Bioorganic & Medicinal Chemistry Letters 20 (2010) 3957–3960

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

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

Laura B. Peterson, Brian S. J. Blagg *

Department of Medicinal Chemistry, The University of Kansas, 1251 Wescoe Hall Drive, Malott 4070, Lawrence, KS 66045-7582, United States

ARTICLE INFO

Article history:
Received 7 April 2010
Revised 28 April 2010
Accepted 30 April 2010
Available online 6 May 2010

Keywords:
Hsp90
Novobiocin
Antiproliferation
Triazole

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

* Corresponding author. Tel.: +1 785 864 2288; fax: +1 785 864 5326.
E-mail address: bblagg@ku.edu (B.S.J. Blagg).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.04.140

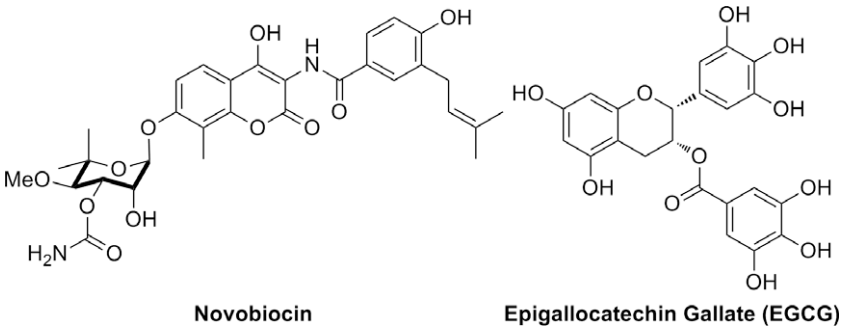
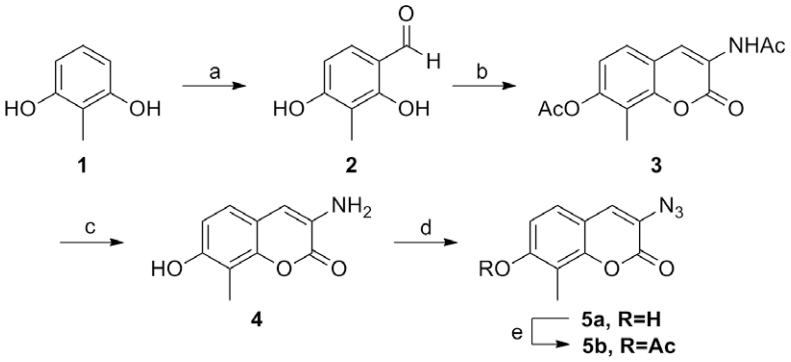


Figure 1. Hsp90 C-terminal inhibitors.

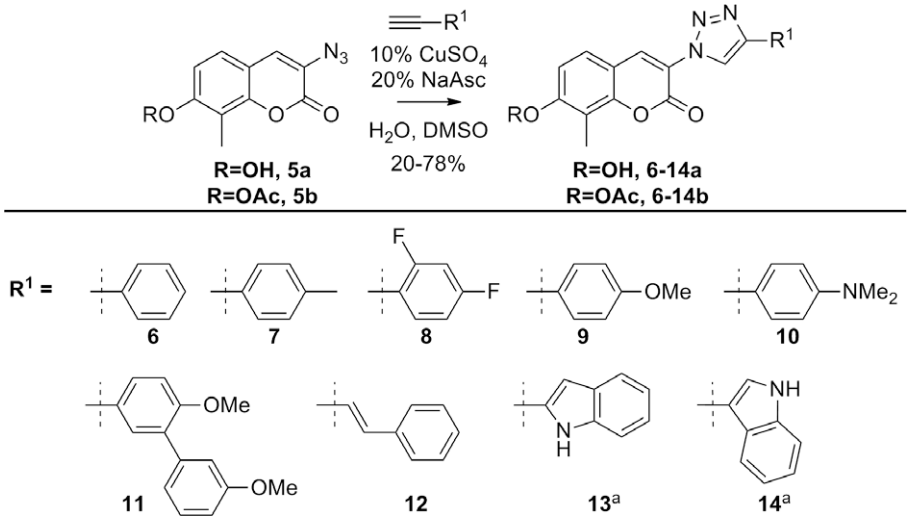


Scheme 1. Reagents: (a) (i) $POCl_3$, DMF, MeCN; (ii) H_2O (55%); (b) *N*-acetyl glycine, NaOAc, Ac_2O; (c) HCl, EtOH; (d) $NaNO_2$, HCl, EtOH, H_2O, then NaN_3 (52% three steps); (e) Ac_2O, pyridine, CH_2Cl_2 (>95%).

