Synthesis and Evaluation of Noviose Replacements on Novobiocin that Manifest Anti-proliferative Activity

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Abstract

Structural modifications to the coumarin core and benzamide side chain of novobiocin have successfully transformed the natural product from a selective DNA gyrase inhibitor into a potent inhibitor of the Hsp90 C-terminus. However, no SAR studies have been conducted on the noviose appendage, which represents the rate-limiting synthon in the preparation of analogues. Therefore, a series of sugar mimics and non-sugar derivatives were synthesized and evaluated to identify simplified compounds that exhibit Hsp90 inhibition. Evaluation against two breast cancer cell lines demonstrated that replacement of the stereochemical complex noviose with simplified alkyl amines increased anti-proliferative activity, resulting in novobiocin analogues that manifest IC_{50} values in the mid nanomolar range.

Keywords

Heat shock protein 90; Hsp90 inhibitors; Novobiocin; Stucture-activity relationships; Breast cancer

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The 90 kDa heat shock proteins (Hsp90) are responsible for the conformational maturation of more than 200 Hsp90-dependent client proteins,[1,2] of which, Her2, Src family kinases, Raf, PLK, RIP, AKT, telomerase and Met are directly associated with the six hallmarks of cancer.[3,4] Consequently, inhibition of the Hsp90 protein folding machinery simultaneously disrupts multiple oncogenic pathways, leading to cell death.[5,6] Since the first proof-of-concept drug, 17-AAG (a synthetic analogue of geldanamycin), entered clinic trials and demonstrated therapeutic benefit at tolerable doses,[7] extensive research has led to more than 20 subsequent clinic trials,[8] highlighting Hsp90 as a promising therapeutic target for the development of anti-cancer agents.[9–11]

Novobiocin, a natural product comprised of a noviose sugar, a coumarin core and a prenylated benzamide side chain, is isolated from *streptomyces* strains[12] and is known to exhibit antimicrobial activity by binding to the DNA gyrase ATP-binding pocket,[13] a unique nucleotide-binding motif shared only by members of the GHKL superfamily.[14] Due to the similar bent conformation exhibited by ADP bound to both DNA gyrase and the Hsp90 N-terminal domain, Neckers and co-workers hypothesized that novobiocin may manifest anti-cancer activity through Hsp90 inhibition.[15] Their pioneering studies revealed novobiocin to

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bind Hsp90, but instead of binding to the well-recognized N-terminal domain, it bound to a previously unrecognized C-terminal region, albeit with low efficiency (~700 μM in SKBr3 cells).[15] Since this study, structural modifications of novobiocin have been pursued to identify molecules that exhibit increased inhibitory activity.[16–22]

The first library of such compounds was designed and synthesized by Yu and co-workers to identify functionalities necessary for Hsp90 inhibition on the coumarin ring and benzamide side chain of novobiocin[17]. Their studies revealed that attachment of the noviose appendage to the 7-position of the coumarin ring and an amide linker at the 3-position are critical, while the 4-hydroxy substituent and the 3′-carbamoyl are detrimental. The most efficacious compound identified from this library was compound **A4**, which induced degradation of Hsp90-dependent client proteins at ~70-fold lower concentration than novobiocin. Intriguingly, compound **A4** induced the heat shock response at concentrations ~1000-fold lower than that required for client protein degradation.[23,24]

To confirm SAR trends observed by Yu and co-workers conformed to the natural product, **DHN2** was synthesized to delineate functionalities responsible for DNA gyrase versus Hsp90 inhibition.[18] This novobiocin analogue confirmed that the 4-hydroxyl and the 3′-carbamate are detrimental to Hsp90 inhibitory activity, but critical for DNA gyrase inhibition, thus confirming the SAR trends observed for **A-4**.

Subsequent structural modifications and SAR studies explored the coumarin ring and benzamide side chain, and several lead-like compounds were identified and remain under investigation.[19,21,22] As shown in Figure 1, the analogues prepared thus far retain the noviose appendage. However, the synthesis of noviose is laborious and hinders analogue development; as it requires more than ten steps to prepare and activate for subsequent coupling with the coumarin phenol.[25,26] Acknowledging the limited SAR for this moiety and its cumbersome preparation, simplified analogues were pursued in an effort to increase activity while simultaneously increasing solubility. In this article, we provide the first biologically-active substitutions for the noviose appendage on novobiocin and the first nonsugar mimics that exhibit increased anti-proliferative activity.

