

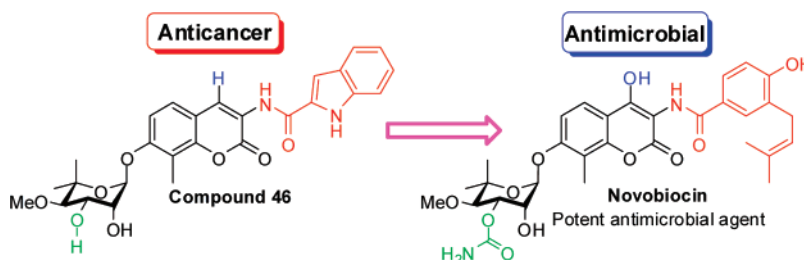
Development of Novobiocin Analogues That Manifest Anti-proliferative Activity against Several Cancer Cell Lines

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Recent studies have shown that the DNA gyrase inhibitor, novobiocin, binds to a previously unrecognized ATP-binding site located at the C-terminus of Hsp90 and induces degradation of Hsp90-dependent client proteins at $\sim 700 \mu\text{M}$. As a result of these studies, several analogues of the coumarin family of antibiotics have been reported and shown to exhibit increased Hsp90 inhibitory activity; however, the monomeric species lacked the ability to manifest anti-proliferative activity against cancer cell lines at concentrations tested. In an effort to develop more efficacious compounds that produce growth inhibitory activity against cancer cell lines, structure–activity relationships were investigated surrounding the prenylated benzamide side chain of the natural product. Results obtained from these studies have produced the first novobiocin analogues that manifest anti-proliferative activity against several cancer cell lines.

Introduction

Although transcription and translation are vital processes, the overall transformation of linear genetic information into three-dimensional proteins that manifest biological activity represents the most remarkable feature of the genetic code.¹ Proteins responsible for the conformational maturation of linear polypeptides into biologically active, three-dimensional enzymes are molecular chaperones. Many chaperones are relatively promiscuous and bind to partially or completely unfolded peptides like those excreted by the ribosome upon translation. However, higher order chaperones appear to be much more selective and fold proteins associated with specific processes, an example of which is the 90 kDa heat shock proteins, Hsp90.² The Hsp90

molecular chaperone is responsible for the conformational maturation of nascent polypeptides associated with cell signaling, steroid hormone receptors, and telomerase.³ Interestingly, it is these proteins/pathways that are most commonly mutated or hijacked in transformed cells to produce immortality.⁴ Not only does Hsp90 fold these wild-type proteins, but also many of the corresponding mutants that contribute to oncogenic phenotypes.^{5,6} In fact, proteins represented in all six hallmarks of cancer have been shown to be dependent upon Hsp90 for conformational maturation.^{7–9} Consequently, Hsp90 has emerged as a promising target for the development of cancer chemotherapeutics.^{10–26}

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(1) Frydman, J. *Annu. Rev. Biochem.* **2001**, *70*, 603–647.

(2) Wegele, H.; Mueller, L.; Buchner, J. *Rev. Physiol. Biochem. Pharmacol.* **2004**, *151*, 1–44.

(3) Blagg, B. S. J.; Kerr, T. D. *Med. Res. Rev.* **2006**, *26*, 310–338.

(4) Zhang, H.; Burrows, F. J. *Mol. Med.* **2004**, *82*, 488–499.

(5) Dai, C.; Whitesell, L. *Future Oncol.* **2005**, *1*, 529–540.

(6) Whitesell, L.; Lindquist, S. L. *Nat. Rev. Cancer* **2005**, *5*, 761–772.

(7) Bishop, S. C.; Burlison, J. A.; Blagg, B. S. J. *Curr. Cancer Drug Targets* **2007**, *7*, 369–388.

(8) McCarty, M. F. *Integr. Cancer Ther.* **2004**, *3*, 349–380.

(9) Hanahan, D.; Weinberg, R. A. *Cell* **2000**, *100*, 57–70.

The Hsp90-mediated protein folding process is dependent upon the hydrolysis of ATP by the N-terminal ATP-binding pocket to provide the requisite source of energy.^{27–28} This nucleotide-binding domain is unique when compared to typical ATP-binding proteins because the nucleotide is bound in a bent conformation as opposed to the extended conformation utilized by most proteins.^{29–32} In fact, only four proteins are known to bind ATP in this manner and they include DNA Gyrase, Hsp90, histidine Kinase, and MutL, all of whom compose the GHKL superfamily.³³ Due to similarities between the ATP-binding sites of DNA gyrase, a well-validated target for bacterial infection and Hsp90, Neckers proposed that a small molecule inhibitor of DNA gyrase may also inhibit Hsp90 function and serve as a lead compound for the development of more efficacious

chemotherapeutics.^{34–37} Upon completion of such an experiment, Neckers and colleagues determined that the DNA gyrase inhibitor, novobiocin,³⁸ was indeed an Hsp90 inhibitor, but instead of binding to the well-documented N-terminal nucleotide binding site, it bound to a previously unrecognized C-terminal ATP-binding motif.^{39–41} Although small molecule inhibitors of the N-terminus (geldanamycin and radicicol) manifest IC₅₀ values in the low nanomolar range,⁴² novobiocin exhibited an IC₅₀ value of ~700 μ M, which is significantly less potent than N-terminal inhibitors. However, novobiocin remained the only known inhibitor of the Hsp90 C-terminal nucleotide binding pocket for several years.

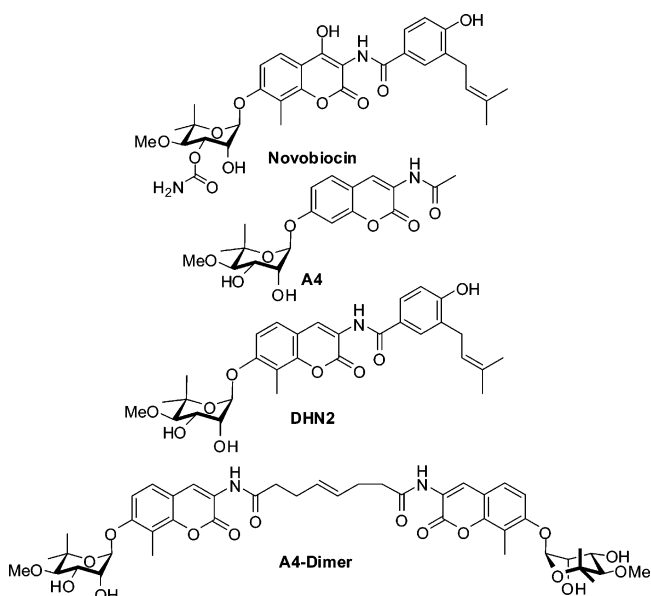


FIGURE 1. Previously reported inhibitors of the Hsp90 C-terminal ATP-binding pocket.

In 2005, the first attempt to improve the inhibitory activity of novobiocin against Hsp90 was reported.⁴³ **A4** was identified as an Hsp90 C-terminal inhibitor that induced degradation of Hsp90-dependent client proteins at ~70-fold lower concentration than novobiocin, Figure 1. In contrast to N-terminal inhibitors that induce Hsp70 and 90 levels at the same concentration that leads to degradation of client proteins, the C-terminal inhibitor, **A4**, induced Hsp90 levels at concentrations 1000–10000-fold

(10) Drysdale, M. J.; Brough, P. A.; Massey, A.; Jensen, M. R.; Schoepfer, J. *Curr. Opin. Drug Discovery Dev.* **2006**, *9*, 483–495.

(11) Chaudhury, S.; Welch, T. R.; Blagg, B. S. J. *Chem. Med. Chem.* **2006**, *1*, 1331–1340.

