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Identification of a New Scaffold for Hsp90 C-Terminal Inhibition

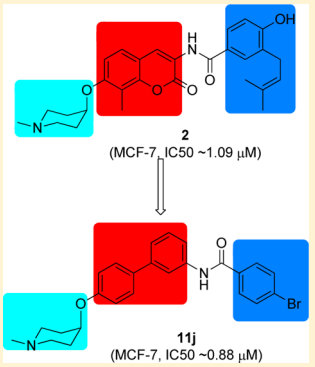
Huiping Zhao,[†] Elisabetta Moroni,[‡] Giorgio Colombo,[‡] and Brian S. J. Blagg*,[†]

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

[‡]Istituto di Chimica del Riconoscimento Molecolare, CNR, Via Mario Bianco 9, 20131 Milano, Italy

Supporting Information

ABSTRACT: Inhibition of Hsp90 C-terminal function is an advantageous therapeutic paradigm for the treatment of cancer. Currently, the majority of Hsp90 C-terminal inhibitors are derived from novobiocin, a natural product traditionally used as an antibiotic. Assisted by molecular docking studies, a scaffold containing a biphenyl moiety in lieu of the coumarin ring system found in novobiocin was identified for development of new Hsp90 C-terminal inhibitors. Initial structure−activity studies led to derivatives that manifest good antiproliferative activity against two breast cancer cell lines through Hsp90 inhibition. This platform serves as a scaffold upon which new Hsp90 C-terminal inhibitors can be readily assembled for further investigation.



KEYWORDS: *Heat shock protein 90, Hsp90 C-terminal inhibitors, novobiocin, biphenyl, breast cancer*

The 90 kDa heat shock protein (Hsp90) is a highly conserved molecular chaperone that plays a pivotal role in the maintenance of protein homeostasis and sustains cell viability during cellular stress.[1] Abnormal expression of Hsp90 has been implicated in a variety of disease states. In cancer, elevated Hsp90 levels are critical for the stabilization and function of oncogenic proteins distributed amongst all six hallmarks of cancer.[2] Therefore, small molecules that inhibit the Hsp90 folding machinery can simultaneously attack multiple signaling pathways that are essential for cancer cell survival, adaptation, and proliferation and provides a unique opportunity for development of cancer therapeutics.[3] In fact, 17 small molecules that inhibit Hsp90 function have entered clinical trials for the treatment of various cancers, demonstrating the viability of this paradigm.[4] Although current drug development efforts focus on small molecules that target the Hsp90 N-terminus, the concomitant heat shock response induced upon administration of such agents compromises their efficacy and allows cancer cell survival, which may lead to resistance and metastasis.[5] Recent studies have demonstrated the existence of a second nucleotide-binding site at the Hsp90 C-terminus, and small molecules such as novobiocin, chlorobiocin, and coumermycin A1 were shown to bind this region and induce a dose-dependent degradation of Hsp90 client proteins in a manner similar to Hsp90 N-terminal inhibitors.[6] In contrast to N-terminal inhibitors, C-terminal inhibitors do not induce the pro-survival heat shock response and therefore provide an alternative model for Hsp90 modulation.[7−9] Although significant progress has been made toward the discovery and development of potent Hsp90 C-terminal inhibitors, most of these small molecules are derived from novobiocin and related natural products.[10−15] The scarcity of scaffolds that bind the Hsp90 C-terminus makes the expansion of chemical space highly desirable for the attainment of new structure−activity relationships.

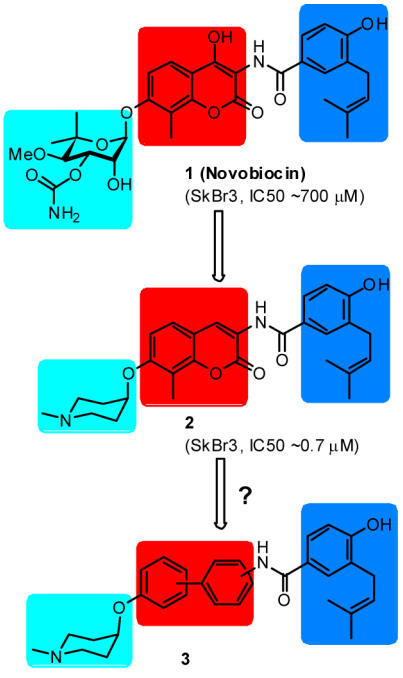


Figure 1. Rationale for proposed coumarin replacement.