It is well understood that sugar moieties in natural products play a key role in solubility, activity and bioavailability for these compounds. Furthermore, the ring size can impart significant affinity towards their cognate protein. With these considerations in mind, a series of mono- and di-hydroxylated furanose and pyranose sugars (**1–5**, Scheme 2) were synthesized according to previously disclosed procedures[26] for incorporation onto the novobiocin scaffold **10**. The preparation of **10** is described in Scheme 1. The coumarin phenol **6** was converted to the methoxymethyl (MOM) ether using methoxymethyl chloride and Hunig's base in Dimethylformamide. The free aniline, liberated through hydrogenolysis with 10% Pd/C and hydrogen in tetrahydrofuran from **7**, was coupled with acid chloride **8** to give benzamide **9**. Subsequent cleavage of the methoxymethyl ether with 4N hydrochloride in dioxane provided phenol **10** in high yield.

Once prepared, the phenol of **10** was coupled with sugars **1–5** under Mitsunobu conditions to give an inseparable diastereomeric mixture of **11–13** and **15** (Scheme 2). In the case of compound **14**, a single diastereomer was formed. Subsequent hydrolysis of the cyclic carbonates and acetyl esters of **11a–b** and **14** with lithium hydroxide in THF/MeOH/H_2O (3:2:2, v/v) afforded a diastereomeric mixture of **16a–b** and **19**, respectively. At this stage, diastereomers **16a** and **16b** were separated by silica chromatography. The assignment of stereochemistry at the anomeric center was established through two-dimensional NMR studies utilizing NOESY. Similarly, hydrolysis of the benzoyl and acetyl ester of **12** with basic methanol yielded **17a** and **17b**, which could be separated by silica chromatography.

The tri-isopropylsilyl (TIPS) of **13** and tert-butyldimethylsilyl (TBDMS) groups of **15** were removed by the addition of tetrabutylammonium fluoride (TBAF) to give separable **18a** and **18b**; and **20a** and **20b**, respectively.

Upon construction of the noviose surrogates, compounds were subjected to evaluation by determination of anti-proliferative activity against SKBr3 (estrogen receptor negative, Her2 over-expressing breast cancer cells) and MCF-7 (estrogen receptor positive breast cancer cells) cell lines. As shown in Table 1, the six-membered sugar mimics (**16a–18a** and **16b–18b**) were found to be more potent than their five-membered counterparts (**19, 20a–b**). Compound **18b** displayed an IC_{50} value of 3.11±0.03μM and 1.56±0.20 against SKBr3 and MCF-7 cell lines respectively, which is ~3–8-fold more active than DHN2 (Table 1) and ~200 times greater than the activity manifested by novobiocin. Surprisingly, the stereochemistry of these sugar mimics was not critical for the observed increase in anti-proliferative activity, as both the α-(**16b**) and β-anomers (**17a**) produced similar activities. Placement of the hydroxyl group (3′-OH or 4′-OH) on the etheral ring also did not impart preferential activity.

Although simplified sugar mimics were found to increase anti-proliferative activity compared to DHN2 and novobiocin, more simplified analogues exhibiting enhanced solubility and activity were desired. N-Heterocycles are found in a variety of biologically active compounds, and in contrast to carbohydrates, are generally ionized at physiological pH.[27] Upon review of the first set of studies, we proposed that the noviose appendage played a siginficant role in solublizing the relatively hydrophobic coumarin core and benzamide side chain. Thus, commercially available amines, **21–27** (Scheme 3, secondary amines were protected with Boc), were selected as potential replacements for the noviose moiety. These alkylamines and heterocyclic analogues contain an ionizable amine located at various positions within the structure to afford potential hydrogen-bonding interactions while simultaneously enhancing solubility through their ionized counterparts.

Originally, coupling of these amines with phenol **10** was expected to easily afford the desired products. However, the acetyl ester on the benzamide side chain was hydrolyzed under these conditions and resulted in an inseparable mixture of mono- or dialkylated products. To circumvent this issue, the amine was first coupled with the coumarin ring and subsequently with the benzamide side chain to afford the desired analogues. The detailed synthesis is described as follows: Tertiary amines or Boc-masked secondary amines were reacted with Cbz-protected coumarin **6** in the presence of two equivalents of triphenylphosphine and diisopropylazodicarboxylate in tetrahydrofuran to give amine-derived coumarins, **28a–28g**. The Cbz-protecting group was removed by hydrogenolysis to give the free amines, which were then coupled with acid chloride **8** to give compounds **29a–29g** in good yield. Removal of the Boc protecting group with trifluoroacetic acid (TFA) in methylene chloride afforded the secondary amine analogues **30b**, **30d** and **30g**. Hydrolysis of the phenolic ester with methanolic triethyl amine gave analogues, **31a–31g**, in good to excellent yields.

