Bioorganic & Medicinal Chemistry 21 (2013) 5118–5129

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and biological evaluation of novobiocin analogues as potential heat shock protein 90 inhibitors

G. M. Kamal B. Gunaherath [a,†], Marilyn T. Marron [a], E. M. Kithsiri Wijeratne [a], Luke Whitesell [b,*], A. A. Leslie Gunatilaka [a,*]

[a] Southwest Center for Natural Products Research and Commercialization, School of Natural Resources and the Environment, College of Agriculture and Life Sciences, University of Arizona, 250 E. Valencia Road, Tucson, AZ 85706, United States
[b] Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142, United States

ARTICLE INFO

Article history:
Received 18 April 2013
Revised 13 June 2013
Accepted 19 June 2013
Available online 27 June 2013

Keywords:
Novobiocin analogues
Heat shock protein 90 (HSP90)
Synthesis
Structure–activity relationship
Biological activity

ABSTRACT

Recent studies have shown that novobiocin (NB), a member of the coumermycin (CA) family of antibiotics with demonstrated DNA gyrase inhibitory activity, inhibits Heat shock protein 90 (HSP90) by binding weakly to a putative ATP-binding site within its C-terminus. To develop more potent HSP90 inhibitors that target this site and to define structure–activity relationships (SARs) for this class of compounds, we have synthesized twenty seven 3-amido-7-noviosylcoumarin analogues starting from NB and CA. These were evaluated for evidence of HSP90 inhibition using several biological assays including inhibition of cell proliferation and cell cycle arrest, induction of the heat shock response, inhibition of luciferase-refolding in vitro, and depletion of the HSP90 client protein c-erbB-2/HER-2/neu (HER2). This SAR study revealed that a substantial increase in biological activity can be achieved by introduction of an indole-2-carboxamide group in place of 4-hydroxy-isopentylbenzamido group at C-3 of NB in addition to removal/derivatization of the 4-hydroxyl group from the coumarin ring. Methylation of the 4-hydroxyl group in the coumarin moiety moderately increased biological activity as shown by compounds **11** and **13**. Our most potent new analogue **19** demonstrated biological activities consistent with known HSP90-binding agents, but with greater potency than NB.

1. Introduction

Heat shock protein 90 (HSP90) is an abundant molecular chaperone that is essential for the post-translational folding and conformational maintenance of over 150 client proteins.[1] Many of these HSP90 client proteins are directly associated with the cancer phenotype including the tumor suppressor p53, receptor-linked tyrosine kinases such as HER2, steroid receptors, and a wide range of other mediators of intracellular signal transduction. As a consequence of constitutive activation of the heat-shock response, levels of HSP90 are frequently elevated in highly malignant tumors.[2] Because HSP90 stabilizes many of the oncoproteins required for cancer survival and growth, inhibition of HSP90 by compounds such as geldanamycin (an N-terminus binding agent) can impair the growth and survival of diverse tumor types. In contrast to the many HSP90 N-terminal inhibitors undergoing clinical evaluation,[3] C-terminal inhibitors have only recently attracted attention as potential anticancer agents. Novobiocin (NB, **1a**) is a well-known natural product inhibitor of bacterial DNA gyrase. Consisting of a coumarin core, an acyl substituent and a noviose sugar moiety, it has been demonstrated to bind the C-terminus of HSP90 at a putative ATP-binding site.[4] However, a very high concentration (~700 μM) of NB is required to impair HSP90 chaperone function and induce degradation of client proteins such as HER2 in human cancer cells.[5] As part of our continuing search for natural product-based HSP90 inhibitors,[6] we prepared twenty seven NB analogues from NB (**1a**) and coumermycin (CA, **2**) and evaluated their activity using a suite of biological assays selected to monitor different aspects of HSP90 function. Herein we report synthetic methods and structure-activity relationships (SARs) for these compounds. The biological activities reported include global effects on proliferation and cell cycle progression as well as more specific

Abbreviations: DAPI, 4′,6-diamidino-2-phenylindole; DCC, dicyclohexylcarbodiimide; EDC, *N*-(3-dimethylaminopropyl)-*N*′-ethyl-carbodiimide; FITC, fluorescein isothiocyanate; NBT/BCIP, nitro-blue tetrazolium chloride and 5-bromo-4-chloro-3′-indolyphosphate *p*-toluidine salt; PBA, phosphate buffered saline with bovine albumin; PFA, formaldehyde in phosphate buffered saline with bovine albumin; PMSF, phenylmethylsulfonyl fluoride; 4-PP, 4-pyrrolidinopyridine; TNES, a buffer