(12) Maroney, A. C.; Marugan, J. J.; Mezzasalma, T. M.; Barnakov, A. N.; Garrabrant, T. A.; Weaner, L. E.; Jones, W. J.; Barnakova, L. A.; Koblish, H. K.; Todd, M. J.; Masucci, J. A.; Deckman, I. C.; Galembo, R. A., Jr.; Johnson, D. L. *Biochemistry* **2006**, *45*, 5678–5685.

(13) Le Brazidec, J.-Y.; Kamal, A.; Busch, D.; Thao, L.; Zhang, L.; Timony, G.; Grecco, R.; Trent, K.; Lough, R.; Salazar, T.; Khan, S.; Burrows, F.; Boehm, M. F. *J. Med. Chem.* **2004**, *47*, 3865–3873.

(14) Shen, G.; Blagg, B. S. J. *Org. Lett.* **2005**, *7*, 2157–2160.

(15) Dymock, B. W.; Barril, X.; Brough, P. A.; Cansfield, J. E.; Massey, A.; McDonald, E.; Hubbard, R. E.; Surgenor, A.; Roughley, S. D.; Webb, P.; Workman, P.; Wright, L.; Drysdale, M. J. *J. Med. Chem.* **2005**, *48*, 4212–4215.

(16) Moulin, E.; Barluenga, S.; Winssinger, N. *Org. Lett.* **2005**, *7*, 5637–5639.

(17) Yang, Z.-Q.; Geng, X.; Solit, D.; Pratilas, C. A.; Rosen, N.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 7881–7889.

(18) He, H.; Zatorska, D.; Kim, J.; Aguirre, J.; Llauger, L.; She, Y.; Wu, N.; Immormino, R. M.; Gewirth, D. T.; Chiosis, G. *J. Med. Chem.* **2006**, *49*, 381–390.

(19) Moulin, E.; Zoete, V.; Barluenga, S.; Karplus, M.; Winssinger, N. *J. Am. Chem. Soc.* **2005**, *127*, 6999–7004.

(20) Wright, L.; Barril, X.; Dymock, B.; Sheridan, L.; Surgenor, A.; Beswick, M.; Drysdale, M.; Collier, A.; Massey, A.; Davies, N.; Fink, A.; Fromont, C.; Aherne, W.; Boxall, K.; Sharp, S.; Workman, P.; Hubbard, R. E. *Chem. Biol.* **2004**, *11*, 775–785.

(21) Llauger, L.; He, H.; Kim, J.; Aguirre, J.; Rosen, N.; Peters, U.; Davies, P.; Chiosis, G. *J. Med. Chem.* **2005**, *48*, 2892–2905.

(22) Biamonte, M. A.; Shi, J.; Hong, K.; Hurst, D. C.; Zhang, L.; Fan, J.; Busch, D. J.; Karjian, P. L.; Maldonado, A. A.; Sensintaffar, J. L.; Yang, Y.-C.; Kamal, A.; Lough, R. E.; Lundgren, K.; Burrows, F. J.; Timony, G. A.; Boehm, M. F.; Kasibhatla, S. R. *J. Med. Chem.* **2006**, *49*, 817–828.

(23) Barril, X.; Beswick, M.; Collier, A.; Drysdale, M. J.; Dymock, B. W.; Fink, A.; Grant, K.; Howes, R.; Jordan, A. M.; Massey, A.; Surgenor, A.; Wayne, J.; Workman, P.; Wright, L. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2543–2548.

(24) Barril, X.; Brough, P.; Drysdale, M.; Hubbard, R. E.; Massey, A.; Surgenor, A.; Wright, L. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5187–5191.

(25) Cheung, K.-M. J.; Matthews, T. P.; James, K.; Rowlands, M. G.; Boxall, K. J.; Sharp, S. Y.; Maloney, A.; Roe, S. M.; Prodromou, C.; Pearl, L. H.; Aherne, G. W.; McDonald, E.; Workman, P. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3338–3343.

(26) Brough, P. A.; Barril, X.; Beswick, M.; Dymock, B. W.; Drysdale, M. J.; Wright, L.; Grant, K.; Massey, A.; Surgenor, A.; Workman, P. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5197–5201.

(27) Prodromou, C.; Roe, S. M.; O'Brien, R.; Ladbury, J. E.; Piper, P. W.; Pearl, L. H. *Cell* **1997**, *90*, 65–75.

(28) Chadli, A.; Bouhouche, I.; Sullivan, W.; Stensgard, B.; McMahon, N.; Catelli, M. G.; Toft, D. O. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 12524–12529.

(29) Roe, S. M.; Prodromou, C.; O'Brien, R.; Ladbury, J. E.; Piper, P. W.; Pearl, L. H. *J. Med. Chem.* **1999**, *42*, 260–266.

(30) Chene, P. *Nat. Rev. Drug Discovery* **2002**, *1*, 665–673.

(31) Jackson, A. P.; Maxwell, A.; Wigley, D. B. *J. Mol. Biol.* **1991**, *217*, 15–7.

(32) Tsai, F. T. F.; Singh, O. M. P.; Skarzynski, T.; Wonacott, A. J.; Weston, S.; Tucker, A.; Pauptit, R. A.; Breeze, A. L.; Poyser, J. P.; et al. *Proteins* **1997**, *28*, 41–52.

(33) Dutta, R.; Inouye, M. *Trends Biochem. Sci.* **2000**, *25*, 24–28.

(34) Marcu, M. G.; Schulte, T. W.; Neckers, L. *J. Natl. Cancer Inst.* **2000**, *92*, 242–248.

(35) Lewis, R. J.; Singh, O. M. P.; Smith, C. V.; Skarzynski, T.; Maxwell, A.; Wonacott, A. J.; Wigley, D. B. *EMBO J.* **1996**, *15*, 1412–20.

(36) Holdgate, G. A.; Tunnicliffe, A.; Ward, W. H. J.; Weston, S. A.; Rosenbrock, G.; Barth, P. T.; Taylor, I. W. F.; Pauptit, R. A.; Timms, D. *Biochemistry* **1997**, *36*, 9663–9673.

(37) Reece, R. J.; Maxwell, A. *Crit. Rev. Biochem. Mol. Biol.* **1991**, *26*, 335–75.

(38) Hengeller, C.; Licciardello, G.; Tudino, V.; Marcelli, E.; Virgilio, A. *Nature* **1965**, *205*, 418–419.

(39) Marcu, M. G.; Chadli, A.; Bouhouche, I.; Catelli, M.; Neckers, L. M. *J. Biol. Chem.* **2000**, *275*, 37181–37186.

(40) Garnier, C.; Lafitte, D.; Tsvetkov, P. O.; Barbier, P.; Leclerc-Devin, J.; Millot, J.-M.; Briand, C.; Makarov, A. A.; Catelli, M. G.; Peyrot, V. J. *Biol. Chem.* **2002**, *277*, 12208–12214.

(41) Marcu, M. G.; Schulte, T. W.; Neckers, L. *J. Natl. Cancer Inst.* **2000**, *92*, 242–248.

(42) Hadden, M. K.; Lubbers, D. J.; Blagg, B. S. J. *Curr. Top. Med. Chem.* **2006**, *6*, 1173–1182.