Anti-proliferative activity manifested by these analogues was assessed against SKBr3 and MCF-7 cell lines. As shown in Table 2, the IC_{50} values for the secondary and tertiary amines varied between 0.4–1.5 μM, making them 1500-fold more potent than novobiocin. Generally, the ester series exhibited comparable anti-proliferative activity to their phenol counterparts, suggesting the ester analogues may rapidly hydrolyze in cells due to esterases. In regards to the piperidine analogues, 4-substituted analogues exhibited greater potency than the 3-substituted analogues against both cell lines. For example, against the SKBr3 cell line, compound **29a** is ~3-fold more active than compound **29c** and compound **30b** and **31b** is ~2 times more active than compound **30d** and **31d**, respectively. The same trend was

observed for the noncyclic amino analogues as well (**29f** vs **29e**, **31f** vs **31e**). These results indicate that the location of the amine is important for binding/manifesting inhibiting activity. A surprising finding of this work was that analogues containing noncyclic amines exhibit equivalent potencies to their piperidine counterparts, specifically, **29a** and **29f**, **31a** and **31f**, indicating that inclusion of a ring structure is not required.

To confirm that replacement of noviose with a sugar or amino surrogate did not alter inhibitory activity against Hsp90, western blot analyses of cell lysates following the administration of **18b** or **31b** were performed. As shown in Figure 2, the Hsp90-dependent client proteins, Her2, Raf and Akt, were degraded in MCF-7 cells in a concentration-dependent manner upon treatment with **18b** or **31b**. The non-Hsp90-dependent protein, actin, was not altered upon administration of **18b** or **31b**, indicating selective degradation of Hsp90-dependent proteins takes place in the presence of **18b** or **31b**. In addition, neither of these two compounds induced the heat shock response, which is a characteristic shared by benzamide-containing novobiocin analogues that bind the Hsp90 C-terminus.[28,29]

In a conclusion, sugar mimics and amino analogues of the noviose appendage on the coumarin ring of novobiocin that exhibit improved solubility and anti-proliferative activity have been produced. The cyclic and acyclic amino surrogates can be synthesized expeditiously and will enable rapid identification of novobiocin analogues that may provide clinical opportunities for the treatment of cancer. The development of improved compounds that exhibit such activity is underway and the results from those studies will be reported in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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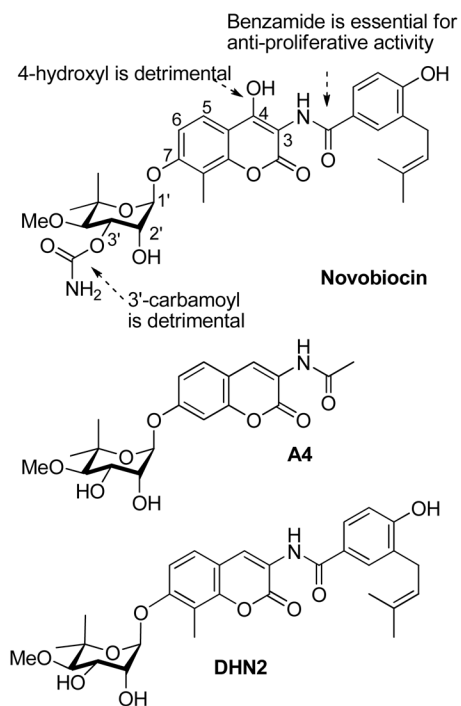
A-4.