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Prior studies have shown that modification to the benzamide side chain and sugar moiety result in a significant enhancement in antiproliferative activity, and lead-like molecules such as **2** were identified (Figure 1).[10] However, modifications to the coumarin core generated a relatively flat SAR trend and analogues containing a naphthalene in lieu of the coumarin ring manifested comparable antiproliferative activity,[16] indicating the coumarin may serve as a backbone for orientation in the binding pocket. In addition, construction of the coumarin moiety is not trivial, and modifications to this ring system are not readily achieved. Biphenyl is a common scaffold found in biologically active compounds, and compounds derived from this scaffold manifest diverse activities, including anticancer activity.[17−19] It was proposed that replacement of the coumarin ring with a biphenyl system may provide a new scaffold upon which Hsp90 C-terminal inhibitors could be developed (Figure 2). In contrast to the coumarin system, the biphenyl group affords the ability to adopt multiple conformations in the binding pocket, which may provide additional interactions with the protein. Although there is no cocrystal structure of a ligand bound to the Hsp90 C-terminus, several computational models have been developed to assist the drug discovery process.[20,21] In combination with prior SAR studies, the distance between the nitrogen atoms on the piperidine ring and the amide were proposed to be important for Hsp90 C-terminal inhibition.[22] Using the computational docking strategy outlined in previous SAR studies,[22] compound **3b**, which contains a *para-meta* substitution pattern on the biphenyl moiety, appeared to overlay with lead molecule **2** (Figures 2 and 2SI, Supporting Information).

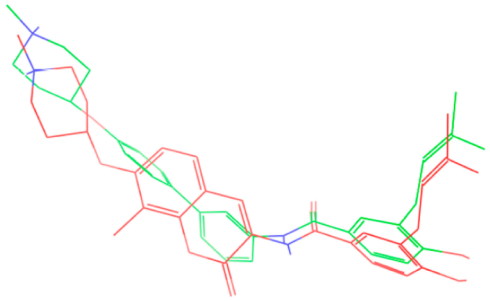
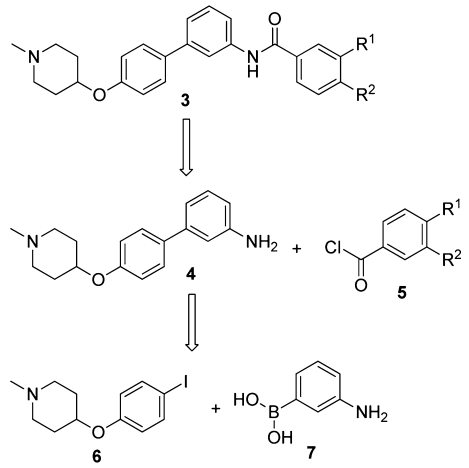


Figure 2. Molecular overlay of compounds **2** (red) and **3b** (green) in the putative Hsp90 C-terminal binding site.

Encouraged by these molecular modeling studies, compounds **3b** and its biaryl counterpart, **3c** (Scheme 2), were synthesized to evaluate this hypothesis. As shown in Scheme 1, compound **3** and its related derivatives were synthesized via an amide coupling reaction between amine **4** and acid chloride **5**. The key intermediate **4** was obtained through a Suzuki coupling reaction between iodide **6** and phenylboronic acid, **7**.

Synthesis of these compounds commenced by a Mitsunobu etherification between 1-methyl-4-hydroxypiperidine (**8**) and 4-iodophenol (**9**) in the presence of triphenylphosphine and diisopropyl azodicarboxylate in tetrahydrofuran, which afforded iodide **6** in good yield. Subsequent Suzuki coupling of iodide **6** with 3-aminophenylboronic acid (**7**) using [1,1′-bis(diphenylphosphino) ferrocene]dichloropalladium(II) as a catalyst gave biphenylamine **4**, which underwent amide coupling with biarylcarbonyl chloride or prenylated phenylcarbonyl chloride to give compounds **3a** and **3c**,[10] respectively. Solvolysis of **3a** in a 10% solution of triethylamine in methanol gave phenol **3b** in excellent yield.