(43) Yu, X. M.; Shen, G.; Neckers, L.; Blake, H.; Holzbeierlein, J.; Cronk, B.; Blagg, B. S. J. *J. Am. Chem. Soc.* **2005**, *127*, 12778–12779.

lower than that required for client protein degradation. On the basis of this observation, **A4** was evaluated for its neuroprotective activity in an experiment in which neurons were exposed to lethal levels of A β , the precursor to amyloid formation in Alzheimer's disease.⁴⁴ In these studies, **A4** manifested neuroprotective activity at the same concentration (EC₅₀ = 6 nM) required to induce heat shock proteins, indicating that increased chaperone levels were required to refold and clear protein aggregates that typically lead to neuronal cell death. In addition to these outstanding neuroprotective properties, **A4** exhibited no toxicity, which is in sharp contrast to Hsp90 N-terminal inhibitors. To determine whether the structural features of **A4** correlated directly with novobiocin, modifications to the natural product were undertaken as represented by **DHN2**, which was found to be more effective than **A4** at inducing client protein degradation and also remained nontoxic.⁴⁵

Since Hsp90 exists as a homodimer and the putative C-terminal ATP binding pocket on each monomeric species is proximal to the dimerization domain, dimers of **A4**, the nontoxic inhibitor, were prepared.⁴⁶ In contrast to the monomeric species, the dimeric molecule (**A4**-Dimer) was found to manifest anti-proliferative activity. The conversion of a nontoxic molecule (**A4**) into a potent anti-proliferative agent (**A4**-Dimer) occurred through modification of the amide side chain, suggesting that further modification of **A4** in a similar manner may produce monomeric species that also exhibit antitumor activity. The identification of such a compound would clearly demonstrate that C-terminal inhibitors function via a mechanism distinct from that of N-terminal inhibitors and thus provide a unique opportunity to produce molecules that can be developed specifically for antitumor or neuroprotective activity via modulation of the same biological target.

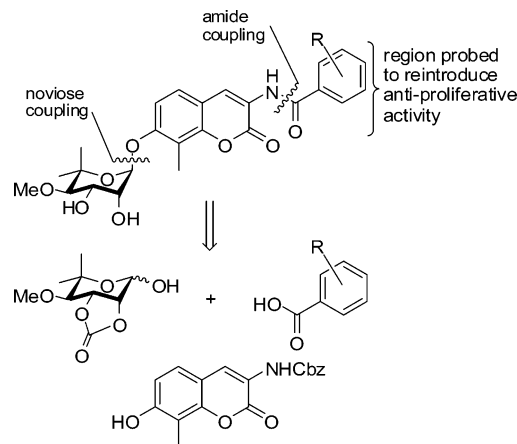
On the basis of these previous studies we proposed to determine whether monomeric compounds based on the **A4** scaffold and the natural product novobiocin could exhibit anti-proliferative activity against various cancer cell lines. In this article we report the rationale utilized toward the successful development of novobiocin analogues that possess potent antitumor activity.

Results and Discussion

Synthesis and Biological Evaluation of Monosubstituted Benzamide Novobiocin Derivatives. For determination of structure–activity relationships for the benzamide side chain of novobiocin, we envisioned the construction of novobiocin analogues with highly substituted benzamides. As shown in Scheme 1, the derivatives were assembled from three components: noviose carbonate,⁴⁷ 8-methylcoumarin,⁴⁸ and a series of substituted benzoic acids. Previously, we demonstrated that the trichloroacetimidate of noviose carbonate couples directly to the coumarin phenol to afford the α -anomer in excellent yield.⁴⁷ Since no cocrystal structure of Hsp90 bound to C-terminal inhibitors exists, commercially available benzoic

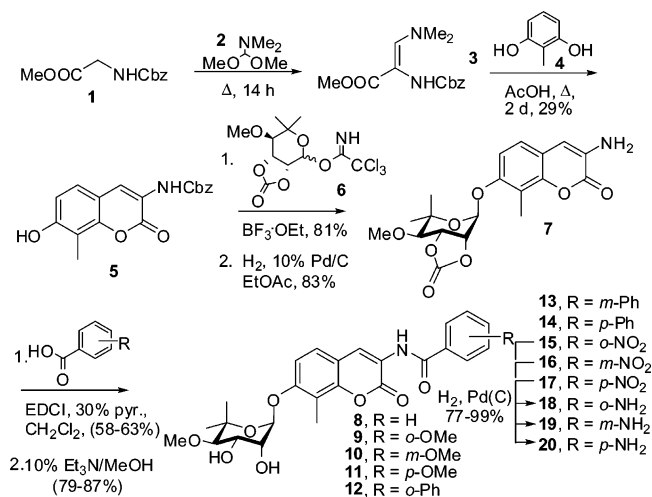
acids were chosen that contained various functionalities in an effort to probe for steric and electronic interactions with the putative hydrophobic pocket that is believed to bind to this region of novobiocin. Structure–activity relationships elucidated from this first library could then be utilized to develop more effective inhibitors.

SCHEME 1. Retrosynthesis of Novobiocin Analogues



Novobiocin analogues were prepared by the condensation of *N,N*-dimethylformamide dimethyl acetal (**2**) with Cbz-protected glycine (**1**) to produce the vinyllogous carbamate, **3** (Scheme 2).⁴⁹

SCHEME 2. Synthesis of Novobiocin Analogues with Monosubstituted Benzamides



The 8-methylcoumarin **5** was prepared by a modified Pechmann condensation of 2-methylresorcinol (**4**) with **3**.⁴⁸ The resulting phenol was noviosylated with the trichloroacetimidate of noviose carbonate (**6**)⁵⁰ in the presence of catalytic boron trifluoride etherate to give **7** in good yield.⁴⁷ The benzyl carbonate was removed via hydrogenolysis to produce aminocoumarin **7**, which proved to be a versatile intermediate throughout this project. The amine was readily coupled to a preselected library of benzoic acids in the presence of *N*-(3-(dimethylamino)propyl)-*N'*-ethylcarbodiimide hydrochloride (EDCI) and 4-DMAP. During the course of our investigation, it was determined that utilization of 4-DMAP led to bisacylation, which proved difficult

(44) Ansar, S.; Burlison, J. A.; Hadden, M. K.; Yu, X. M.; Desino, K. E.; Bean, J.; Neckers, L.; Audus, K. L.; Michaelis, M. L.; Blagg, B. S. J. *Bioorg. Med. Chem. Lett.* **2007**, 17, 1984–1990.

(45) Burlison, J. A.; Neckers, L.; Smith, A. B.; Maxwell, A.; Blagg, B. S. J. *J. Am. Chem. Soc.* **2006**, 128, 15529–15536.

(46) Burlison, J. A.; Blagg, B. S. J. *Org. Lett.* **2006**, 8, 4855–4858.

(47) Shen, G.; Yu, X. M.; Blagg, B. S. J. *Bioorg. Med. Chem. Lett.* **2004**, 14, 5903–5906.

(48) Toplak, R.; Svete, J.; Stanovnik, B.; Grdadolnik, S. G. *J. Heterocycl. Chem.* **1999**, 36, 225–235.

(49) Robinson, A. J.; Lim, C. Y.; He, L.; Ma, P.; Li, H.-Y. *J. Org. Chem.* **2001**, 66, 4141–4147.

(50) Yu, X. M.; Shen, G.; Blagg, B. S. J. *J. Org. Chem.* **2004**, 69, 7375.