Figure 1.
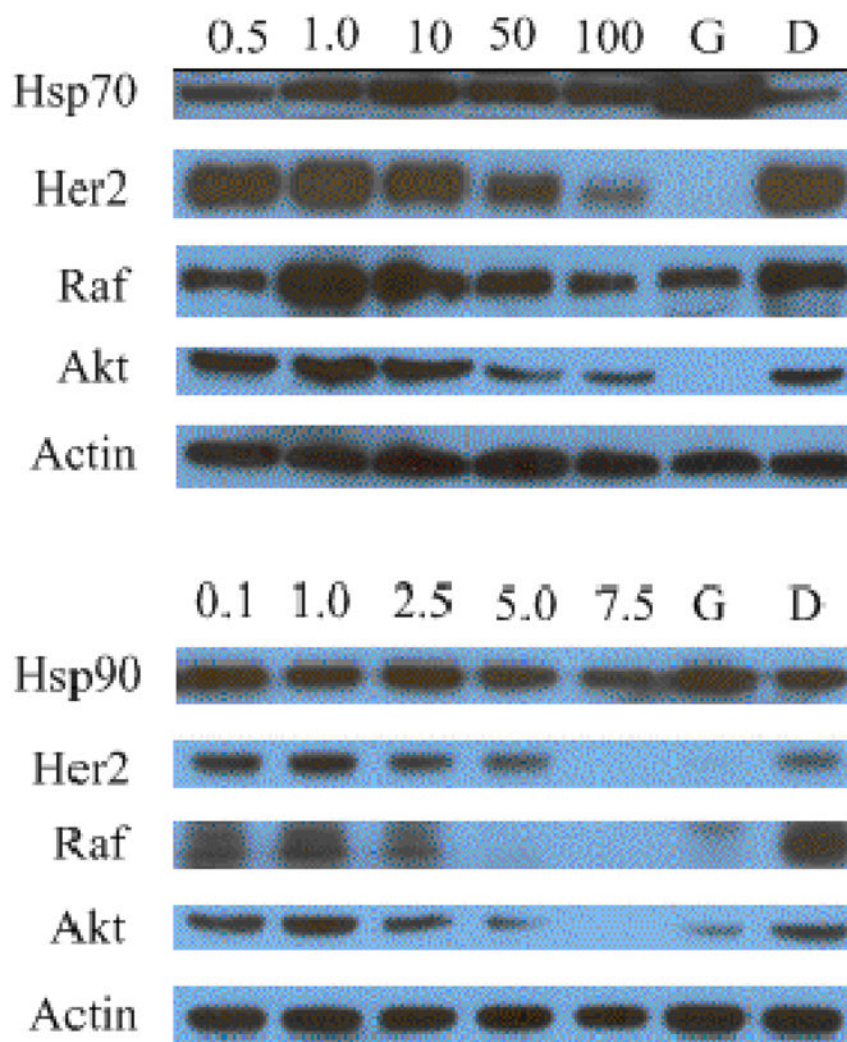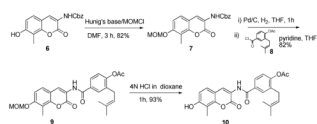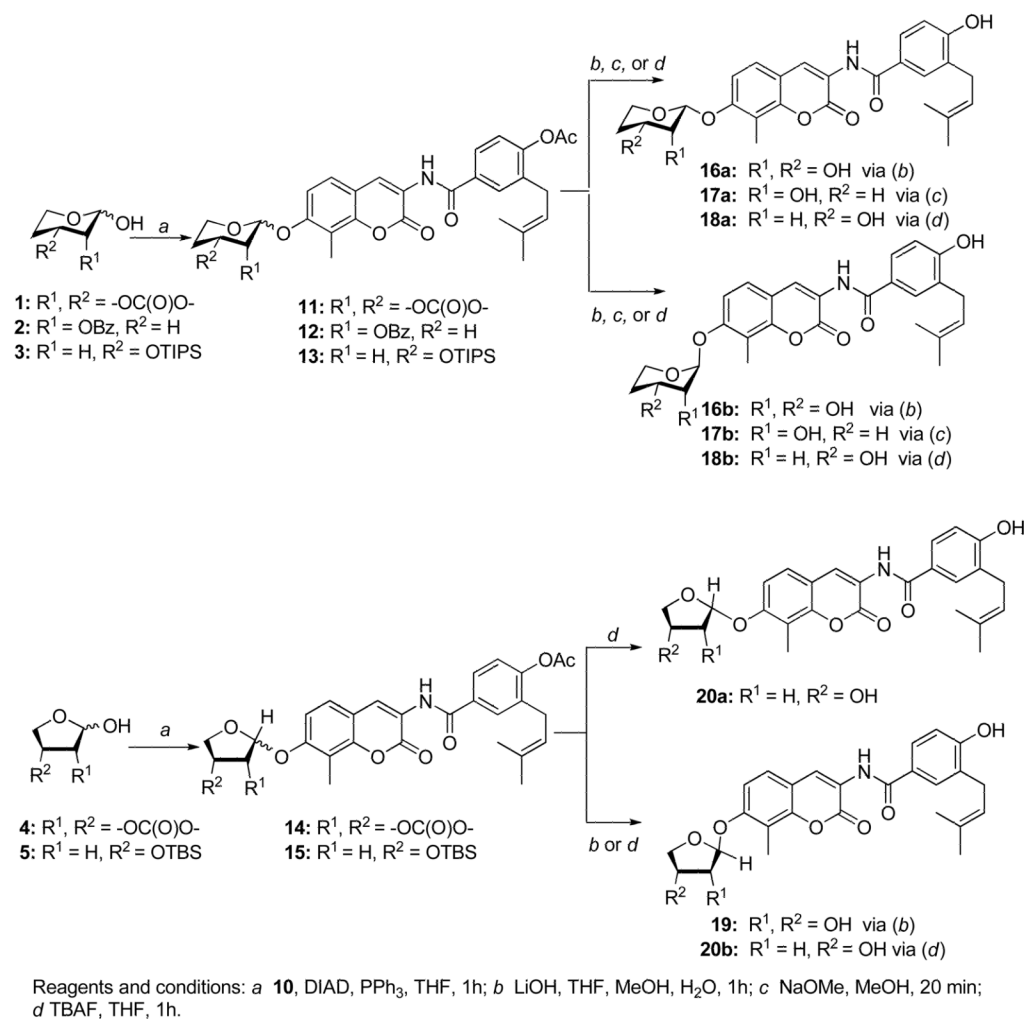SAR generated from previous investigations.