Scheme 1. Retrosynthetic Analysis of Biphenyl Derivatives



Antiproliferative studies with compounds **3a−c** were performed against the SKBr3 (estrogen receptor negative, HER2 overexpressing breast cancer cells) and MCF-7 (estrogen receptor positive breast cancer cells) breast cancer cell lines. As shown in Table 1, all three compounds manifested low micromolar activity against both breast cancer cell lines. These activities are in close proximity to their coumarin counterparts, suggesting the biphenyl moiety represents an attractive surrogate for the coumarin ring system. However, in contrast to the novobiocin derivatives, which usually manifest better antiproliferative activities against the SKBr3 cell line, these biphenyl derivatives were more efficacious against the MCF-7 cell line.

Table 1. Antiproliferative Activity of Biphenyl Derivatives

entry	SKBr3 (IC_{50}, μM)	MCF-7 (IC_{50}, μM)
1	~700	
2	0.76 ± 0.14[a]	1.09 ± 0.08
3a	3.65 ± 0.14	1.45 ± 0.02
3b	2.94 ± 0.11	2.21 ± 0.06
3c	3.47 ± 0.47	2.71 ± 0.40

[a]Values represent mean ± standard deviation for at least two separate experiments performed in triplicate.

Confirmation that these compounds manifested their antiproliferative activity through Hsp90 inhibition was performed by Western blot analyses of several Hsp90 client protein levels in MCF-7 cell lysates. Actin, a protein that is not dependent on Hsp90 for its function, was chosen as a control. As shown in Figure 3, two Hsp90 client proteins, Her2 and Raf-1, were degraded upon treatment with **3a** or **3c** at concentrations that mirror their antiproliferative IC_{50} values. Concentration-dependent analysis of MCF-7 cell lysates upon the administration of **3b** showed the degradation of Her2, Raf-1, and *p*-Akt (Figure 3, **B**). These high-low and gradient-concentration Western blot analyses suggest that inhibition of the Hsp90 protein folding machinery is responsible for the observed antiproliferative activity, suggesting that the biphenyl moiety can serve as a replacement for the coumarin system.

Structure−activity relationship interrogation suggested that modifications to the benzamide side chain of novobiocin produced analogues that exhibit improved antiproliferative activities against various cancer cell lines.[22,23] Therefore, initial SAR

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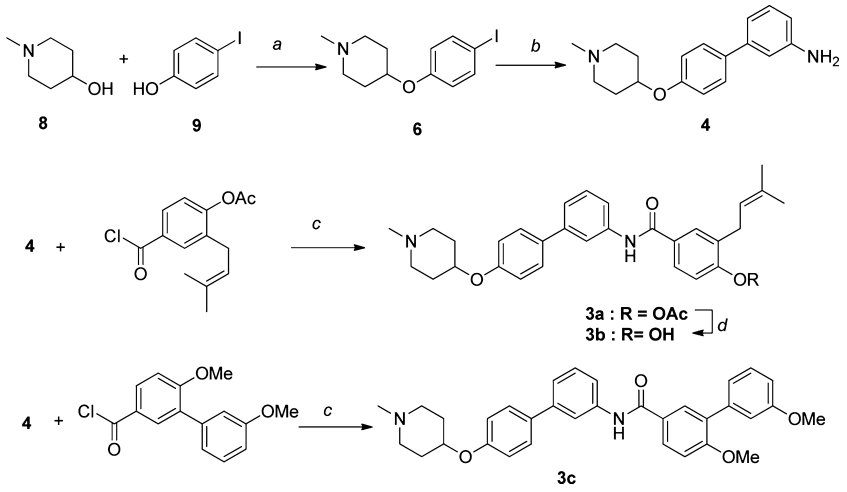
Scheme 2. Synthesis of Biphenyl Amides[a]



[a]Reagents and conditions: a. Ph_3P, DIAD, THF; b. **7**, $Pd(dppf)_2Cl_2$, 2 M K_2CO_3, 1,4-dioxane; c. pyridine, DCM; d. Et_3N, MeOH.

