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Syntheses of photolabile novobiocin analogues

Gang Shen, Xiao ming Yu and Brian S. J. Blagg*

Department of Medicinal Chemistry and The Center for Protein Structure and Function, The University of Kansas, 1251 Wescoe Hall Drive, Malott Hall 4070, Lawrence, KS 66045-7562, United States

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Abstract—Novobiocin was recently shown to inhibit Hsp90 through a previously unrecognized *C*-terminal ATP binding site. Although the *N*-terminal region of Hsp90 has been solved by X-ray crystallography, the *C*-terminal region has not. In an effort to elucidate the *C*-terminal binding site of Hsp90, four photolabile analogues of novobiocin were prepared.

The coumarin antibiotics novobiocin, clorobiocin, and coumermycin A1 (Fig. 1) have been isolated from several *streptomyces* strains and exhibit potent activity against Gram-positive bacteria.[1–4] These compounds bind to type II topoisomerases including DNA gyrase and inhibit the enzyme catalyzed hydrolysis of ATP.[5–8] As a result, novobiocin analogues have garnered the attention of numerous researchers as an attractive agent for the treatment of bacterial infection. Unlike norfloxacin, pipemidic acid, and nalidixic acid, which are clinically used DNA gyrase inhibitors, novobiocin produces cytotoxicity in a number of cancer cell lines.[9–12]

The crystal structure of topoisomerase II has not been solved, but the co-crystal structure of DNA gyrase bound to novobiocin was reported in 1991.[13–15] There were little similarities between novobiocin and adenine triphosphate, but the structure did indicate that ATP is bound in an unusual, bent conformation, as opposed to the typical extended form. Like DNA gyrase, the *N*-terminal region of the 90 kDa heat shock proteins (Hsp90) also bind ATP in a very similar bent conformation.[16] Because novobiocin binds to a similarly shaped ATP binding site in DNA gyrase and has cytotoxicity in cancer cell lines, it was proposed that this molecule may be binding to the analogous ATP binding pocket in Hsp90. Elegant studies by Neckers and co-workers demonstrated that novobiocin did bind to Hsp90, but not to the *N*-terminal region.[17] Instead, novobiocin was found to bind a previously unrecognized *C*-terminal nucleotide-binding region. Subsequent studies have shown that inhibition of Hsp90 by novobiocin leads to a decrease in Hsp90 client proteins in various cancer cell lines,[18] an effect that is similar to *N*-terminal inhibitors, geldanamycin, and radicicol.[19–26]

Hsp90 is a molecular chaperone responsible for the conformational maturation of nascent polypeptides into biological active, three-dimensional structures.[16,27–36] Hsp90 dependent client proteins represented in the six hallmarks of cancer include Her-2,[37] Src family kinases,[38] Raf,[39] PLK,[40] RIP,[41] AKT,[42] telomerase,[43] and MET.[44] Inhibition of the Hsp90 protein folding machinery results in the simultaneous disruption of multiple oncogenic pathways and consequently, Hsp90 has emerged as a promising target for the development of cancer chemotherapeutics.[45,46]

Critical to the discovery of new Hsp90 inhibitors is elucidation of the *C*-terminal ATP binding site. In an effort to elucidate the location of this nucleotide-binding pocket, four photolabile novobiocin analogues were prepared by convergent syntheses.

The syntheses of photolabile novobiocin analogues began by the preparation of known compounds 4,7-dihydroxy-8-methyl-2*H*-1-benzopyran-2-one[47] (**4**, Scheme 1) and diazonium salt **5**.[48] Upon treatment of **4** with **5**, a yellow precipitate was immediately observed and subsequent characterization confirmed this material was the desired diaza coumarin derivative **6**. Previous synthetic efforts aimed at the preparation of novobiocin analogues have shown that a sulfone analogue of the coumarin ring could be coupled directly with trichloracetoamidate **7** in the presence of a catalytic amount of boron trifluoride

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* Corresponding author. Tel.: +1 785 864 2288; fax: +1 785 864 5326; e-mail: bblagg@ku.edu

