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Synthesis and biological evaluation of arylated novobiocin analogs as Hsp90 inhibitors

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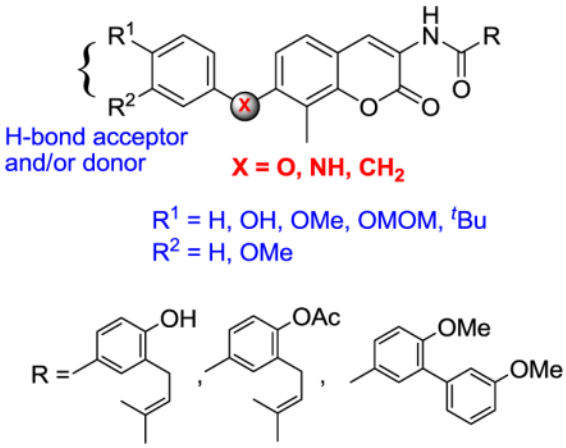
Abstract

Novobiocin analogs lacking labile glycosidic ether have been designed, synthesized and evaluated for Hsp90 inhibitory activity. Replacement of the synthetically complex noviose sugar with simple aromatic side chains produced analogs that maintain moderate cytotoxic activity against MCF7 and SkBR3 breast cancer cell-lines. Rationale for the preparation of *des*-noviose novobiocin analogs in addition to their synthesis and biological evaluation are presented herein.



Heat shock protein 90 kDa (Hsp90) is a remarkably versatile molecular chaperone that plays a key role in homeostasis.[1,2] However, Hsp90 is unregulated in malignancies and facilitates the maturation, stabilization and/or activation of more than 50 oncogenic proteins, including several kinases and transcription factors.[3–6] Thus, Hsp90 has attracted the attention of both industrial and academic laboratories as a promising target for the development of cancer chemotherapeutics.

Hsp90 exists as an obligate homodimer and possesses two nucleotide binding regions: 1) an N-terminal ATP-binding pocket that manifests ATPase activity, and produces the requisite energy for client protein maturation, and 2) a C-terminal nucleotide binding pocket that exhibits allosteric control over substrate and co-chaperones. [2,7–10] Traditional inhibitors of

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Hsp90 disrupt the N-terminal ATP binding pocket, resulting in client protein degradation and inhibition of cell proliferation. At present, no N-terminal Hsp90 inhibitor has been approved by the FDA. Detriments related to N-terminal inhibition have include; induction of the pro-survival heat shock response, as well as undesired toxicity profiles. [1,11,12] Thus, new opportunities for Hsp90 modulation, including C-terminal inhibition, have been sought after for Hsp90 disruption.[13–21]

Natural products identified as C-terminal Hsp90 inhibitors include the coumarin family of antibiotics such as novobiocin, clorobiocin and coumermycin A1 (Figure 1).[22,23] Structure-activity relationship (SAR) studies on novobiocin, which was originally identified as a bacterial DNA gyrase inhibitor, transformed this broad spectrum antibiotic into a potent cytotoxic Hsp90 inhibitor.[13] In addition, SAR studies revealed a class of C-terminal Hsp90 inhibitors that manifest cytotoxic activity at concentrations significantly lower than that which is needed for induction of the heat shock response, unlike the N-terminal inhibitors in clinical trials.[24,25] Furthermore, evidence suggests that C-terminal inhibition may improve selective client protein degradation, producing a potential improvement over N-terminal inhibitors.[24] Thus, C-terminal inhibitors of Hsp90 represent a promising option for Hsp90 modulation and may possess attributes that overcome limitations manifested with the N-terminal inhibitors.

In pursuit of the development of more potent novobiocin analogs, we recently reported derivatives that contain sugar surrogates in lieu of the stereochemically complex noviose (Figure 2). These studies resulted in the attainment of compounds that exhibit nanomolar inhibitory activity against both breast and prostate cancer cell-lines.[22,25–27] In continuation of previous optimization efforts; it was desired to replace the noviose sugar with substituted phenyl rings *via* various linkages (-O, -NH and $-CH_2$). Rationale for the design of these analogs was three-fold: 1) Aryl ethers of novobiocin represent a novel class of uninvestigated analogs; 2) Elimination of the stereochemically complex sugar moiety (which requires 11-steps to prepare[28]), and 3) Removal of the hydrolytic sensitive glycosidic linkage, which may limit its utility for therapeutic applications.[29,30] As a result of these studies, a non-labile and synthetically accessible class of novobiocin analogs was pursued.