TABLE 1. Anti-proliferation Activities of Novobiocin Analogues (in μM) ($n = 3$)

entry (IC ₅₀)	SkBr3	MCF-7	HCT-116	PL45	LNCaP	PC-3
8	21.5 $\pm$ 1.4	20.6 $\pm$ 0.4	13.0 $\pm$ 2.1	3.4 $\pm$ 0.6	72.0 $\pm$ 4.0	67.6 $\pm$ 9.7
9	>100	5.3 $\pm$ 1.3	>100	35.8 $\pm$ 3.4	N/T	9.1 $\pm$ 0.5
10	>100	5.6 $\pm$ 2.5	1.9 $\pm$ 0.6	2.8 $\pm$ 0.8	21.7 $\pm$ 2.0	N/T
11	15.6 $\pm$ 4.2	10.3 $\pm$ 0.9	15.9 $\pm$ 1.9	5.9 $\pm$ 2.1	7.3 $\pm$ 0.9	17.1 $\pm$ 4.3
12	39.1 $\pm$ 4.1	18.9 $\pm$ 7.0	32.7 $\pm$ 1.6	14.4 $\pm$ 2.4	17.3 $\pm$ 5.2	65.3 $\pm$ 5.6
13	13.0 $\pm$ 1.4	18.0 $\pm$ 3.8	12.8 $\pm$ 2.3	1.6 $\pm$ 0.2	1.6 $\pm$ 0.5	11.6 $\pm$ 1.4
14	16.3 $\pm$ 1.6	8.1 $\pm$ 6.0	3.6 $\pm$ 2.0	1.6 $\pm$ 0.2	44.9 $\pm$ 31.6	19.3 $\pm$ 5.1
15	21.8 $\pm$ 0.8	28.7 $\pm$ 4.8	44.3 $\pm$ 4.8	20.1 $\pm$ 4.7	16.8 $\pm$ 0.8	N/T
16	>100	>100	>100	>100	N/T	>100
17	>100	>100	>100	>100	69.3 $\pm$ 4.1	>100
18	17.7 $\pm$ 1.4	17.2 $\pm$ 3.3	14.4 $\pm$ 0.8	6.2 $\pm$ 1.3	12.7 $\pm$ 0.8	57.9 $\pm$ 10.1
19	61.4 $\pm$ 4.7	30.1 $\pm$ 3.4	21.2 $\pm$ 2.2	12.5 $\pm$ 0.9	24.8 $\pm$ 9.7	14.7 $\pm$ 2.6
20	33.1 $\pm$ 1.1	14.0 $\pm$ 0.5	24.4 $\pm$ 5.8	8.4 $\pm$ 0.3	26.4 $\pm$ 15.8	65.2 $\pm$ 2.8

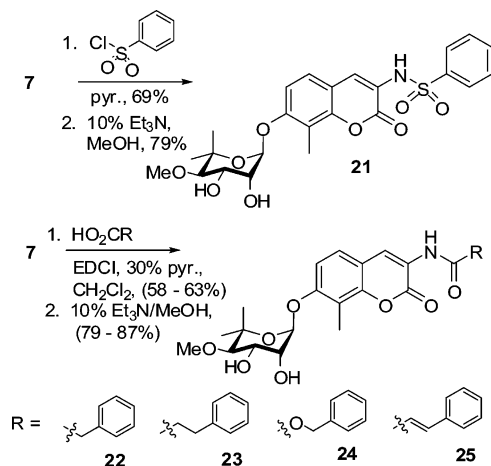
TABLE 2. Anti-proliferation Activities of Novobiocin Analogues (in μM) ($n = 3$)

entry (IC ₅₀)	SkBr3	MCF-7	HCT-116	PL45	LNCaP	PC-3
21	>100	>100	>100	>100	>100	>100
22	21.4 $\pm$ 2.2	16.4 $\pm$ 0.4	13.2 $\pm$ 0.6	8.5 $\pm$ 1.7	10.4 $\pm$ 0.2	43.3 $\pm$ 13.4
23	10.2 $\pm$ 2.3	6.9 $\pm$ 0.3	5.4 $\pm$ 0.6	9.8 $\pm$ 0.2	92.8 $\pm$ 0.9	22.0 $\pm$ 5.8
24	17.8 $\pm$ 2.4	12.1 $\pm$ 0.1	13.0 $\pm$ 0.2	11.4 $\pm$ 0.6	N/T	N/T
25	2.6 $\pm$ 0.6	4.0 $\pm$ 0.3	3.2 $\pm$ 0.5	3.0 $\pm$ 1.0	4.5 $\pm$ 0.7	3.9 $\pm$ 0.05

to separate from the monoacylated product. Therefore pyridine was employed as the base and provided exclusively monoacylated products. With the desired benzamides in hand, the cyclic carbonates underwent solvolysis with triethylamine in methanol to give the diol in excellent yield. To complete our small library of inhibitors, aryl nitro compounds (**15–17**) were subjected to hydrogenation to afford the corresponding anilines.

Simultaneous with the investigation of aryl substitutes, modification of the amide and tether functionalities was also explored (Scheme 3). Sulfonamide **21** was assembled by

SCHEME 3. Preparation of Novobiocin Derivatives with Various Linkers



sulfonylation of amine **7** with benzenesulfonyl chloride, the carbonate of which was subjected to solvolysis to provide the resulting diol. The Cbz-containing product **24** was obtained by direct solvolysis of **5**. Amides **22**, **23**, and **25** were prepared by coupling the appropriate acid with amine **7** in the presence of EDCI and pyridine, followed by solvolysis of the cyclic carbonate.

Upon completion of this initial library, compounds were evaluated for anti-proliferative activity against SkBr3 (Her2 overexpressing breast cancer cells), MCF-7 (estrogen receptor

positive breast cancer cells), HCT-116 (colon cancer cells characterized by wild-type p53), PL45 (pancreatic cancer cells), LNCaP (androgen sensitive prostate cancer cells), and PC-3 (androgen independent prostate cancer cells) cell lines. As shown in Table 1, the simplified benzamide **8** manifested anti-proliferative activity. This is in stark contrast to **A4** (Figure 1), which does not.⁴⁴ The monosubstituted benzamide variants improved activity. In fact, the most potent anti-proliferative agents identified were the methoxy (**9–11**) and phenyl (**12–14**) derivatives that produced activities against most cell lines. Thus, our data suggest that a *p*-hydrogen bond acceptor and an *m*-aryl side chain on the benzamide were most effective. In the nitro (**15–17**) and aniline (**18–20**) series, the ortho-derivatives in each respective series were more active than the corresponding regioisomers, which indicates hydrogen bond interactions may be important in this region of the molecule. To our surprise, activity was abolished upon replacement of the amide with a sulfonamide (**21**, Table 2). This suggests that key hydrogen-bonding interactions also exist between the amide and the protein target that are critical for manifesting anti-proliferative activity. Concerning the spatial requirements of the hydrophobic pocket, we found that a two-carbon spacer between the amide and phenyl ring (**23**) results in compounds that are more potent than those that contain a methylene linker (**22**), a benzyl carbamate (**24**), or a simplified benzamide (**8**). Furthermore, as seen with *trans*-cinnamide **25**, increasing the rigidity in the ethyl linker provided an additional ~3-fold increase in inhibitory activity versus the saturated derivative (**23**). These results suggest that the hydrophobic pocket into which the benzamide projects may accommodate larger aromatic systems that exhibit increased affinity.

Overall, we were pleased to have identified novobiocin analogues that manifest anti-proliferative activity against multiple transformed cells, in particular, the drug resistant pancreatic ductal adenocarcinoma (PL45), a very aggressive cancer that is associated with high mortality rates in patients.⁵¹

(51) Greenlee, R. T.; Hill-Harmon, M. B.; Murray, T.; Thun, M. *Ca-Cancer J. Clin.* **2001**, 51, 15–36.

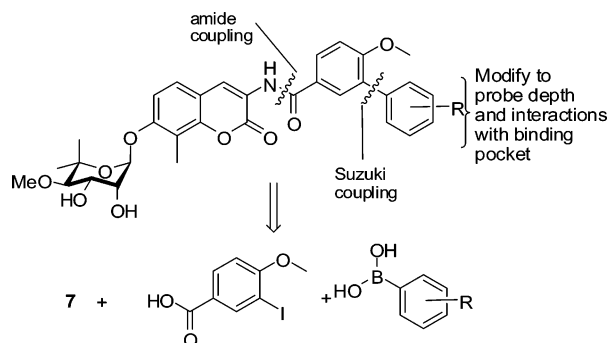
(52) Greenfield, A. A.; Butera, J. A.; Caufield, C. E. *Tetrahedron Lett.* **2003**, 44, 2729–2732.