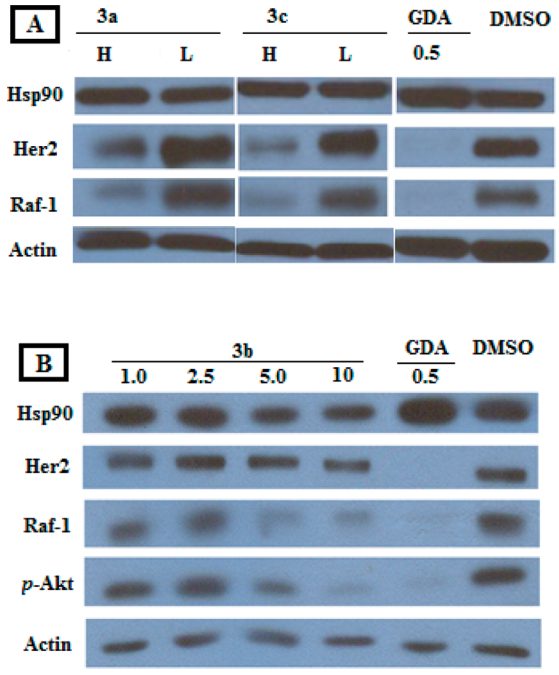
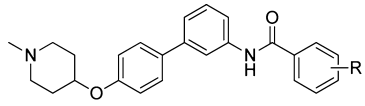


Figure 3. Western blot analyses of Hsp90-dependent client proteins from MCF-7 breast cancer cell lysate upon treatment with biphenyl derivatives. Concentrations (in μM) were indicated above each line. H represents a concentration equal to 5-fold of the antiproliferative activity. L represents a concentration equal to 0.5-fold of the antiproliferative activity. Geldanamycin (GDA, 0.5 μM) and dimethylsulfoxide (DMSO, 100%) were employed as positive and negative controls.

studies with these biphenyl derivatives began by modification to this region of the molecule. Electron-donating, electron-withdrawing, and sterically bulky substitutions were installed onto the phenylamide side chain by a one-step amide coupling between aniline **4** and substituted benzoyl chlorides (**10a**−**10m**), in the presence of pyridine to give **11a**−**11m** (Scheme 3). Compound **11n** and **11o** were assembled in the similar manner.

Upon construction of this library, the corresponding biphenyl derivatives were evaluated for antiproliferative activity against SKBr3 and MCF-7 breast cancer cell lines. As shown in Table 2,

Table 2. Antiproliferative Activity of Biphenyl Derivatives with Various Phenyl Substitutions



Entry	R	SKBr3 (IC_{50}, μM)	MCF-7 (IC_{50}, μM)
11a	H	18.86 ± 0.95	12.02 ± 0.57
11b	*p*-CH_3	5.27 ± 0.29[a]	3.92 ± 0.13
11c	*m*-CH_3	11.38 ± 1.37	7.73 ± 1.90
11d	*p*-*t*-butyl	1.51 ± 0.31	3.45 ± 0.02
11e	*p*-methoxy	10.1 ± 0.93	5.52 ± 0.01
11f	*m*-methoxy	8.36 ± 1.35	4.50 ± 0.46
11g	*p*-Cl	3.63 ± 1.03	2.23 ± 0.05
11h	*m*-Cl	4.29 ± 0.43	2.11 ± 0.42
11i	*o*-Cl	7.87 ± 0.48	5.17 ± 0.49
11j	*p*-Br	1.94 ± 0.11	0.88 ± 0.07
11k	3,4-dichloro	2.24 ± 0.11	2.17 ± 0.37
11l	2,4-dichloro	5.91 ± 0.15	3.93 ± 0.47
11m	3,5-dichloro	4.23 ± 0.09	3.72 ± 0.15
11n	(2-naphthoyl)	2.09 ± 0.34	1.66 ± 0.27
11o	(1-naphthoyl)	1.64 ± 0.13	1.10 ± 0.17

[a]Values represent mean ± standard deviation for at least two separate experiments performed in triplicate.