Syntheses of aryl ethers **13–21** were initiated from previously reported coumarin scaffold **1**,[22] *via* the oxidative coupling with commercially available boronic acids **2–4** in the presence of $Cu(OAc)_2$ and pyridine to yield intermediates **5**–**7** in excellent yields.[31,32] Removal of the carboxybenzyl (Cbz) protecting group and subsequent coupling with acid chloride **11** or **12**[25–27] provided compounds **13**, **15**, **17** or **19–21**, respectively. Finally, hydrolysis of **13, 15** and **17** under mild conditions produced the free phenols **14**, **16** and **18**.

In parallel, a series of NH-linked aryl analogs **30–32** were synthesized for direct comparison to their aryl ether counterparts. Furthermore, these analogs incorporated a hydrogen bond donor in lieu of the ether acceptor and may therefore provide enhanced interactions with the C-terminal binding pocket.

The NH-linked aryl analogs **30–32**, were synthesized from commercially available 3-nitro-2-methyl phenol **23**, which was transformed to the corresponding amino phenol **24**, upon reduction of the nitro functionality. Intermediate **26** was afforded in reasonable yield *via* a Buchwald N-arylation cross coupling[33,34] of compound **24** with 4-iodo anisole **25** in the presence of $Pd(dppf)Cl_2$ and KO^tBu in dioxane. Phenol **26** was condensed with enamine **27**[25–27] in acetic acid under reflux conditions to provide N-arylated coumarin **28** in acceptable yield. Hydrogenolysis of the Cbz protecting group and subsequent coupling with acid chlorides **11** or **12**, provided analogs **30** and **32**, in good yields, respectively. Finally, methanolysis of acetate **30** yielded the corresponding phenol, **32**.

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To further investigate the influence of surrogates on the activity of aryl-containing replacements, linkers containing of a methylene in lieu of the ether or amine were also pursued. These analogs were prepared in an effort to provide direct comparison the aryl ethers/amines previously described. Methylene linked analogues **43–54** were prepared in 3–5 steps commencing with the coumarin **1**, which was converted to triflate **33** in the presence of Tf_2O and pyridine. Utilizing Pd-mediated borylation chemistry,[35] intermediate **34** was obtained in one-step. Interestingly, the vinylogous amide was deprotected simultaneously under the enlisted conditions, which was required for benzamide formation. Subsequent coupling of amine **34** with acid chloride **11** or **12** provided analogs **35** and **36** respectively, in excellent yield.

Finally, palladium-catalyzed Sp^2-Sp^3 coupling[36–38] of **35** with benzyl bromides **37–42** in the presence of K_2CO_3 yielded compounds **43, 45, 47, 49** and **51**, respectively, in respectable yields. Methanolysis of aryl acetates **43, 45,** and **47** yielded compounds **44**, **46** and **48**, respectively. In addition, analogs **50** and **52** were produced *via* acid mediated hydrolysis of the acetate and MOM groups present in **49** and **51** in single step. Intermediate **36** was also coupled with benzyl bromides **37** and **38** to yield compounds **53** and **54**, respectively.

Upon preparation of these non-hydrolysable novobiocin analogs with various linkers (-O, -NH and $-CH_2$), the compounds were evaluated for anti-proliferative activity against SkBR3 and MCF7 breast cancer cell-lines. In general, the ether analogs containing a prenylated aryl side chain (**13–18**) maintained moderate efficacy, whereas compounds exhibiting the biaryl side chain (**19–21**) displayed IC_{50} values >100 μM. This observation is noteworthy, as the presence of the prenylated functionality in noviosylated novobiocin analogs attenuates the antiproliferative activity, suggesting that simple replacement of the sugar moiety with an aryl appendage significantly alters the mode of binding. Furthermore, analysis of analogs **13–18** suggests a cell-line sensitivity to the appended methyl ether. The 3′-OMe analogs (**15** and **16**) were slightly more active than the corresponding 4′-OMe analogs (**13** and **14**) against SKBr3 cell lines; however, **13** and **14** were more active against MCF-7 cell lines. The aryl ether analogs also indicate that steric bulk at the 4′-position attenuates activity, as all aryl ethers containing a 4′*t*-BuOH moiety (**17, 18,** and **21)** exhibited inferior anti-proliferative activities, suggesting spacial limitations at this region of the binding pocket.