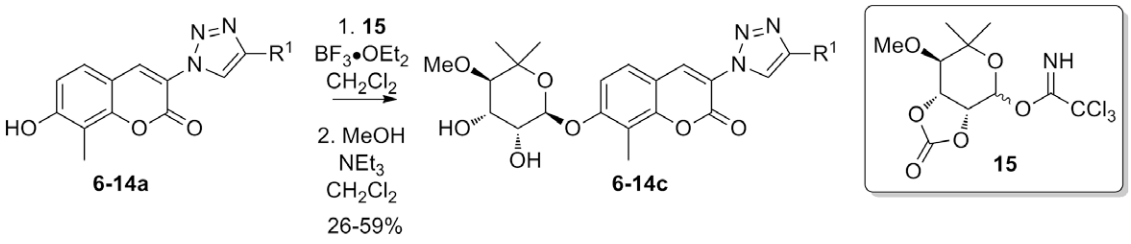
design, syntheses, and biological evaluation of these compounds are described herein.

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.



Scheme 3. Noviosylation of triazole appended coumarins.

to investigate the electronic nature of the aryl side chain, including neutral (Me), electron-withdrawing (F) and electron-donating (OMe, NMe_2) properties as originally identified during classical novobiocin SAR studies.[13] Alkynes **11–14** were chosen based on prior observations that the analogous amide derivatives manifested good anti-proliferative activities against multiple cell lines,[13] and thus provide direct comparison between amide and triazole analogues.

Completion of the triazole-containing novobiocin analogues was achieved by incorporation of the noviose sugar into **6–14a** (Scheme 3). The phenols of compounds **6–14a** were noviosylated with the trichloroacetimidate of noviose carbonate (**15**) in the presence of boron trifluoride etherate.[21] Solvolysis of the cyclic carbonate with methanolic triethylamine afforded novobiocin analogues **6–14c**.

Upon preparation of compounds **6–14a, b** and **c**, their growth inhibitory activities against MCF-7 (ER+) and SKBr-3 (ER-, HER2 overexpressing) breast cancer cell lines were determined (Table 1). These results provide evidence that compounds **14a**–**14c**, and **11c** are the most potent analogues prepared in this series. The structure–activity relationships suggest that analogues bearing sterically demanding side chains exhibit greater potency, as compounds with biaryl, indole, or homologated aryl groups (**11–14**) were more efficacious than substituted aryl compounds (**6–10**). In addition, comparison of compounds **6–10** suggests that *para*-substitution of the aryl ring with Me, OMe, NMe_2 or F is not well tolerated, as these compounds displayed minimal anti-proliferative activity. These results suggest that the C-terminal binding pocket has a large hydrophobic pocket that can easily accommodate substituents as in compounds **11–14**.

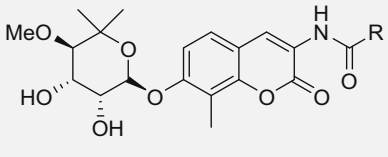
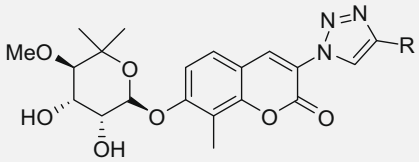
Additionally, comparison between the amide-containing analogues and triazole-containing analogues provides further SAR (Table 2). As evident from the data presented in Table 2, the triazole moiety has little effect on anti-proliferative activity. Both the triazole and amide analogues containing the biaryl and 3-indole side chains indicate the activities for both sets of compounds

Table 1
Anti-proliferative activities of triazole-containing novobiocin analogues[a]