these derivatives are efficacious against both MCF-7 and SKBr3 cells. In general, all substitutions on the phenyl ring were determined beneficial, consistent with the existence of a hydrophobic pocket in this region of the Hsp90 C-terminal binding site. However, electron-deficient groups were more beneficial than their electron-rich counterparts (**11g**−**h** vs **11b**−**c** and **11e**−**f**). Substitution at the para position resulted in increased activity compared to substitutions at the meta position (**11b** vs **11c**), while substitution at the ortho position was detrimental (**11i** vs **11g** and **11h**, and **11l** vs **11k**). It appears that bulky substitutions (**11d**) at the para position can also produce compounds that exhibit increased antiproliferative activity. However, it was surprising that 1-naphthoyl derivative (**11o**) is more active than 2-naphthoxyl derivative (**11n**), considering the relatively detrimental effect of ortho-substitution (**11i** vs **11h** and **11g**).

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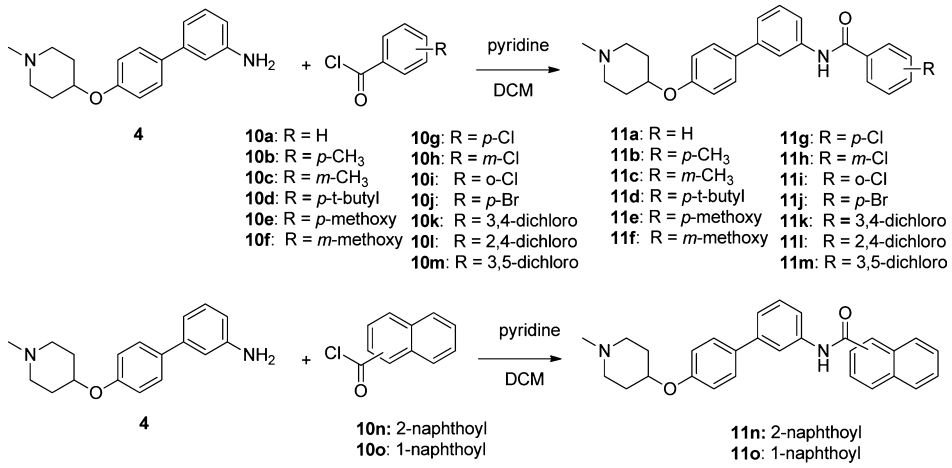
Scheme 3. Synthesis of Biphenyl Derivatives with a Modified Phenylamide Side Chain



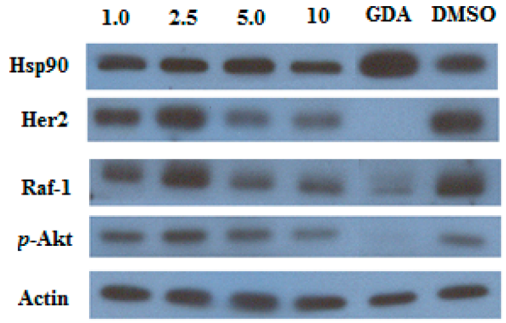


Figure 4. Western blot analyses of Hsp90-dependent client proteins from MCF-7 breast cancer cell lysate upon treatment with compound **11j**. Concentrations (in μM) were indicated above each line, and geldanamycin (GDA, 0.5 μM) and dimethylsulfoxide (DMSO) were employed as positive and negative controls.

To confirm these compounds manifested their antiproliferative activity through Hsp90 inhibition, Western blot analyses of several Hsp90 client protein levels were examined in MCF-7 cell lysates treated with the most active compound, **11j**. As shown in Figure 4, the Hsp90-dependent client proteins Her2, Raf-1, and *p*-Akt, were degraded in a concentration-dependent manner, while Actin levels remained constant. Hsp90 levels also remained unchanged, a characteristic feature shared by Hsp90 C-terminal inhibitors.[22,24−26]

In conclusion, the biphenyl moiety was identified as a suitable replacement for the coumarin ring system of novobiocin. Molecular modeling and Western blot analyses suggest these compounds manifest antiproliferative activity through Hsp90 inhibition. A library of small molecules containing the biphenyl moiety was designed, synthesized, and evaluated against two breast cancer cell lines. Initial structure−activity relationships for the amide appendage were investigated, and compound **11j** was shown to exhibit lead-like activity as demonstrated by Western blot analyses. The discovery of biphenyl as a coumarin surrogate not only simplifies the synthesis, but also allows rapid access to modifications that should enable succinct assembly of structure−activity relationships. Investigations are currently underway to further develop this scaffold and to unveil previously unobtainable structure−activity relationships, which will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures for the synthesis and characterization of new compounds (^{1}H and ^{13}C NMR, HRMS). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(B.S.J.B.) 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.