Methylene linked analogs, **43, 44, 46** and **48–54,** were also evaluated for anti-proliferative activity. With the exception of **54** and **48**, methylene linked compounds exhibited a relatively flat SAR, manifesting equipotency against both MCF-7 and SkBR3 cell lines. Compound **48**, exhibits moderate activity (~13 μM) against MCF-7 cells, but is inactive up to 100 μM against SkBR3 cells. Although, novobiocin-based analogs are typically slightly more active against MCF-7 cells, the ~10-fold sensitivity to **48** is intriguing. Compound **53** represents the most active aryl linked compound identified in this series, and manifested IC_{50} values of ~4 μM and ~7 μM against SkBR3 and MCF-7 cancer cells, respectively. Surprisingly, the NH-linked analogs exhibit no anti-proliferative activity against MCF-7 or SkBR3 cell lines up to 100 μM, suggesting that replacement of the ether linker with an NH moiety precludes binding. This result suggests that a hydrogen bond donor at this location and/or the conformational induced *via* the NH linker may be detrimental to binding and novobiocin analogs containing such features should not be pursed.

In order to confirm inhibition of Hsp90, two representative compounds, **14** and **43**, were evaluated for their ability to induce Hsp90 client protein degradation. As expected for a C-terminal Hsp90 inhibitor, client protein degradation was observed and indicative of C-terminal inhibition, no Hsp90 induction was seen. This is in contrast to N-terminal Hsp90 inhibitors, which induce the heat shock response at the same concentration needed to induce client protein degradation.

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In conclusion, a series of non-hydrolyzable novobiocin analogs that contain non-hydrolysable linkers (-O, -NH and $–CH_2$) was designed, synthesized and evaluated for Hsp90 inhibitory activity against SkBR3 and MCF7 breast cancer cell-lines. Replacement of the synthetically complex noviose sugar with simple ether or methylene linked aromatic side chains resulted in moderate anti-proliferative activity that is attributable to Hsp90 C-terminal inhibition. These non-hydrolyzable novobiocin analogs provide a class of C-terminal Hsp90 inhibitors that can be obtained through an expedient process, as the synthetically complex noviose has been eliminated.

Acknowledgments

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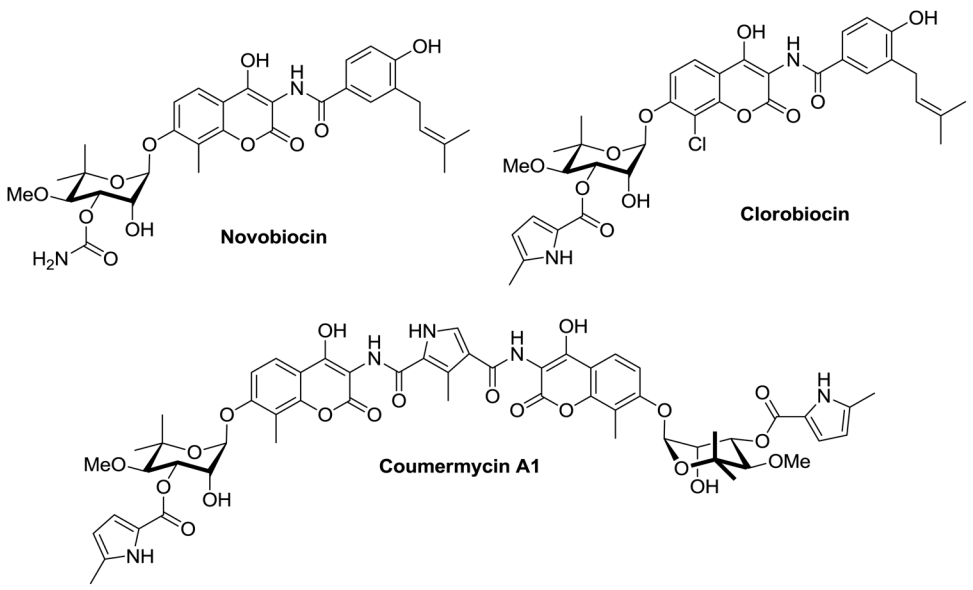

Figure 1.
Representative Natural Product Hsp90 C-terminal inhibitors.

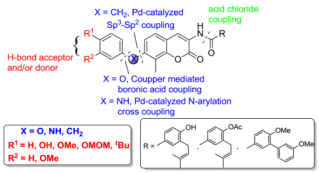

Figure 2.
Non-hydrolysable novobiocin analogues.

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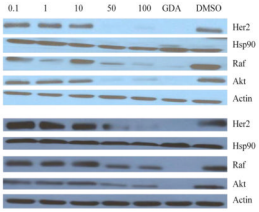

Figure 3.
Western blot analyses of Hsp90 client protein degradation assays against MCF-7 cells. Concentrations (in μM) of **14** (top) and **43** (bottom) are indicated above each lane. GDA (geldanamycin, 500 nM) and DMSO were respectively employed as positive and negative controls.

