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Development of Noviomimetics as C-Terminal Hsp90 Inhibitors

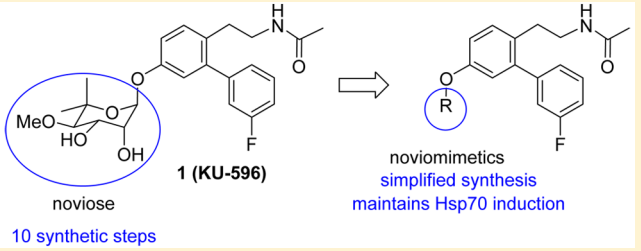
Mercy Anyika,[†] Mason McMullen,[‡] Leah K. Forsberg,[†] Rick T. Dobrowsky,[‡] and Brian S. J. Blagg*[,†]

[†]Department of Medicinal Chemistry, The University of Kansas, 1251 Wescoe Hall Drive, Malott 4070, Lawrence, Kansas 66045-7563, United States

[‡]Department of Pharmacology and Toxicology Department, The University of Kansas, Lawrence, Kansas 66045, United States

Supporting Information

ABSTRACT: KU-32 and KU-596 are novobiocin-derived, C-terminal heat shock protein 90 (Hsp90) modulators that induce Hsp70 levels and manifest neuroprotective activity. However, the synthetically complex noviose sugar requires 10 steps to prepare, which makes translational development difficult. In this study, we developed a series of "noviomimetic" analogues of KU-596, which contain noviose surrogates that can be easily prepared, while maintaining the ability to induce Hsp70 levels. Both sugar and sugar analogues were designed, synthesized, and evaluated in a luciferase reporter assay, which identified compound **37**, a benzyl containing noviomimetic, as the most potent inducer of Hsp70.



KEYWORDS: *Heat Shock Protein 90, Heat Shock Protein 70, C-terminal inhibition, neuroprotection, noviomimetics*

Heat shock protein 90 (Hsp90) is a 90 kDa molecular chaperone that represents a promising biological target for the treatment of cancer and/or neurodegenerative diseases. It exhibits a wide range of functions stemming from its ability to assist in the folding, stability, and rematuration of proteins. Hsp90 interacts with more than 200 client proteins, many of which are oncoproteins that contribute to cancer growth and/or resistance.[1−6]

There has been a tremendous focus to develop Hsp90 inhibitors for the treatment of cancer. In fact, 17 small molecules that bind the N-terminus of Hsp90 have entered clinical trials.[7,8] These drugs preferentially inhibit Hsp90 and induce client protein degradation in malignant versus normal cells.[9−11] Although this selectivity aids the clinical efficacy of N-terminal Hsp90 inhibitors, enthusiasm for their use has been dampened because they also induce the pro-survival heat shock response at the same concentration needed to inhibit client protein folding, which may limit their clinical potential.[2] In contrast to N-terminal inhibitors, we have developed novobiocin-based C-terminal inhibitors such as KU-32 and A4 (Figure 1) that can segregate induction of the heat shock response (and subsequent cytoprotection) from client protein degradation (and cytotoxicity).[12−19] In fact, KU-32 was found to protect against neuronal glucotoxicity and to reverse clinical end points of diabetic peripheral neuropathy in mice.[20,21]

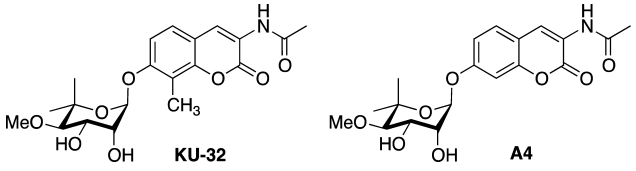


Figure 1. Chemical structures of KU-32 and A4.