TABLE 3. Anti-proliferation Activities of Novobiocin Biaryl Analogues (in μM) ($n = 3$)

entry (IC ₅₀)	SkBr3	MCF-7	HCT-116	PL45	LNCaP	PC-3
28	16.5 $\pm$ 4.7	19.5 $\pm$ 2.3	11.4 $\pm$ 0.0	2.8 $\pm$ 0.4	1.9 $\pm$ 0.2	25.4 $\pm$ 5.2
29	32.4 $\pm$ 5.1	18.9 $\pm$ 2.7	37.5 $\pm$ 3.4	6.8 $\pm$ 1.5	22.6 $\pm$ 3.3	64.7 $\pm$ 17.1
30	20.4 $\pm$ 3.5	20.4 $\pm$ 0.8	26.4 $\pm$ 0.6	4.2 $\pm$ 1.0	4.2 $\pm$ 1.2	50.7 $\pm$ 4.3
31	25.4 $\pm$ 1.7	16.7 $\pm$ 6.3	2.4 $\pm$ 1.0	1.4 $\pm$ 0.1	3.7 $\pm$ 0.9	31.9 $\pm$ 10.3
32	7.1 $\pm$ 0.6	15.6 $\pm$ 6.3	5.2 $\pm$ 2.1	3.8 $\pm$ 1.7	6.0 $\pm$ 1.0	52.3 $\pm$ 34.7
33	7.5 $\pm$ 1.0	18.7 $\pm$ 1.8	5.1 $\pm$ 1.1	2.0 $\pm$ 0.6	2.1 $\pm$ 0.3	53.3 $\pm$ 4.5
34	7.8 $\pm$ 0.5	37.9 $\pm$ 2.3	24.0 $\pm$ 0.3	2.6 $\pm$ 0.5	3.3 $\pm$ 0.6	44.0 $\pm$ 20.3
35	1.5 $\pm$ 0.1	1.5 $\pm$ 0.1	4.7 $\pm$ 1.4	1.4 $\pm$ 0.2	2.6 $\pm$ 0.6	16.6 $\pm$ 4.4
36	2.9 $\pm$ 1.2	5.3 $\pm$ 1.5	4.5 $\pm$ 1.2	1.1 $\pm$ 0.0	2.5 $\pm$ 1.2	14.7 $\pm$ 2.4
37	1.6 $\pm$ 0.2	2.3 $\pm$ 0.8	1.4 $\pm$ 0.1	1.9 $\pm$ 0.8	2.6 $\pm$ 0.5	22.3 $\pm$ 3.6
38	>100	>100	>100	>100	8.8 $\pm$ 0.6	1.0 $\pm$ 0.1

In an effort to incorporate the initial structure–activity relationships into more efficacious inhibitors, two small libraries of novobiocin derivatives were prepared. The first library explored optimization of the benzamide that contained a *p*-methoxy and a *m*-phenyl substituent. It was proposed that such a compound may exhibit synergistic or additive activity and lead to more effective compounds. The second library focused on the incorporation of heterocycles into the benzamide region in order to investigate hydrogen bond donor/acceptor interactions and the effects of rigidity as suggested by our initial findings. It was believed that this set of compounds could be expeditiously prepared by the coupling of a 3-iodo-4-methoxy benzoic acid with **7**, to produce an intermediate (**27**) upon which the incorporation of additional phenyl substituents could be pursued for elucidation of structure–activity relationships (Scheme 4).

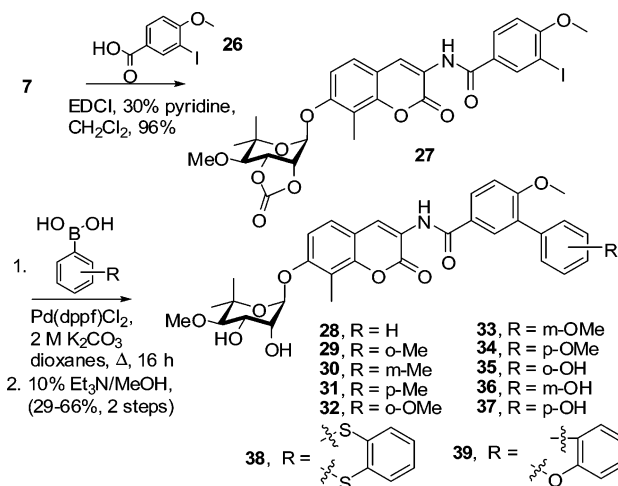
SCHEME 4. Retrosynthetic Analysis of Biaryl Novobiocin Analogues



Synthesis and Biological Evaluation of the Biaryl Novobiocin Library. The Suzuki precursor **27** was prepared by coupling aminocoumarin **7** with benzoic acid **26** in the presence of EDCI and pyridine (Scheme 5). The biaryl substituents also included various hydrogen bond acceptors and donors to further probe key binding interactions with Hsp90. After an extensive survey of experimental conditions, it was found that dichloro-[1,1'-bis(diphenylphosphino)ferrocene]palladium(II) dichloromethane [Pd(dppf)Cl₂] in the presence of substituted phenyl boronic acids and 2 M potassium carbonate in dioxane at 50 °C provided the most reproducible cross-coupling conditions.⁵² To complete the synthesis, the carbonates were removed upon solvolysis with methanolic triethylamine.

Upon completion of the biaryl derivatives, the compounds were evaluated for anti-proliferative activity against the same cell lines described above. Unfortunately, as presented in Table 3, combinations of the *p*-methoxy and *m*-phenyl substituents on the benzamide (**28**) did not produce compounds that inhibit cell growth more effectively as originally proposed (**28** com-

SCHEME 5. Preparation of the Biaryl Novobiocin Derivatives



pared to **11** and **13**). Likewise, the tolyl derivatives (**29–31**) also manifested lower growth inhibition than biaryl **28**, and complete activity was lost against most cell lines upon incorporation of dihydrothianthrene, **38**. Dihydrodibenzofuran **39** lacked reasonable solubility in DMSO and was therefore not evaluated in our studies. However, as seen in the methoxy series (**32–34**) and the phenol series (**35–37**), activity increased as polarity and hydrogen bond donor/acceptor properties of the inhibitor increased. For example, introduction of an *o*-OMe (**32**) improved inhibition ~2-fold when compared to **28**, but the corresponding phenol (**35**) was ~8 times more effective. Key structure–function relationships observed for the biaryl benzamide novobiocin derivatives are summarized in Figure 2.

Synthesis and Biological Evaluation of Heterocyclic Novobiocin Derivatives. With the initial structure–function relationships for novobiocin in hand, we wished to introduce

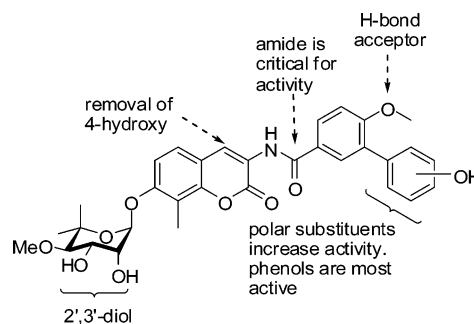
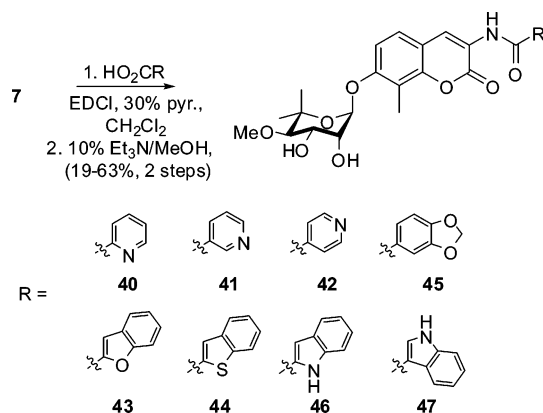
**FIGURE 2.** SAR for the biaryl novobiocin derivatives and Hsp90.