■ REFERENCES

(1) Taipale, M.; Jarosz, D. F.; Lindquist, S. Hsp90 at the hub of protein homeostasis: Emerging mechanistic insights. *Nat. Rev. Mol. Cell Biol.* **2010**, *11*, 515−528.

(2) Hanahan, D.; Weinberg, R. A. Hallmarks of cancer: The next generation. *Cell* **2011**, *144*, 646−674.

(3) Blagg, B. S. J.; Kerr, T. D. Hsp90 inhibitors: Small molecules that transform the Hsp90 protein folding machinery into a catalyst for protein degradation. *Med. Res. Rev.* **2006**, *26*, 310−338.

(4) Neckers, L.; Workman, P. Hsp90 molecular chaperone inhibitors: Are we there yet? *Clin. Cancer Res.* **2012**, *1*, 64−76.

(5) Whitesell, L.; Santagata, S.; Lin, N. U. Inhibiting Hsp90 to treat cancer: A strategy in evolution. *Curr. Mol. Med.* **2012**, *12*, 1108−1124.

(6) Marcu, M. G.; Schulte, T. W.; Neckers, L. M. Novobiocin and related coumarins and depletion of heat shock protein 90-dependent signaling proteins. *J. Natl. Cancer Inst.* **2000**, *92*, 242−248.

(7) Eskew, J. D.; Sadikot, T.; Morales, P.; Duren, A.; Dunwiddie, I.; Swink, M.; Zhang, X. Y.; Hembruff, S.; Donnelly, A.; Rajewski, R. A.; Blagg, B. S. J.; Manjarrez, J. R.; Matts, R. L.; Holzbeierlein, J. M.; Vielhauer, G. A. Development and characterization of a novel C-terminal inhibitor of Hsp90 in androgen dependent and independent prostate cancer cells. *BMC Cancer* **2011**, *11*, 468.

(8) Shelton, S. N.; Shawgo, M. E.; Comer, S. B.; Lu, Y.; Donnelly, A. C.; Szabla, K.; Tanol, M.; Vielhauer, G. A.; Rajewski, R. A.; Matts, R. L.; Blagg, B. S.; Robertson, J. D. KU135, a novel novobiocin-derived C-terminal inhibitor of Hsp90, exerts potent antiproliferative effects in human leukemic cells. *Mol. Pharmacol.* **2009**, *76*, 1314−1322.