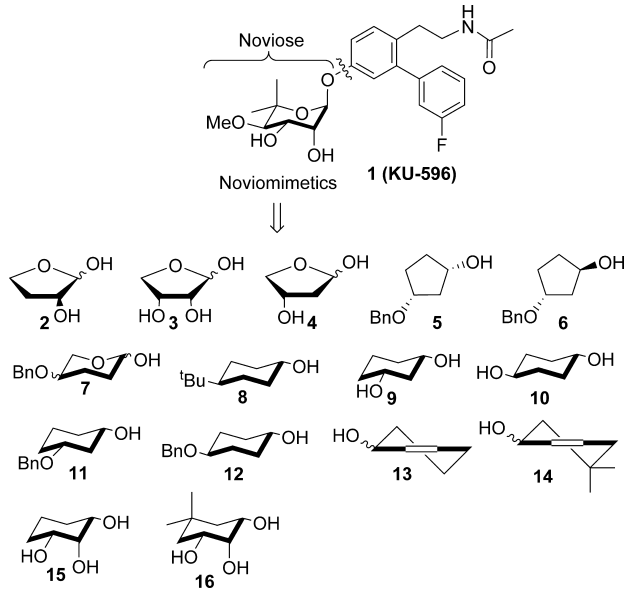


Figure 2. Structure of KU-596 (**1**) and sugar analogues selected for noviose replacement.

In an ongoing effort to develop C-terminal Hsp90 inhibitors that manifest neuroprotective activity, KU-32 has undergone modifications to produce a second generation of noviosylated

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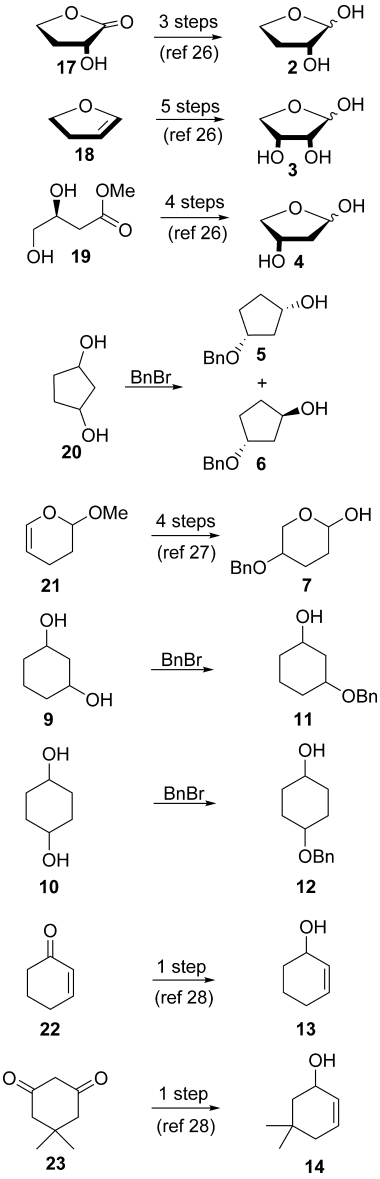
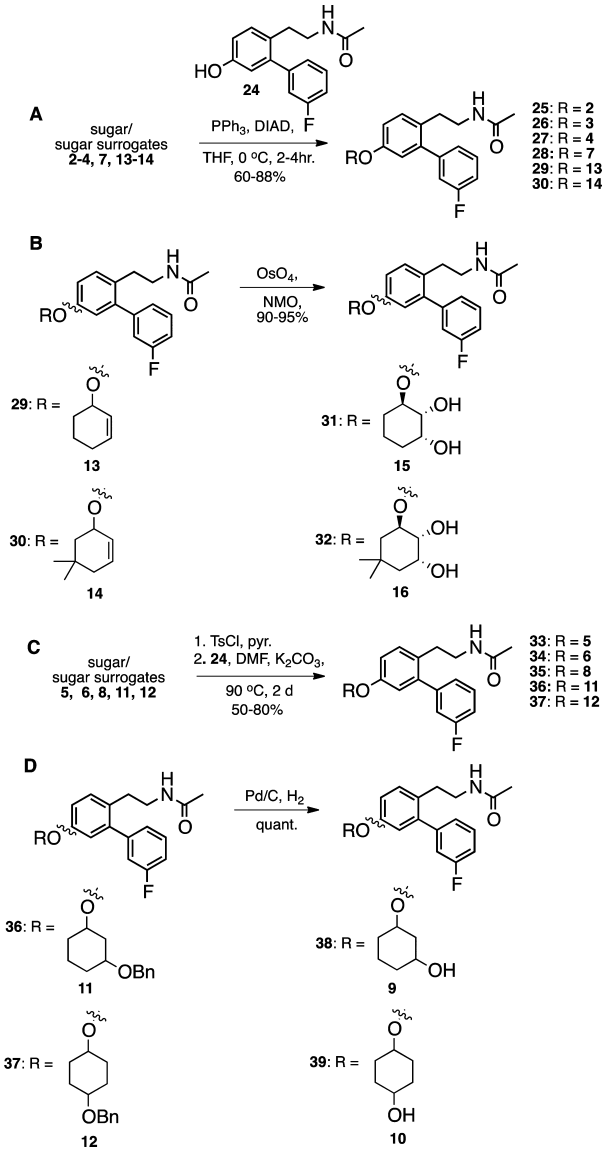
Scheme 1. Synthesis of 5- and 6-Membered Ring Analogues