* Corresponding authors. Tel.: +1 520 621 9932; fax: +1 520 621 8378 (A.A.L.G.); tel.: +1 617 452 3542; fax: +1 617 258 7228 (L.W.).
E-mail addresses: whitesell@wi.mit.edu (L. Whitesell), leslieg@cals.arizona.edu (A.A. Leslie Gunatilaka).
† Present address: Department of Chemistry, The Open University of Sri Lanka, P.O. Box 21, Nugegoda, Sri Lanka.

0968-0896/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2013.06.042

HSP90-related effects on heat-shock induction, protein refolding in vitro, and depletion of HER2 in breast cancer cells.

In a previous SAR study of NB analogues, Blagg and co-workers have shown that the noviose moiety at C-7 of the coumarin ring is critical for biological activity based on depletion of the HSP90 client protein phospo-AKT.[7a] The most active analogue in this study lacked the C-4 OH group in the coumarin moiety and contained an *N*-acetyl side chain in place of the substituted benzamide group of NB. In a subsequent study this group found that analogues containing both the modified sugar and coumarin moieties such as 3′-descarbamoyl-4-deoxynovobiocin were more potent in depleting cellular levels of the HSP90 client proteins, HER2 and p53.[7b] In a more recent report they have suggested that replacement of the substituted benzoyl moiety by a 2-indolecarbonyl group in 3′-descarbamoyl-4-deoxynovobiocin results in a compound with improved activity as measured by depletion of the HSP90 client proteins HER2,RAF, and AKT and induction of the heat-shock response.[7c] Renoir and co-workers in their SAR study involving a series of denoviosyl NB analogues found that the presence of an OH group at C-4 or C-7 of the coumarin substructure is essential fordegradation of HSP90 client proteins.[8a] In a subsequent study they showed that introduction of a tosyl group at C-4 along with the removal of substituents at C-7 and C-8 of the coumarin moiety enhanced the activity of NB.[8b] It is also noteworthy that Blagg's group has recently investigated NB analogues with an array of structural diversity[9] while Huang et al. have designed new NB derivatives with higher HSP90 inhibitory activities based on a three-dimensional quantitative SAR (3D QSAR) study and confirmed by molecular simulation.[10] In an attempt to develop novenamine-based antibacterial agents, Berkov-Zrihen et al. have recently studied acylation of NB by carboxylic acid anhydrides.[11]

2. Results and discussion

2.1. Chemical synthesis

Fifteen 3-*N*-acyl analogues of NB **6a**–**6o** (Scheme 1) were prepared from **1a** via 2′-noviosyloxazolocoumarin (**3**) and 2′-acetylnoviosyl-3-aminocoumarin (**4**). Compound **3** was obtained by refluxing **1a** with Ac_2O and pyridine[12] which on subsequent treatment with AcCl in refluxing EtOH yielded **4**.[13] Acylation of **4** with a variety of carboxylic acids in the presence of DCC/4-PP or EDC gave a series of NB analogues **5a**–**5j** and **5l**–**5p**. Hydrogenolysis of **5j** afforded 5**k**. A series of NB analogues **6a**–**6o** were obtained from **5a**–**5i** and **5k**–**5p** via deacetylation with aq. MeOH/NaCN (Scheme 1). Analogues **10a**–**10e** containing a noviose moiety similar to CA were obtained from commercially available **2** via the 2′-acetylnoviosyloxazolocoumarin (**7**) following a similar procedure described for **3** above.[13] The 2′-acetylnoviosyl-3-aminocoumarin derivative **8**, obtained by the treatment of **7** with AcCl in refluxing EtOH, was acylated with a variety of carboxylic acids in the presence of EDC to yield **9a**–**9e**, which on deacetylation with aq. MeOH/NaCN yielded the NB analogues **10a**–**10e** (Scheme 1).

