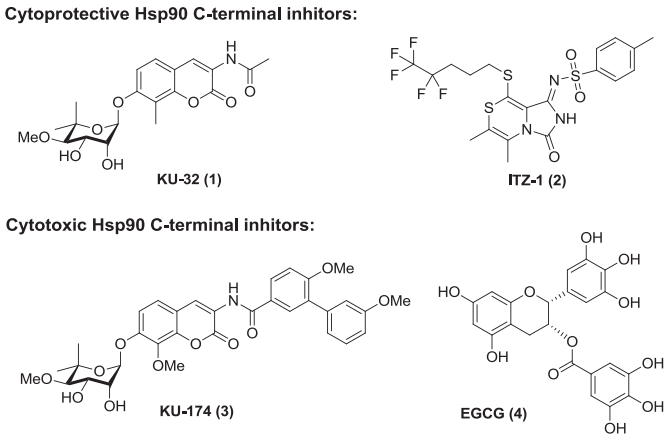

Fig. 1. Small molecules that target the Hsp90 C-terminus.

chemistry efforts have been most focused on analogs of novobiocin, which was the first Hsp90 C-terminal inhibitor identified [17]. The identification of new chemical scaffolds that target the Hsp90 C-terminal domain is needed to dissect the role played by Hsp90 C-terminal inhibitors during the Hsp90 protein folding cycle as well as to improve upon inhibitory activity.

2. Result and discuss

Prior modifications to novobiocin have revealed some structure–activity relationships and identified analogs that exhibit improved inhibitory activity [14,18–23]. As summarized in Fig. 2, these studies identified the benzamide side chain as critical for anti-proliferative activity, and modifications to this region can further increase inhibitory activity. The noviose sugar contributes to solubility and efficacy, however, replacement with ionizable amines results in analogs that also exhibit improved inhibitory activity, but do not induce the HSR (**6**, Fig. 2). The amide linker not only provides important hydrogen bonding interactions, but it also serves to orient the aromatic side chain for interactions with the binding site. Recently, it was discovered that replacement of the amide with urea led to analogs that manifest greater anti-proliferative activity (**7**, Fig. 2), presumably due to an extended hydrogen bonding network [24,25]. In contrast to these modifications, studies on the coumarin ring system have produced only minor effects. Moreover, substitutions on the coumarin ring did not produce compounds with significantly altered activity, suggesting that the coumarin ring may serve to orient of the sugar and benzamide side chains within the binding pocket. Therefore, it was proposed that the coumarin ring could be replaced without compromising activity [19,26].

Recently, it was observed that the optimum distance between the piperidine nitrogen and the hydrogen-bonding network of the amide/urea is critical for inhibitory activity [25,27]. Based on this observation, it was hypothesized that replacement of the coumarin core with scaffolds that maintain this distance may provide compounds upon which new inhibitors could be developed. Attempts to replace the coumarin with fused ring systems did not produce improved inhibitory activities [19,26], suggesting that a flexible ring system may be beneficial for projection of the amino and benzamide side chain. The biphenyl ring system is relatively flexible and could therefore adopt different conformations within the binding pocket, which may present additional interactions with the protein. As a privileged-structure, compounds derived from this scaffold are known to manifest diverse activities, including anti-tumor activity [28]. In addition, the substitution pattern on this moiety can be modified and the distance between the ionizable amine and amide tuned. Therefore, molecules enlisting biphenyl as a coumarin replacement were

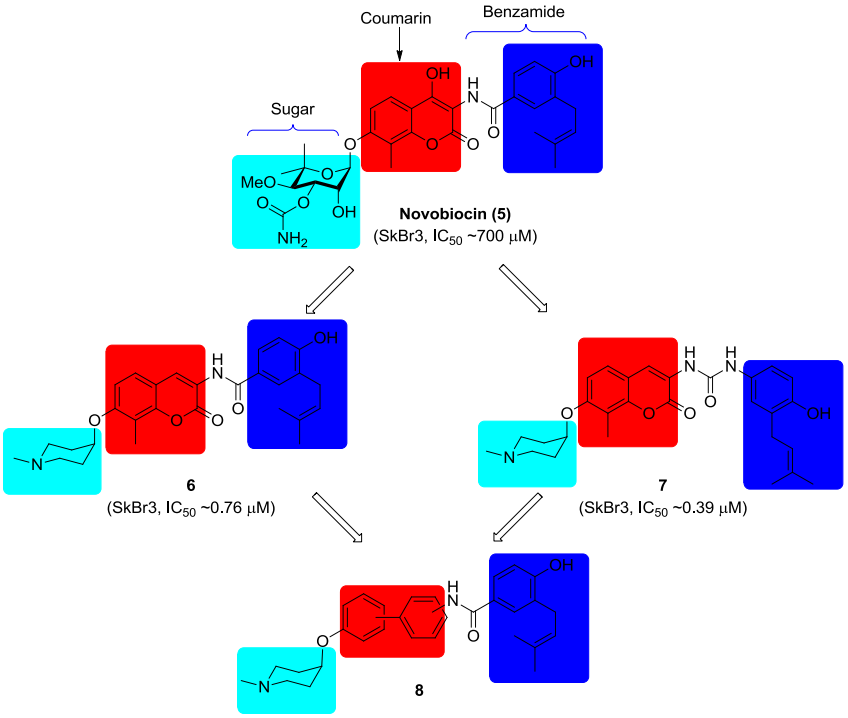

Fig. 2. Rationale for proposed coumarin replacements.