Scheme 2. Synthesis of Noviomimetics 25−39



analogues, termed novologues. Novologues are small molecules that replaced the coumarin core of KU-32 with a biphenyl ring system and flexible side chain such as KU-596 (**1**), which manifests an enhancement in neuroprotective activity upon biological evaluation against primary sensory neurons.[22] Since novologues contain noviose, a synthetically complex sugar that requires ten steps to prepare, this sugar represents a potential impediment for the translational advancement of such compounds.[23−25] To circumvent this concern, a library of succinctly prepared KU-596 analogues containing both sugar and nonsugar surrogates was generated. Previous molecular modeling studies suggested that **1** and KU-32 bind the C-terminal binding site in a manner that projects the noviose sugar into a pocket that could accommodate additional substitutions.[22] Therefore, the design of noviose replacements that extend into this pocket and introduce additional interactions within the binding site were pursued. The ability of the non-noviosylated compounds to function as "noviomimetics" by promoting the induction of Hsp70 was assessed.

The noviose surrogates chosen for this study are shown in Figure 2 and include a series of simplified pyranoses that closely mimic the noviose chair conformation. Ring contracted furanose analogues in the envelope conformation that can project substituents into unexplored regions of the Hsp90 C-terminal binding pocket were also investigated.

As the syntheses of sugars often require many steps, simplified analogues containing a cyclohexyl or cyclopentyl ring were also investigated to determine whether a carbocyclic analogue could exhibit beneficial activity. Analogues containing an alkyl or aryl substituent were also probed to determine constraints within the binding pocket. A racemic mixture of the compounds were used in these studies.

As shown in Scheme 1, furanose derivatives **2**, **3**, and **4**, were synthesized from commercially available (*S*)-(−)-α-hydroxy-γ-butyrolactone, **17**, 2,3-dihydrofuran, **18**, and (*S*)-methyl 3,4-dihydroxybutanoate, **19**, respectively, via reported procedures,[26] whereas cyclopentanes **5** and **6** were obtained from monobenzylation of commercially available 1,3-cyclopentadiol, **20**. The resulting *syn*- and *anti*-isomers were easily separated by column chromatography. Sugar **7** was obtained from

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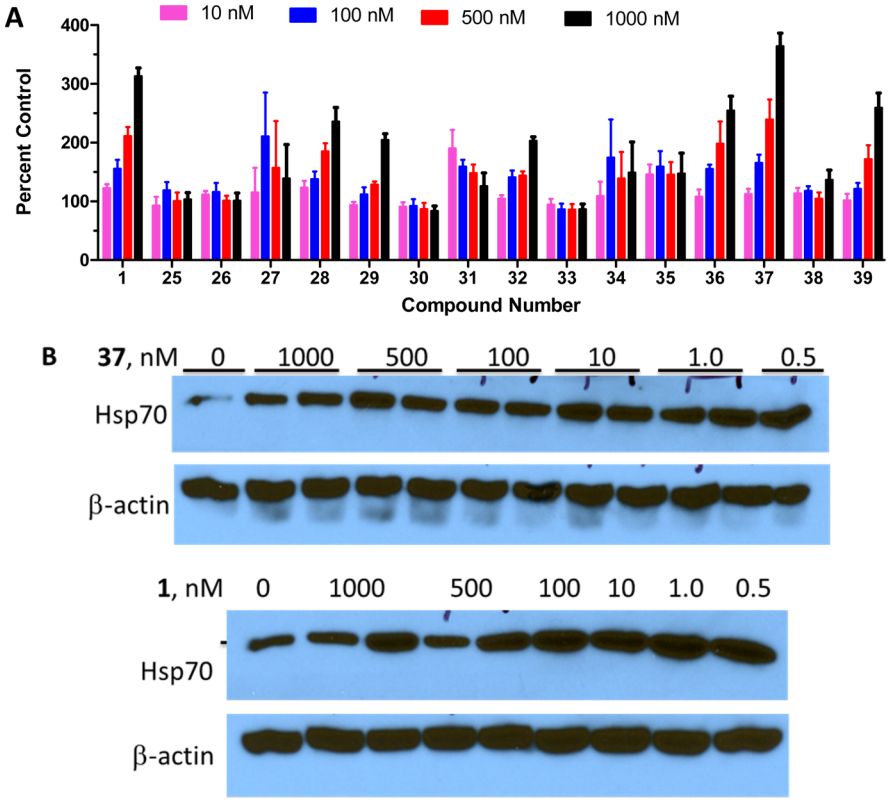