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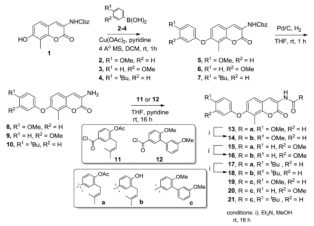

Scheme 1.
Synthesis of O-linked non-labile novobiocin analogs.

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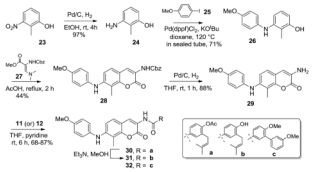

Scheme 2.
Synthesis of N-linked non-labile novobiocin analogs.

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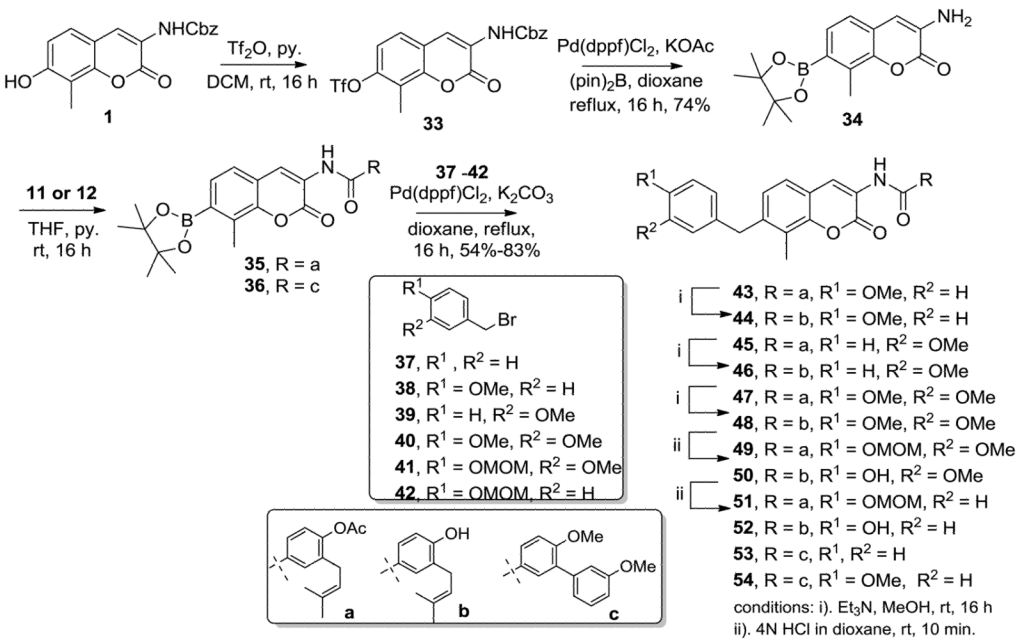

Scheme 3.
Synthesis of methylene linked novobiocin analogs.

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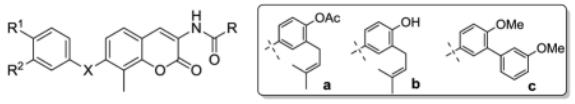
Table 1

Antiproliferative activity of non-labile novobiocin analogs.



Entry	X	R	R^1	R^2	SkBR3	MCF7
13	O	a	OMe	H	19.51±1.8	18.20±2.1
14	O	b	OMe	H	42.72±2.1	6.51±0.7
15	O	a	H	OMe	13.68±1.1	15.69±2.3
16	O	b	H	OMe	33.14±0.9	62.8±4.4
17	O	a	^{t}Bu	H	72.39±1.9	74.99±4.9
18	O	b	^{t}Bu	H	68.27±3.8	20.55±0.8
19	O	c	OMe	H	>100	>100
20	O	c	H	OMe	>100	>100
21	O	c	^{t}Bu	H	>100	>100
30	NH	a	OMe	H	>100	>100
31	NH	b	OMe	H	>100	>100
32	NH	c	OMe	H	>100	>100
43	CH_2	a	OMe	H	23.49±0.7	11.5±0.9
44	CH_2	b	OMe	H	11.08	13.25±1.2
46	CH_2	b	H	OMe	14.83±1.3	19.65±2.0
48	CH_2	b	OMe	OMe	>100	13.94±3.3
49	CH_2	a	OMOM	OMe	14.24±0.4	14.88±0.9
50	CH_2	b	OH	OMe	10.69±1.1	14.62±1.4
51	CH_2	a	OMOM	H	13.50±0.7	22.67±1.7
52	CH_2	b	OH	H	21.46±0.5	32.11±3.2
53	CH_2	c	H	H	4.09±0.2	7.11±1.6
54	CH_2	c	OMe	H	>100	>100