TABLE 4. Anti-proliferation Activities of Novobiocin Heterocyclic Analogues (in μM) ($n = 3$)

entry (IC ₅₀)	SkBr3	MCF-7	HCT-116	PL45	LNCaP	PC-3
40	28.4 $\pm$ 2.0	20.3 $\pm$ 4.1	23.3 $\pm$ 5.9	7.9 $\pm$ 0.7	44.9 $\pm$ 31.1	15.1 $\pm$ 2.1
41	65.9 $\pm$ 15.8	47.8 $\pm$ 4.2	37.3 $\pm$ 1.0	13.2 $\pm$ 1.0	50.1 $\pm$ 3.3	67.0 $\pm$ 20.2
42	>100	>100	>100	19.2 $\pm$ 2.5	34.8 $\pm$ 16.0	N/T
43	N/T	28.6 $\pm$ 4.1	9.6 $\pm$ 0.3	3.8 $\pm$ 0.4	7.9 $\pm$ 1.1	18.8 $\pm$ 4.4
44	N/T	96.3 $\pm$ 3.7	78.9 $\pm$ 3.5	33.2 $\pm$ 0.7	>100	7.4 $\pm$ 2.1
45	>100	>100	>100	5.2 $\pm$ 1.1	9.3 $\pm$ 0.6	10.9 $\pm$ 2.3
46	0.37 $\pm$ 0.06	0.57 $\pm$ 0.07	0.17 $\pm$ 0.01	0.47 $\pm$ 0.34	12.2 $\pm$ 0.0	22.3 $\pm$ 10.1
47	12.2 $\pm$ 1.5	5.3 $\pm$ 0.3	3.5 $\pm$ 0.5	1.8 $\pm$ 0.3	2.3 $\pm$ 0.1	4.8 $\pm$ 2.4

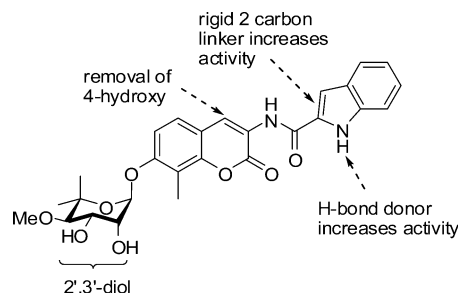
heterocycles into the benzamide region of novobiocin to further increase both solubility and polarity. We proposed that heterocycles may increase desired pharmacological properties and provide additional opportunities for hydrogen-bonding interactions within the binding pocket, as was observed in the nitro (**15–17**) and aniline (**18–20**) series reported in Table 1. To this end, heterocyclic novobiocin derivatives were prepared as described above by coupling commercially available carboxylic acids with aminocoumarin **7** via treatment with EDCI and pyridine, and the carbonates of the resulting molecules were then solvolized with methanolic triethylamine to afford the requisite diols, **40–47** (Scheme 6).

SCHEME 6. Preparation of Heterocyclic Novobiocin Derivatives

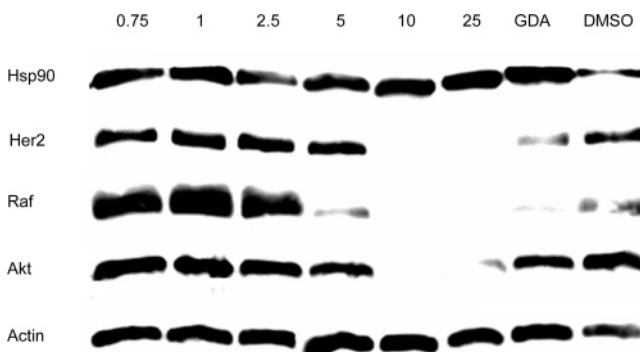


The inhibitory values were obtained by evaluation against our panel of cancer cell lines. In the nicotinic (**40–42**) series, the *ortho*-analogue was found to represent the most active regioisomer, consistent with the trend observed for the nitro (**15–17**) and aniline (**18–20**) derivatives (Table 4). Likewise, we examined benzofuran **43** and benzothiophene **44**. We postulated that because these structures contain a hydrogen bond acceptor in the same proximity as the other *ortho* derivatives they would therefore introduce a rigid two-carbon spacer between the amide and the phenyl ring. Unfortunately, benzofuran (**43**) was less active than the *o*-OMe variant (**9**), and the bioisoelectronic benzothiophene (**44**) was even worse. The 2-indole was included for its potential to provide a hydrogen bond donor as was observed for *o*-anilines. Anti-proliferation-activity was significantly increased against the majority of our cell lines indicating that the indole does indeed properly account for the activities observed for a *trans* two-carbon tether and an *o*-aniline. Substitution of the benzamide with a 2-indoleamide increased the activity >500-fold against SkBr3 cells when compared to the natural product, novobiocin. Interestingly, the position of the nitrogen on the indole is critical for activity, as the 3-indoleamide (**47**) was approximately 3–10 times less

effective than compound **46**. A summary of the observed trends for **46** is provided in Figure 3.

**FIGURE 3.** Structure–activity relationships observed for **46** and novobiocin.

Finally, to provide additional evidence that the growth inhibitory activity manifested by **46** resulted from Hsp90 inhibition, **46** was evaluated by its ability to induce degradation of Hsp90-dependent client proteins. As seen in Figure 4, Hsp90 client proteins such as Her2, Raf, and Akt were degraded in a concentration-dependent manner while Hsp90 was induced in the presence of **46**. Since non-Hsp90-dependent substrates such as actin remained unchanged, the inhibition of cell growth was directly correlated to the degradation of Hsp90-dependent client proteins.

**FIGURE 4.** Western blot analyses of Hsp90 client protein degradation assays against MCF-7 breast cancer cells. Concentration of **46** (in μM) is denoted above each lane. GDA (geldanamycin) and DMSO were used as positive and negative controls, respectively.

Conclusions

In conclusion, we have identified key structure–activity relationships for novobiocin analogues. Two small molecule libraries were prepared and evaluated for anti-proliferative activity against several cancer cell lines. The 2-indoleamide (**46**) was identified as the most potent inhibitor resulting from these studies, which resulted from rational development through

observed structure–activity trends. Studies are currently underway with this and other novobiocin analogues to confirm their Hsp90 inhibitory activity and to identify the location of the C-terminal nucleotide-binding region. The results from such studies will be reported in due course along with optimized derivatives of **46**.