Figure 3. (A) Luciferase assay assessing Hsp70 induction by analogues **25**−**39**. Results are mean ± SEM (n = 5−13). (B) Western Blot analysis of Hsp70 induction for **37** and **1** in nontransfected 50B11 cells.

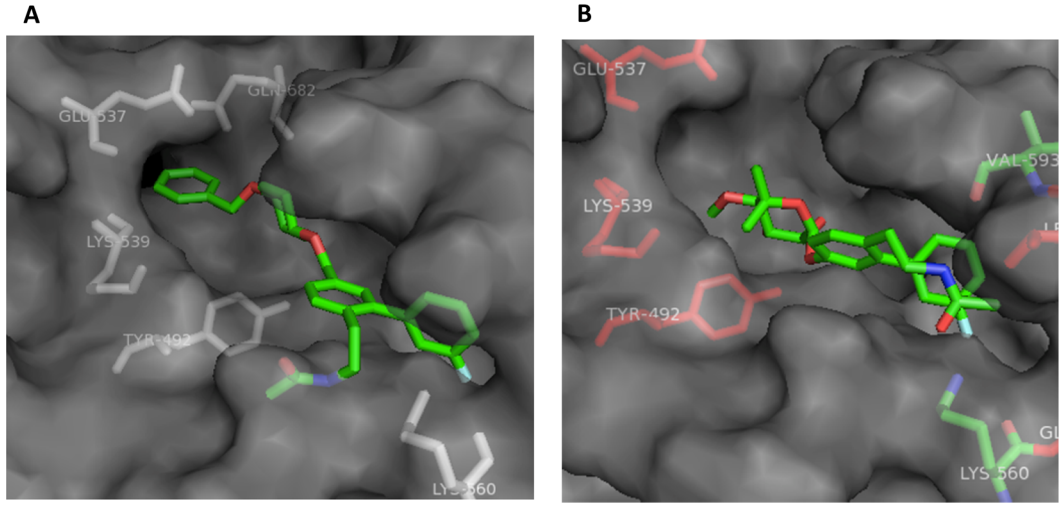

Figure 4. (A) Compound **37** docked to Hsp90 C-terminal binding site. (B) KU-596, **1**, docked to the Hsp90 C-terminal binding site.

commercially available 3,4-dihydro-2-methoxypyran, **21**, via a four-step procedure.[27] Cyclohexane derivatives **8**, **9**, and **10** are commercially available, whereas **11** and **12** were obtained from monobenzylation of **9** and **10**, respectively. Cyclohexene derivatives **13** and **14** were synthesized via published procedures from commercially available 2-cyclohexen-1-one, **22**, and 5,5-dimethyl-1,3-cyclohexanedione, **23**, respectively (Scheme 1).[28] Aglycone **24** was synthesized via our previously reported procedure.[22]

Sugars and sugar surrogates **2**, **3**, **4**, **7**, **13**, and **14** were linked to aglycone **24** via a Mitsunobu reaction, employing triphenyl phosphine and DIAD, to obtain compounds **25**−**30** (Scheme 2A). Noviomimetics **29** and **30** were subsequently subjected to an osmium tetroxide-catalyzed dihydroxylation reaction in order to obtain the corresponding diols, **31** and **32**, which contain the sugar surrogates, **15** and **16**, respectively (Scheme 2B). To obtain compounds **33**−**37**, sugar surrogates **5**, **6**, **8**, **11**, and **12** were first converted to the corresponding toluenesulfonates using 4-toluenesulfonyl chloride, after which they were subjected to an S_N2 substitution reaction with aglycone **24** to obtain compounds **33**−**37** (Scheme 2C). The benzyl ether-containing noviomimetics **36** and **37**, were cleaved via hydrogenolysis to afford compounds **38** and **39**, which contain the sugar surrogates, **9** and **10**, respectively (Scheme 2D).