Novobiocin	MCF-7[b] (IC_{50}, μM) 481.3 ± 1.5			SKBr-3[b] (IC_{50}, μM) 474.7 ± 13.9		
	a	b	c	a	b	c
6	25.16 ± 4.52	40.83 ± 8.39	64.44 ± 5.79	39.02 ± 3.11	76.37 ± 2.31	>100
7	23.15 ± 0.82	>100	>100	>100	90.82 ± 9.19	>100
8	33.47 ± 1.64	>100	>100	34.18 ± 4.12	>100	>100
9	>100	>100	>100	>100	>100	>100
10	>100	>100	>100	>100	>100	>100
11	28.00 ± 0.60	42.37 ± 4.96	13.16 ± 3.85	44.91 ± 3.35	50.81 ± 0.71	21.22 ± 5.99
12	33.68 ± 3.22	28.46 ± 2.20	14.62 ± 0.44	33.92 ± 6.72	65.12 ± 12.70	51.91 ± 2.98
13	38.30 ± 5.95	76.13 ± 8.37	NT	42.53 ± 0.88	50.39 ± 1.77	NT
14	8.17 ± 0.02	7.45 ± 0.69	18.33 ± 4.67	13.30 ± 2.88	6.52 ± 0.76	8.17 ± 0.11

NT = not tested.
[a] Values represent mean ± standard error for at least two separate experiments performed in triplicate.
[b] IC_{50} is defined as the concentration of compound necessary to inhibit cellular growth by 50%.

Table 2
Comparison of anti-proliferative activities: amide-containing versus triazole-containing novobiocin analogues

		
	R = Biaryl	R = Biaryl (**11c**)
IC_{50} (MCF-7)	18.7 ± 1.8[a]	13.16 ± 3.85
IC_{50} (SKBr-3)	7.5 ± 1.0[a]	21.22 ± 5.99
	R = 3-Indole	R = 3-Indole (**14c**)
IC_{50} (MCF-7)	5.3 ± 0.3[a]	18.33 ± 4.67
IC_{50} (SKBr-3)	12.2 ± 1.5[a]	8.17 ± 0.11

[a] Biological data taken from Ref. 13.

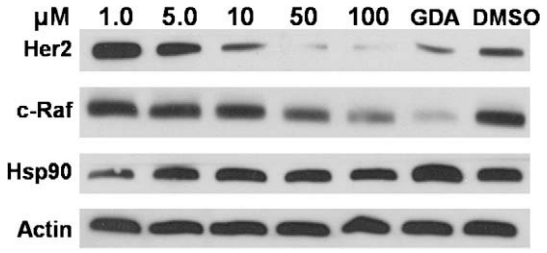


Figure 2. Western blot analysis for compound **14b**.

are comparable against the two cell lines tested. One discrepancy observed between the amide and triazole analogues is that in which simple aryl side chains (**6–10**) manifest IC_{50} values in the 10–20 μM range for the amide-containing molecules while the triazole compounds **6** and **9** display IC_{50} values above 50 μM.[13] These results suggest that the triazole moiety affects biological activity in two ways: the availability of a hydrogen bond donor in the amide linkage and the steric bulk of the side chain. This hydrogen bond contact is lost upon introduction of the triazole moiety, but can be overcome by the introduction of steric bulk in the side chain. A preference for steric bulk has been observed in several series of novobiocin analogues, and appears to be a contributing factor to biological activity.

After analysis of the triazole-containing analogues anti-proliferative activity, Western blot analysis was preformed for the most active compound, **14b**, to confirm anti-proliferative activity results from Hsp90 inhibition (Fig. 2). Western blot analysis of MCF-7 cells treated with increasing concentrations of **14b**, induce Hsp90-dependent client protein degradation in a dose-dependent manner, with an apparent IC_{50} value that correlates directly to the anti-proliferative IC_{50} value. Her2 and c-Raf are client proteins of Hsp90, and pharmacological inhibition of Hsp90 leads to their degradation via ubiquitinylation and proteome-mediated hydrolysis. Additionally, compound **14b** appears to have little effect on the heat shock response, as unaltered levels of Hsp90 were observed, consistent with inhibition of the Hsp90 C-terminus. In contrast, client protein degradation and heat shock induction was not observed for the inactive compound **10b** (data not shown). Western blot analysis indicates that these compounds are interfering with the Hsp90-mediated protein folding process, and that the anti-proliferative activities for these compounds are directly related to Hsp90 inhibition.