Experimental Section

Benzyl 7-((3*aR*,4*R*,7*R*,7*aR*)-7-methoxy-6,6-dimethyl-2-oxotetrahydro-3*aH*-[1,3]dioxolo[4,5-*c*]pyran-4-yloxy)-8-methyl-2-oxo-2*H*-chromen-3-ylcarbamate (7*a*). Boron trifluoride etherate (61 μ L, 69 mg, 0.49 mmol, 30 mol %) was added dropwise to a solution of (3*aR*,4*S*,7*R*,7*aR*)-7-methoxy-6,6-dimethyl-2-oxo-tetrahydro-3*aH*-[1,3]dioxolo[4,5-*c*]pyran-4-yl 2,2,2-trichloroacetimidate (**6**, 588 mg, 1.62 mmol) and benzyl 7-hydroxy-8-methyl-2-oxo-2*H*-chromen-3-yl carbamate (**5**, 527 mg, 1.62 mmol) in CH_2Cl_2 (16 mL) at rt. The mixture was stirred for 14 h, before Et_3N (150 μ L) was added and the mixture concentrated. The residue was purified by chromatography (SiO_2 , DCM $\rightarrow$ 100:1 CH_2Cl_2 :acetone) to afford **7a** (670 mg, 81%) as a yellow foam: $[\alpha]_D^{25}$ –19.7 (*c* 1.54, 20% MeOH in CH_2Cl_2); ^1H NMR (CDCl_3 , 400 MHz) δ 8.27 (s, 1H), 7.85 (s, 1H), 7.55–7.35 (m, 5H), 7.29 (d, *J* = 2.9 Hz, 1H), 7.11 (d, *J* = 8.7 Hz, 1H), 5.77 (d, *J* = 1.9 Hz, 1H), 5.23 (s, 2H), 5.05 (d, *J* = 1.9 Hz, 1H), 4.95 (t, *J* = 7.7 Hz, 1H), 3.59 (s, 3H), 3.30 (d, *J* = 7.6 Hz, 1H), 2.27 (s, 3H), 1.34 (s, 3H), 1.19 (s, 3H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 159.0, 155.2, 153.6, 153.6, 149.2, 136.0, 129.1 (2C), 129.0, 128.7 (2C), 125.8, 122.6, 122.1, 115.2, 115.1, 111.6, 94.8, 83.3, 78.4, 77.6, 77.0, 67.9, 61.0, 27.9, 22.6, 8.8; IR (film) ν_{max} 3402, 3319, 3063, 3034, 2984, 2939, 2839, 1817, 1709, 1634, 1609, 1587, 1522, 1456, 1383, 1366, 1331, 1296, 1263, 1229, 1205, 1175 cm^{-1} ; HRMS (ESI+) *m/z* 526.1688 (*M* + H^+ , $\text{C}_{27}\text{H}_{28}\text{NO}_{10}$ requires *m/z* 526.1713).

3-Amino-7-((3*aR*,4*R*,7*R*,7*aR*)-7-methoxy-6,6-dimethyl-2-oxotetrahydro-3*aH*-[1,3]dioxolo[4,5-*c*]pyran-4-yloxy)-8-methyl-2-oxo-2*H*-chromen-2-one (7). Palladium on carbon (10%, 67 mg) was added to a solution of carbamate **5** (670 mg, 1.31 mmol) in THF (13 mL). The suspension was stirred for 6 h under a hydrogen atmosphere before it was filtered through a plug of SiO_2 and eluted with THF (20 mL). The eluent was concentrated and the residue purified by chromatography (SiO_2 , 100:1 $\rightarrow$ 50:1 CH_2Cl_2 :acetone) to afford **7** (425 mg, 83%) as a yellow foam: $[\alpha]_D^{25}$ –26.4 (*c* 0.780, 20% MeOH in CH_2Cl_2); ^1H NMR (CDCl_3 , 400 MHz) δ 7.10 (d, *J* = 8.6 Hz, 1H), 7.05 (d, *J* = 8.6 Hz, 1H), 6.68 (s, 1H), 5.73 (d, *J* = 2.0 Hz, 1H), 5.04 (dd, *J* = 2.0, 7.9 Hz, 1H), 4.95 (t, *J* = 7.7 Hz, 1H), 4.11 (s, 2H), 3.54 (s, 3H), 3.29 (d, *J* = 7.6 Hz, 1H), 2.28 (s, 3H), 1.34 (s, 3H), 1.21 (s, 3H); ^{13}C NMR (CDCl_3 , 200 MHz) δ 159.6, 153.3, 153.0, 148.1, 130.2, 122.7, 116.1, 114.8, 111.9, 111.0, 94.5, 83.0, 78.0, 77.3, 76.4, 60.6, 27.5, 22.2, 8.6; IR (film) ν_{max} 3462, 3362, 2984, 2937, 2839, 1807, 1707, 1636, 1595, 1497, 1387, 1371, 1331, 1263, 1169, 1109, 1078, 1036 cm^{-1} ; HRMS (ESI+) *m/z* 392.1357 (*M* + H^+ , $\text{C}_{19}\text{H}_{22}\text{NO}_8$ requires *m/z* 392.1346).

2-Methoxy-*N*-(7-((3*aR*,4*R*,7*R*,7*aR*)-7-methoxy-6,6-dimethyl-2-oxotetrahydro-3*aH*-[1,3]dioxolo[4,5-*c*]pyran-4-yloxy)-8-methyl-2-oxo-2*H*-chromen-3-yl)benzamide (9*a*). General EDCI coupling procedure **A**: *N*-(3-(Dimethylamino)propyl)-*N'*-ethylcarbodiimide hydrochloride (3 equiv) was added to a solution of aminocoumarin **7** (1 equiv), benzoic acid (3 equiv), and 4-DMAP (2.0 equiv) in CH_2Cl_2 at rt. The solution was stirred for 14 h then concentrated, and the residue was purified via preparative TLC or column chromatography (SiO_2 , 40:1 CH_2Cl_2 :acetone) to afford the benzamide as a colorless, amorphous solid (66%): $[\alpha]_D^{24}$ –29.6 (*c* 0.61, 20% MeOH in CH_2Cl_2); ^1H NMR (400 MHz, CDCl_3) δ 8.77 (s, 1H), 8.18 (d, *J* = 7.8 Hz, 1H), 7.46 (t, *J* = 7.8 Hz, 1H), 7.27 (d, *J* = 8.3 Hz, 1H), 7.09–7.02 (m, 2H), 6.99 (d, *J* = 8.3 Hz, 1H), 5.71 (s, 1H), 4.99 (d, *J* = 7.9 Hz, 1H), 4.91 (t, *J* = 7.9 Hz, 1H), 4.05 (s, 3H), 3.53 (s, 3H), 3.24 (d, *J* = 7.9 Hz, 1H), 2.23 (s, 3H), 1.29 (s, 3H), 1.14 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 164.5,

159.6, 158.1, 155.3, 153.6, 149.5, 134.3, 132.6, 126.4, 124.4, 123.5, 121.9, 121.3, 115.5, 115.1, 112.0, 111.5, 94.8, 83.3, 78.4, 77.4, 77.1, 61.0, 56.6, 27.9, 22.6, 8.8; IR (film) ν_{max} 3308, 3055, 2986, 2939, 2930, 1817, 1807, 1707, 1655, 1603, 1533, 1481, 1466, 1367, 1263, 1232 cm^{-1} ; HRMS (ESI+) *m/z* 526.1691 (*M* + H^+ , $\text{C}_{27}\text{H}_{28}\text{NO}_{10}$ requires *m/z* 526.1713).