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We have previously shown that the cytoprotective activity manifested by KU-32 is dependent upon expression of Hsp70, which occurs upon Hsp90 inhibition.[21] Therefore, upon construction of the library of noviomimetics, we determined their ability to induce Hsp70 via a luciferase reporter assay. In this assay, an Hsp70 promoter, which contains a heat shock binding element, is subcloned in front of a luciferase reporter gene and the resulting vector transfected into an immortalized sensory neuronal cell line (50B11 cells). The transformed cells are subsequently treated with Hsp90 inhibitors, which displace the transcription factor, heat shock factor 1 (HSF1), from the Hsp90 complex. Upon activation, HSF1 translocates to the nucleus, wherein it binds the Hsp70 promoter and leads to increased luciferase activity that is easily quantified. An increase in luciferase activity represents activation of the Hsp70 promoter.

Based on the results shown in Figure 3A, the derivatives that closely mimicked the chair conformation of the noviose sugar, resulted in greater Hsp70 induction. The furanose novologues **25**, **26**, and **27** were relatively inactive in the luciferase reporter assay, which suggests that the conformation manifested by the furanose derivatives does not project substituents into a favorable region of the binding pocket and, consequently, minimizes Hsp70 induction. Similarly cyclopentanes **33** and **34** did not result in significant induction. The novologues with constrained rings, **29** and **30**, were also relatively inactive in the luciferase reporter assay, and do not mimic the chair conformation exhibited by the noviose sugar. These results collectively suggest that the novologues that mimic the chair conformation of the noviose sugar is required for Hsp70 induction.

Novologues that contain simplified pyranose derivatives produced varying degrees of luciferase induction. Generally, the more simplified the pyranose, the greater the Hsp70 induction. Compounds **36** and **37** were most active in the luciferase assay and represent simplified pyranose derivatives. Their non-benzylated derivatives **38** and **39** were less active but exhibit the same trend wherein para substituents result in greater luciferase induction when compared to the meta substituent. These results suggest that not only is the chair conformation of the pyranose important for activity but larger substituents improve activity. As shown in Figure 3A, compound **37**, which contains the 4-benzyl ether on the carbocyclic ring, induced the highest level of luciferase in the 50B11 transformed cellular assay and, consequently, was further investigated.

Using nontransfected 50B11 cells, compound **37** was shown to increase Hsp70 levels at concentrations similar to KU-596 (Figure 3B). In fact, **37** continued to induce a robust heat shock response even at subnanomolar concentrations.

As depicted in Figure 4A, compound **37** is suspected to bind the Hsp90 C-terminal binding site and project the aryl ring further into the pocket, which is left unoccupied in the case of KU-596, Figure 4B. Studies are currently underway to probe for additional interactions between substitutions on the aryl ring of **37** and the binding site.

In conclusion, a library of noviomimetics was designed to replace the synthetically complex noviose sugar of **1** with various sugar surrogates. It was determined that a cyclohexyl derivative containing a 4-benzyl ether (**37**) manifested equipotent activity as KU-596, which significantly simplifies the preparation of such compounds. Furthermore, these studies suggest that noviomimetics can successfully retain the ability to induce Hsp70, a key mechanistic feature that is associated with the neuroprotective activity manifested by the novologue class of compounds, such as **1**, as well as the novobiocin-based compounds (e.g., KU-32). Thus, noviomimetics may represent a new series of synthetically simple neuroprotective compounds for the treatment of neurodegenerative diseases.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.5b00331.

Preparation and evaluation of reported compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Phone: (785) 864-2288. Fax: (785) 864-5326. E-mail: bblagg@ku.edu.

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Notes

The authors declare no competing financial interest.

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