Figure 2.
Western blot analyses of Hsp90-depedent client proteins from MCF-7 breast cancer cell lysates upon treatment with **18b** (top) or **31b** (bottom). Concentrations (in μM) were indicated above each line, geldanamycin (G, 0.5 μM) and dimethyl sulfoxide (D) were employed as positive and negative controls.

Scheme 1.
Preparation of coumarin phenol 10.

Reagents and conditions: *a* **10**, DIAD, PPh₃, THF, 1h; *b* LiOH, THF, MeOH, H₂O, 1h; *c* NaOMe, MeOH, 20 min; *d* TBAF, THF, 1h.

Scheme 2.
Synthesis of novobiocin analogues containing mono- and di-hydroxylated furanoses and pyranoses.
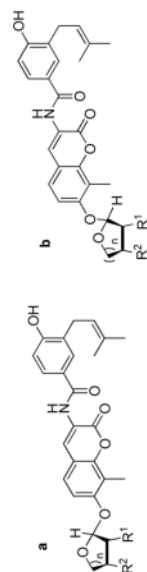Reagents and conditions: *a* **10**, DIAD, PPh₃, THF, 1h; *b* LiOH, THF, MeOH, H₂O, 1h; *c* NaOMe, MeOH, 20 min; *d* TBAF, THF, 1h.

Scheme 3.
Synthesis of amine analogues.
Reagents and Conditions: *a*. **6**, PPh$_3$, DIAD, THF *b*. H$_2$,10% Pd/C *c*. **8**, Pyridine, THF *d*. 10% TFA/CH$_2$Cl$_2$ *e*. 10% Et$_3$N/MeOH

Table 1
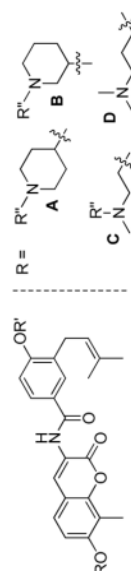
Anti-proliferation activities of sugar derived novobiocin analogues

Compound	n	R²	R¹	SKBr3 (µM)	MCF7 (µM)
16a	2	OH	OH	10.68±0.05	10.04±0.03
16b	2	OH	OH	6.96±0.06	13.30±0.21
17a	2	H	OH	7.87±0.04	6.45±0.13
17b	2	H	OH	29.98±2.07	10.24±0.21
18a	2	OH	H	5.07±0.26	1.34±0.18
18b	2	OH	H	3.11±0.03	1.56±0.20
19	1	OH	OH	14.37±0.52	14.31±0.40
20a	1	OH	H	22.16±0.94	>100
20b	1	OH	H	21.46±2.28	22.50±0.40
DHN2	--	--	--	10.86±0.47	11.29±0.41

Table 2

Anti-proliferative activities of amine analogues.

Compound	R	R'	R"	SKBr3 (μM)	MCF-7 (μM)
29a	A	Ac	Me	0.58±0.05	1.18±0.20
29c	B	Ac	Me	1.42±0.02	1.57±0.05
29e	C	Ac	Me	1.32±0.23	4.76±0.52
29f	D	Ac	--	0.46±0.19	1.18±0.03
30b	A	Ac	H	0.56±0.05	1.53±0.14
30d	B	Ac	H	1.23±0.00	1.54±0.41
30g	C	Ac	H	0.91±0.21	2.08±0.13
31a	A	H	Me	0.76±0.17	1.09±0.10
31b	A	H	H	0.47±0.10	0.85±0.09
31c	B	H	Me	4.69±0.16	10.12±0.17
31d	B	H	H	0.79±0.11	1.68±0.25
31e	C	H	Me	9.45±0.22	13.48±0.38
31f	D	H	--	0.44±0.02	1.35±0.38
31g	C	H	H	0.75±0.12	1.33±0.01