In conclusion, a series of triazole-containing novobiocin analogues was prepared and evaluated against two breast cancer cell lines. Western blot analysis affirmed Hsp90 inhibition by this class of compounds. The compounds described herein exhibit comparable activities to the corresponding amide-containing analogues. These results indicate that the amide moiety can be replaced by the triazole functionality, however, in some cases the loss of the hydrogen bond donor appears detrimental, but can be overcome by the inclusion of steric bulk in the triazole substituent.

Acknowledgements

The authors gratefully acknowledge the support of this project by NIH CA120458 and the NIH Training Grant (T32 GM008545) on Dynamic Aspects in Chemical Biology (L.B.P.).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.04.140.

References and notes

1. Bishop, S. C.; Burlison, J. A.; Blagg, B. S. J. *Curr. Cancer Drug Targets* **2007**, *7*, 369.
2. Blagg, B. S. J.; Kerr, T. D. *Med. Res. Rev.* **2006**, *26*, 310.
3. Hanahan, D.; Weinberg, R. A. *Cell* **2000**, *100*, 57.
4. Zhang, H.; Burrows, F. *J. Mol. Med.* **2004**, *82*, 488.
5. Soti, C.; Nagy, E.; Giricz, Z.; Vigh, L.; Csermely, P.; Ferdinandy, P. *Brit. J. Pharmacol.* **2005**, *146*, 769.
6. Chiosis, G.; Vilenchik, M.; Kim, J.; Solit, D. *Drug Discovery Today* **2004**, *9*, 881.
7. Walter, S.; Buchner, J. *Angew. Chem., Int. Ed.* **2002**, *41*, 1098.
8. Biamonte, M. A.; Van de Water, R.; Arndt, J. W.; Scannevin, R. H.; Perret, D.; Lee, W. *J. Med. Chem.* **2010**, *53*, 3.
9. Prodromou, C.; Panaretou, B.; Chohan, S.; Siligardi, G.; O'Brien, R.; Ladbury, J. E.; Roe, S. M.; Piper, P. W.; Pearl, L. H. *EMBO J.* **2000**, *19*, 4383.
10. Donnelly, A.; Blagg, B. S. J. *Curr. Med. Chem.* **2008**, *15*, 2702.
11. Marcu, M. G.; Chadli, A.; Bouhouche, I.; Catelli, M. G.; Neckers, L. *J. Biol. Chem.* **2000**, *2000*, 37181.
12. Marcu, M. G.; Schulte, T. W.; Neckers, L. *J. Natl. Cancer Inst.* **2000**, *92*, 242.
13. Burlison, J. A.; Avila, C.; Vielhauer, G.; Lubbers, D. J.; Holzbeierlein, J.; Blagg, B. S. J. *J. Org. Chem.* **2008**, *73*, 2130.
14. Burlison, J. A.; Blagg, B. S. J. *Org. Lett.* **2006**, *8*, 4855.
15. Burlison, J. A.; Neckers, L.; Smith, A. B.; Maxwell, A.; Blagg, B. S. J. *J. Am. Chem. Soc.* **2006**, *128*, 15529.
16. Donnelly, A.; Mays, J. R.; Burlison, J. A.; Nelson, J. T.; Vielhauer, G.; Holzbeierlein, J.; Blagg, B. S. J. *J. Org. Chem.* **2008**, *73*, 8901.
17. Yu, X. M.; Shen, G.; Neckers, L.; Blake, H.; Holzbeierlein, J.; Cronk, B.; Blagg, B. S. J. *J. Am. Chem. Soc.* **2005**, *127*, 12778.
18. Kolb, H. C.; Sharpless, K. B. *Drug Discovery Today* **2003**, *8*, 1128.
19. Tron, G. C.; Pirali, T.; Billington, R. A.; Canonico, P. L.; Sorba, G.; Genazzani, A. A. *Med. Res. Rev.* **2008**, *28*, 278.
20. Sivakumar, K.; Xie, F.; Cash, B. M.; Long, S.; Barnhill, H. N.; Wang, Q. *Org. Lett.* **2004**, *6*, 4603.
21. Shen, G.; Yu, X. m.; Blagg, B. S. J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5903.