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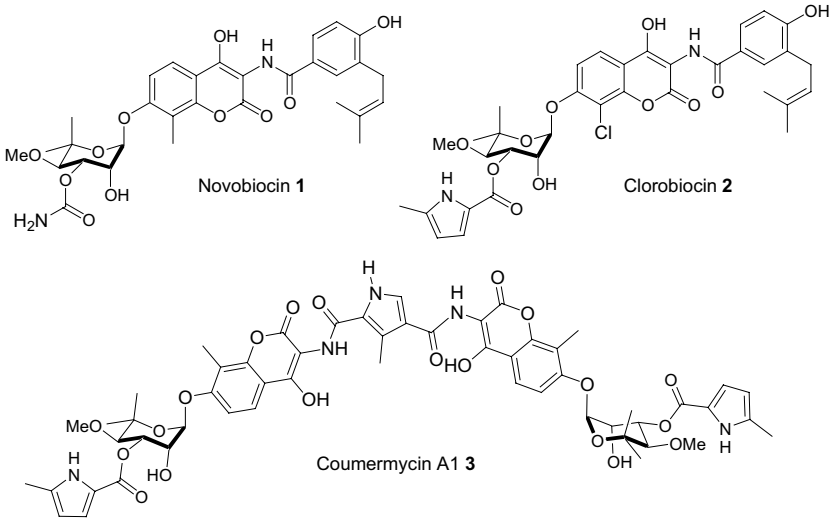
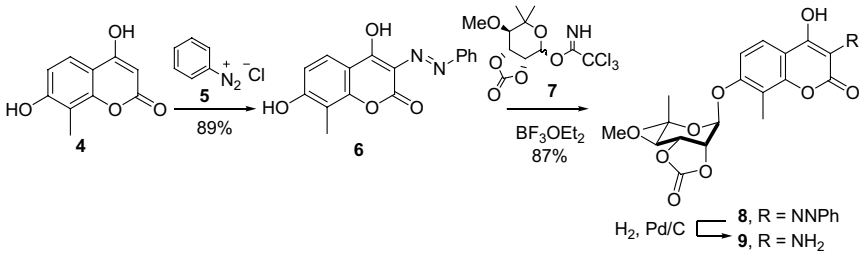
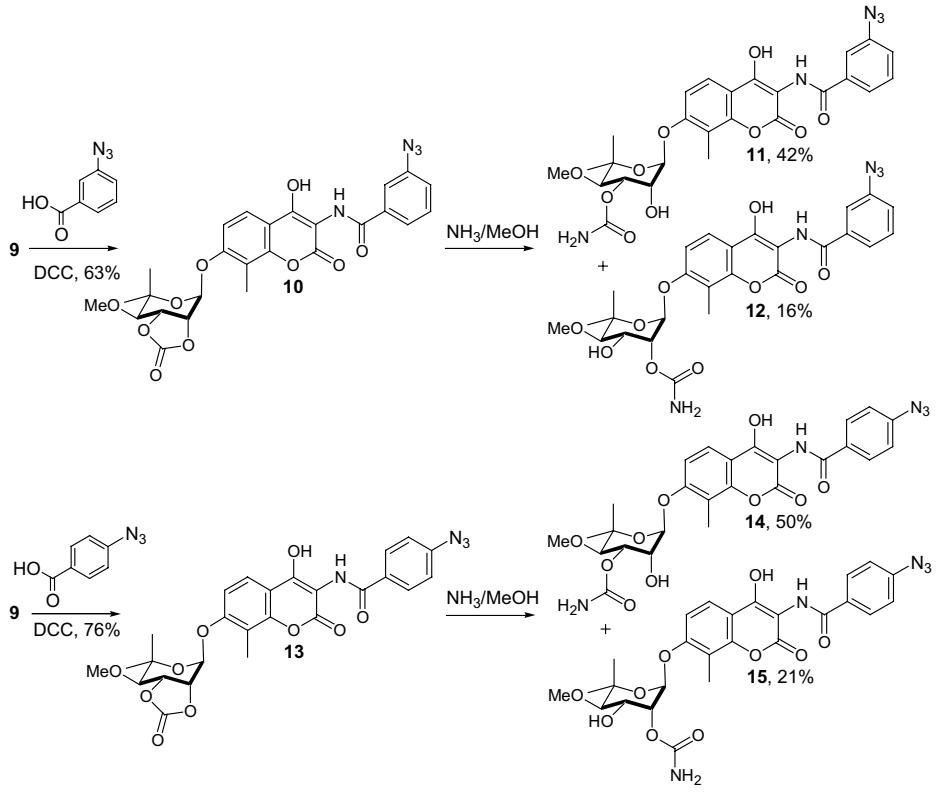


Figure 1. Coumarin antibiotics.



Scheme 1. Synthesis of aminocoumarin.