***N*-(7-((2*R*,3*R*,4*S*,5*R*)-3,4-Dihydroxy-5-methoxy-6,6-dimethyltetrahydro-2*H*-pyran-2-yloxy)-8-methyl-2-oxo-2*H*-chromen-3-yl)-2-methoxybenzamide (9).** General procedure for solvolysis of the cyclic carbonate: Et_3N (10% total volume) was added dropwise to a solution of cyclic carbonate in methanol. The resulting mixture was stirred for 14 h, and then concentrated. The residue was purified via preparative TLC or column chromatography (SiO_2 , 4:1 CH_2Cl_2 :acetone) to yield a colorless, amorphous solid (74%): $[\alpha]_D^{25}$ –16.1 (*c* 0.16, 20% MeOH in CH_2Cl_2); ^1H NMR (400 MHz, 20% CD_3OD in CD_2Cl_2) δ 8.79 (s, 1H), 8.15 (d, *J* = 7.9 Hz, 1H), 7.57 (t, *J* = 7.8 Hz, 1H), 7.38 (d, *J* = 8.7 Hz, 1H), 7.20 (d, *J* = 8.7 Hz, 1H), 7.16 – 7.10 (m, 2H), 5.55 (s, 1H), 4.20–4.14 (m, 2H), 4.11 (s, 3H), 3.58 (s, 3H), 3.35 (d, *J* = 8.0 Hz, 1H), 2.28 (s, 3H), 1.34 (s, 3H), 1.11 (s, 3H); ^{13}C NMR (100 MHz, 20% CD_3OD in CD_2Cl_2) δ 164.9, 160.2, 158.4, 156.7, 149.8, 134.6, 132.4, 126.3, 125.3, 123.0, 122.9, 121.9, 121.2, 114.7, 112.5, 111.7, 99.1, 84.8, 79.2, 71.9, 69.0, 62.1, 56.8, 29.1, 22.9, 8.5; IR (film) ν_{max} 3373, 2947, 2835, 2525, 1641, 1630, 1610, 1448, 1412, 1398 cm^{-1} ; HRMS (ESI+) *m/z* 500.1893 (*M* + H^+ , $\text{C}_{26}\text{H}_{30}\text{NO}_9$ requires *m/z* 500.1921).

***N*-(7-((2*R*,3*R*,4*S*,5*R*)-3,4-Dihydroxy-5-methoxy-6,6-dimethyltetrahydro-2*H*-pyran-2-yloxy)-8-methyl-2-oxo-2*H*-chromen-3-yl)-6-methoxybiphenyl-3-carboxamide (28).** General procedure for Suzuki coupling and solvolysis of the cyclic carbonate: Aryl iodide **27** (1.0 equiv), 2 M $\text{K}_2\text{CO}_3(\text{aq})$ (3.0 equiv), and the aryl boronic acid were dissolved in dioxane before $\text{PdCl}_2(\text{dppf})\cdot\text{CHCl}_3$ (3 mol %) was added to the solution at rt. The resulting solution was stirred at rt for 30 min and then warmed to 55 $^\circ\text{C}$ for 3–16 h, after which the mixture was concentrated, filtered through a pad of silica gel (eluted with 40:1 CH_2Cl_2 :acetone), and purified via preparative TLC (SiO_2 , 40:1 CH_2Cl_2 :acetone). The resulting product was dissolved in methanol containing 10% Et_3N and stirred for 14 h before being concentrated. The residue was purified by preparative TLC (4:1 CH_2Cl_2 :acetone) to afford the corresponding diol as a colorless, amorphous solid (46%, 2 steps): $[\alpha]_D^{25}$ –8.7 (*c* 0.23, CH_2Cl_2); ^1H NMR (800 MHz, CD_2Cl_2) δ 8.78 (s, 1H), 8.69 (s, 1H), 7.93 (dd, *J* = 4.4, 8.0 Hz, 1H), 7.87 (d, *J* = 2.4 Hz, 1H), 7.55 (d, *J* = 8.0 Hz, 2H), 7.45 (t, *J* = 8.0 Hz, 2H), 7.93 (dd, *J* = 4.4, 8.0 Hz, 2H), 7.21 (d, *J* = 8.8 Hz, 1H), 7.10 (d, *J* = 8.8 Hz, 2H), 5.60 (s, 1H), 4.27–4.20 (m, 2H), 3.90 (s, 3H), 3.59 (s, 3H), 3.36 (d, *J* = 9.6 Hz, 1H), 2.73 (s, 2H), 2.28 (s, 3H), 1.36 (s, 3H), 1.13 (s, 3H); ^{13}C NMR (200 MHz, CD_2Cl_2) δ 167.2, 161.7, 161.2, 157.9, 151.0, 139.4, 133.0, 131.8, 131.5 (3C), 130.0 (2C), 129.4, 128.1, 127.6, 125.8, 124.0, 116.1, 116.0, 113.0 (2C), 99.7, 86.1, 80.4, 73.1, 70.5, 63.7, 57.8, 30.8, 24.2, 10.1; IR (film) ν_{max} 3402, 3086, 3055, 3028, 2974, 2934, 2849, 2837, 1709, 1670, 1607, 1526, 1504, 1489, 1367, 1265, 1231, 1095 cm^{-1} ; HRMS (ESI+) *m/z* 576.2231 (*M* + H^+ , $\text{C}_{32}\text{H}_{34}\text{NO}_9$ requires *m/z* 576.2234).

***N*-(7-((2*R*,3*R*,4*S*,5*R*)-3,4-Dihydroxy-5-methoxy-6,6-dimethyltetrahydro-2*H*-pyran-2-yloxy)-8-methyl-2-oxo-2*H*-chromen-3-yl)-1*H*-indole-3-carboxamide (47).** General EDCI Coupling Procedure **B**: *N*-(3-(Dimethylamino)propyl)-*N'*-ethylcarbodiimide hydrochloride (2.5 equiv) was added to a solution of aminocoumarin **7** (1.0 equiv) and carboxylic acid (2.0 equiv) in CH_2Cl_2 containing 30% pyridine at rt. The solution was stirred for 14 h, then concentrated, and the residue was purified via preparative TLC (SiO_2 , 40:1 CH_2Cl_2 :acetone) to afford the amide. The resulting product was dissolved in methanol containing 10% Et_3N and stirred for 14 h at rt. The mixture was concentrated and the residue was purified by preparative TLC (10:1 CH_2Cl_2 :methanol or 4:1 CH_2Cl_2 :actone) to afford the corresponding diol as a colorless, amorphous solid (19%, 2 steps): $[\alpha]_D^{26}$ –11.4 (*c* 0.18, 20% MeOH

in CH_2Cl_2); ^1H NMR (500 MHz, 20% CD_3OD in CD_2Cl_2) δ 8.75 (s, 1H), 8.12 (dd, $J = 2.0, 6.5$ Hz, 1H), 7.97 (s, 1H), 7.49 (dd, $J = 2.0, 6.5$ Hz, 1H), 7.38 (d, $J = 8.5$ Hz, 1H), 7.31–7.24 (m, 2H), 7.20 (d, $J = 8.5$ Hz, 1H), 5.55 (d, $J = 2.5$ Hz, 1H), 4.16 (dd, $J = 4.5, 9.5$ Hz, 1H), 4.12 (t, $J = 4.5$ Hz, 1H), 3.57 (s, 3H), 3.33–3.31 (m, 1H), 2.28 (s, 3H), 1.33 (s, 3H), 1.10 (s, 3H); ^{13}C NMR (125MHz, 20% CD_3OD in CD_2Cl_2) δ 165.7, 161.3, 157.6, 150.6, 138.4, 131.0, 127.1, 126.4, 125.1, 124.5, 123.8, 123.5, 121.4, 115.8 (2C), 113.9, 112.8, 112.7, 100.0, 85.9, 80.1, 72.9, 70.1, 63.2, 30.2, 23.9, 9.6; IR (film) ν_{max} 3439, 3418, 3394, 2957, 2924, 2853, 1636, 1529, 1437, 1379, 1261, 1180, 1128, 1082, 1020 cm^{-1} ; HRMS

(ESI+) m/z 509.1924 ($\text{M} + \text{H}^+$, $\text{C}_{27}\text{H}_{29}\text{N}_2\text{O}_8$ requires m/z 509.1924).

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Supporting Information Available: Full experimental procedures and characterization for all compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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