(9) Conde, R.; Belak, Z. R.; Nair, M.; O'Carroll, R. F.; Ovsenek, N. Modulation of Hsf1 activity by novobiocin and geldanamycin. *Biochem. Cell Biol.* **2009**, *87*, 845−851.
(10) Zhao, H. P.; Donnelly, A. C.; Kusuma, B. R.; Brandt, G. E. L.; Brown, D.; Rajewski, R. A.; Vielhauer, G.; Holzbeierlein, J.; Cohen, M. S.; Blagg, B. S. J. Engineering an antibiotic to fight cancer: Optimization of the novobiocin scaffold to produce anti-proliferative agents. *J. Med. Chem.* **2011**, *54*, 3839−3853.
(11) Burlison, J. A.; Neckers, L.; Smith, A. B.; Maxwell, A.; Blagg, B. S. J. Novobiocin: Redesigning a DNA gyrase inhibitor for selective inhibition of Hsp90. *J. Am. Chem. Soc.* **2006**, *128*, 15529−15536.
(12) Zhao, H. P.; Kusuma, B. R.; Blagg, B. S. J. Synthesis and evaluation of noviose replacements on novobiocin that manifest antiproliferative activity. *ACS Med. Chem. Lett.* **2010**, *1*, 311−315.
(13) Donnelly, A. C.; Zhao, H. P.; Kusuma, B. R.; Blagg, B. S. J. Cytotoxic sugar analogues of an optimized novobiocin scaffold. *Med. Chem. Commun.* **2010**, *1*, 165−170.
(14) Le Bras, G.; Radanyi, C.; Peyrat, J. F.; Brion, J. D.; Alami, M.; Marsaud, V.; Stella, B.; Renoir, J. M. New novobiocin analogues as antiproliferative agents in breast cancer cells and potential inhibitors of heat shock protein 90. *J. Med. Chem.* **2007**, *50*, 6189.
(15) Audisio, D.; Messaoudi, S.; Cegielkowski, L.; Peyrat, J. F.; Brion, J. D.; Methy-Gonnot, D.; Radanyi, C.; Renoir, J. M.; Alami, M. Discovery and biological activity of 6BrCaQ as an inhibitor of the Hsp90 protein folding machinery. *ChemMedChem* **2011**, *6*, 804.
(16) Donnelly, A. C.; Mays, J. R.; Burlison, J. A.; Nelson, J. T.; Vielhauer, G.; Holzbeierlein, J.; Blagg, B. S. J. The design, synthesis, and evaluation of coumarin ring derivatives of the novobiocin scaffold that exhibit antiproliferative activity. *J. Org. Chem.* **2008**, *73*, 8901−8920.
(17) Welsch, M. E.; Snyder, S. A.; Stockwell, B. R. Privileged scaffolds for library design and drug discovery. *Curr. Opin. Chem. Biol.* **2010**, *14*, 1−15.
(18) Horton, D. A.; Bourne, G. T.; Smythe, M. L. The combinatorial synthesis of bicyclic privileged structures or privileged substructures. *Chem. Rev.* **2003**, *103*, 893−930.
(19) Turrado, C.; Puig, T.; Garcia-Carceles, J.; Artola, M.; Benhamu, B.; Ortega-Gutierrez, S.; Relat, J.; Oliveras, G.; Blancafort, A.; Haro, D.; Marrero, P. F.; Colomer, R.; Lopez-Rodriguez, M. L. New synthetic inhibitors of fatty acid synthase with anticancer activity. *J. Med. Chem.* **2012**, *55*, 5013−5023.
(20) Matts, R. L.; Dixit, A.; Peterson, L. B.; Sun, L.; Voruganti, S.; Kalyanaraman, P.; Hartson, S. D.; Verkhivker, G. M.; Blagg, B. S. J. Elucidation of the Hsp90 C-terminal inhibitor binding site. *ACS Chem. Biol.* **2011**, *6*, 800−807.
(21) Morra, G.; Neves, M. A. C.; Plescia, C. J.; Tsustsumi, S.; Neckers, L.; Verkhivker, G.; Altieri, D. C.; Colombo, G. Dynamics-based discovery of allosteric inhibitors: Selection of new ligands for the C-terminal domain of Hsp90. *J. Chem. Theory Comput.* **2010**, *6*, 2978−2989.
(22) Zhao, H. P.; Moroni, E.; Yan, B.; Colombo, G.; Blagg, B. S. J. 3D-QSAR assisted design, synthesis and evaluation of novobiocin analogue. *ACS Med. Chem. Lett.* **2013**, *4*, 57−62.
(23) Burlison, J. A.; Avila, C.; Vielhauer, G.; Lubbers, D. J.; Holzbeierlein, J.; Blagg, B. S. J. Development of novobiocin analogues that manifest anti-proliferative activity against several cancer cell lines. *J. Org. Chem.* **2008**, *73*, 2130.
(24) Zhao, H. P.; Brandt, G. E.; Galam, L.; Matts, R. L.; Blagg, B. S. J. Identification and initial SAR of silybin: An Hsp90 inhibitor. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2659−2664.
(25) Zhao, H. P.; Yan, B.; Peterson, L. B.; Blagg, B. S. J. 3-Arylcoumarin derivatives manifest anti-proliferative activity through Hsp90 inhibition. *ACS Med. Chem. Lett.* **2012**, *3*, 327−331.
(26) Tran, P.; Kim, S. A.; Choi, H. S.; Yoon, J. H.; Ahn, S. G. Epigallocatechin-3-gallate suppresses the expression of HSP70 and HSP90 and exhibits anti-tumor activity in vitro and in vivo. *BMC Cancer* **2010**, *10*, 276.