Scheme 2. Syntheses of photolabile novobiocin analogues.

etherate.[49] When initial conditions were surveyed with **6** and **7**, we observed that both the 4- and 7-phenolic moieties were noviosylated in low to moderate yields. Further experimentation proved that in the presence of excess coumarin, the amount of dinoviosylated product was drastically reduced and the desired 7-noviose product, **8**, was provided in good yield. The diaza-bridge connecting the phenyl and coumarin rings served as a masked amine, which could be cleaved under reducing conditions to provide the 3-amino coumarin **9**. However, **9** turned out to be unstable under a variety of conditions, including chromatography.

Consequently, a crude mixture containing both the aniline product and aminocoumarin **9** was treated with either 3-azido or 4-azidobenzoic acid to furnish the desired amides **10** or **13**, respectively. During the total synthesis of novobiocin by Vaterlaus et al., the cyclic carbonate of novobiocin was treated with liquid ammonia to produce a 3:1 mixture of carbamates with a combined yield of 33%.[50] To optimize the amount of carbamate produced by ammoniaolysis of the similar carbonate in our system, a number of conditions were explored. After careful optimization, we found the cyclic carbonate could be opened with methanolic ammonia at room temperature to provide the desired 3-carbamate noviose products, **11**[51] and **14**[52] in good yields and with good regioselectivity (Scheme 2). 2-Carbamate products (**12** and **15**) were also isolated in small quantities.

In this letter, we described the syntheses of four photolabile analogues of novobiocin, an inhibitor of not only type II topoisomerases, but also Hsp90. These photolabile analogues are currently under biological investigation for their ability to covalently modify the *C*-terminal ATP binding site of Hsp90. The outcome of these studies will be reported in due course.

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51. For **11**: $[\alpha]_D^{25}$ −45.3 (*c* 0.23, 50% MeOH in CH_2Cl_2); ^1H NMR (CD_2Cl_2/CD_3OD, 400 MHz): 7.83 (d, $J = 8.9$ Hz, 1H), 7.76 (d, $J = 7.6$ Hz, 1H), 7.68 (s, 1H), 7.54 (t, $J = 7.8$ Hz, 1H), 7.29 (d, $J = 7.9$ Hz, 1H), 7.23 (d, $J = 8.9$ Hz, 1H), 5.59 (s, 1H), 5.36 (d, $J = 9.9$ Hz, 1H), 4.27 (s, 1H), 3.57 (d, $J = 9.9$ Hz, 1H), 3.55 (s, 3H), 2.32 (s, 3H), 1.36 (s, 3H), 1.16 (s, 3H); ^{13}C NMR (CD_2Cl_2/CD_3OD, 125 MHz): δ 167.5, 162.5, 158.0, 157.8, 150.8, 141.4, 134.7 (2C), 130.5, 124.2, 123.1, 122.5, 118.8, 114.2, 111.7, 110.8, 102.1, 98.9, 81.7, 79.2, 72.1, 69.9, 61.3, 28.4, 22.5, 8.2; IR (film) ν_{max} 2115, 1736, 1718, 1701, 1684, 1652, 1607, 1580, 1540, 1523, 1509, 1369, 1323, 1270, 1118, 1093 cm^{-1}; HRMS 570.1887 (FAB^+) *m/z* ($M+H^+$, $C_{26}H_{27}N_5O_{10}$ requires 570.1836).
52. For **14**: $[\alpha]_D^{25}$ −36.6 (*c* 0.06, 50% MeOH in CH_2Cl_2); ^1H NMR (CD_2Cl_2/CD_3OD, 400 MHz): 8.04 (m, 2H), 7.84 (d, $J = 8.9$ Hz, 1H), 7.22 (m, 3H), 5.59 (d, $J = 2.2$ Hz, 1H), 5.36 (dd, $J = 3.1$, 9.8 Hz, 1H), 4.20 (dd, $J = 2.2$, 3.1 Hz, 1H), 3.58 (d, $J = 9.8$ Hz, 1H), 3.56 (s, 3H), 2.33 (s, 3H), 1.37 (s, 3H), 1.17 (s, 3H); ^{13}C NMR (CD_2Cl_2/CD_3OD, 125 MHz): δ 167.6, 162.7, 162.3, 157.9 (2C), 150.9, 144.9, 141.0, 129.9 (2C), 129.3, 122.5, 119.3 (2C), 114.1, 110.7, 102.0, 98.9, 81.7, 79.1, 72.1, 69.9, 61.2, 28.3, 22.5, 8.1; IR (film) ν_{max} 2119, 1735, 1690, 1629, 1600, 1496, 1371, 1280, 1251, 1193, 1097, 937 cm^{-1}; HRMS 570.1841 (FAB^+) *m/z* ($M+H^+$, $C_{26}H_{27}N_5O_{10}$ requires 570.1836).