It has recently been reported that 4-OH and 3′-carbamoyl groups in NB are detrimental to its ability to inhibit HSP90.[7b] Prompted by this report and in keeping with our strategy of using NB (**1a**) and CA (**2**) as starting materials to obtain NB analogues required for this SAR study, we examined the effect of alkylating the 4-OH group as an alternative to its removal. Thus, novobiocin dimethyl ether (**11**) was prepared from **1a** by treatment with Me_2SO_4/K_2CO_3 (Scheme 2). To determine if the 3′-carbamoyl group of the noviose moiety of NB is essential for its activity, 3′-descarbamoylnovobiocin (**12**) was prepared by refluxing NB monosodium salt (**1b)** with aq. MeOH/NaCN, followed by acidification with aq. 2 M HCl. Subsequent methylation of **12** as above gave 3′-descarbamoylnovobiocin-4,4″-dimethyl ether (**13**). Further modification to the noviosyl moiety was carried out by linking **13** with indole-2-carboxylic acid yielding the NB analogues **14** and **15**. Treatment of **15** with imidazole in DMF afforded **16** resulting from migration of the axial 2′-acyl group to a less hindered equatorial 3′-position (Scheme 2). Treatment of **1b** with chlorobis(cyclopentadienyl)hydridozirconium (Cp_2ZrHCl) under anhydrous conditions in DME yielded 4-deoxynovobiocin (**17**) (Scheme 2).[14] Although the yield of this nonoptimized reaction was low, it is noteworthy that this report constitutes the first application of this facile reductive enolate deoxygenation methodology to a complex molecule such as NB. The 4-deoxy-3-(indole-2-carboxamide) analogue **19** was prepared by deoxygenation of the lithium enolate of **5n** with Cp_2ZrHCl under anhydrous conditions yielding **18** followed by deacetylation with aq. MeOH/NaCN (Scheme 3).

2.2. Biological evaluation

2.2.1. Depletion of cell surface HER2

Destabilization and subsequent depletion of the receptor-linked tyrosine kinase HER2 is a hallmark of HSP90 inhibition.[15] As an initial screen for biological activity, all NB analogues were evaluated for their ability to deplete HER2 in a flow cytometry-based assay using the human breast adenocarcinoma cell line SKBR3. DMSO and geldanamycin (GA, 0.5 μM) served as negative and positive controls, respectively. NB (500 μM) served as a benchmark for purposes of comparison. When SKBR3 cells were incubated overnight with each of the 27 analogues at a concentration of 100 μM, only compounds **10e**, **11**, **13**, **17**, and **19** depleted cell surface HER2 by ~40% (Fig. 1A). NB demonstrated no activity at this concentration. To begin correlating HER2 depletion by compounds with their antiproliferative activity, we measured the number of viable SKBR3 cells after overnight exposure to compounds (Supplementary Fig. S36). This experiment was performed in the same SKBR3 cells and under the same exposure conditions as the flow cytometry experiment measuring HER2 levels (Fig. 1A). Only those compounds which induced HER2 depletion demonstrated anti-proliferative activity in this assay.

To avoid potential confounding effects on kinase depletion due just to cytotoxicity, the active analogues **10e**, **11**, **13**, **17**, and **19** were assayed for HER2 depletion using the MCF-7 cell line. These cells express the HER2 kinase, but are far less dependent on it for their short-term survival, In this follow-up experiment, the EC_{50} values of analogues **11**, **13**, **17** and **19** were 38 ± 2, 41 ± 8, 24 ± 5, and 20 ± 5, respectively, demonstrating that **17** and **19** have the greatest potency among those tested, whereas analogue **10e** showed the weakest activity (EC_{50} > 50.0 μM).

2.2.2. Depletion of total cellular HER2

The NB analogues **11**, **13**, **17**, and **19** demonstrating significant activity by flow cytometry-based HER2 depletion assay, were evaluated for their ability to deplete total cellular HER2 by western blot analysis. MCF-7 cells were treated overnight with analogues prior to preparation of lysates. Analogues **11**, **13**, **17**, and **19** exhibited ~50% reduction of total HER2 at 50 μM (data not shown). However, **17** and **19** were more potent with depletion of HER2 evident at concentrations of 12.5 and 25.0 μM, respectively (Figs. 1B and C), correlating well with the observations from flow cytometric analysis. To confirm equal loading across their respective lanes, each membrane was stained with Ponceau S prior to blotting to visualize total protein (Supplementary Fig. S37). Because analogue **17** was cytotoxic to MCF-7 cells at concentrations over 25.0 μM, it could not be reliably evaluated at higher concentrations. Western blots were subsequently re-probed for an inducible isoform of HSP70 to evaluate heat-shock induction. Only the highest concentration of NB analogue **19** induced any detectable increase in the level of this protein. This result further confirms equal protein loading across lanes.