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Modified Biphenyl Hsp90 C-terminal Inhibitors for the Treatment of Cancer

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Abstract

Heat shock protein 90 (Hsp90) is a chaperone under investigation for the treatment of neurodegenerative diseases and cancer. Neuroprotective Hsp90 C-terminal inhibitors (novologues) contain a biaryl ring system, and include KU-596, which was modified and investigated for potential anti-cancer activity. Incorporation of a benzamide group on the biaryl novologues in lieu of the acetamide yields compounds that manifest anti-cancer activity against breast cancer cell lines. Further exploration on the central phenyl ring led to compounds with enhanced anti-proliferation activity. The design, synthesis, and evaluation of these new analogs against breast and prostate cancer cell lines is reported herein, where it was found that **8b** and **10** manifest potent anti-proliferative activity and a robust degradation of Hsp90 client dependent proteins.

Graphical Abstract

Keywords

Breast Cancer; Prostate Cancer; Heat Shock Protein 90; Hsp90 inhibitors; Novobiocin Analogs

Heat Shock Protein 90 (Hsp90) is a molecular chaperone that is responsible for the maturation of more than 300 nascent polypeptides.^{1–5} Hsp90-dependent client proteins are associated with all 10 hallmarks of cancer, making it an excellent therapeutic target for the treatment of cancer.^{6–8} As a result, 17 Hsp90 inhibitors have been investigated in clinical trials for the treatment of cancer.^{9–13} Hsp90 exists as a homodimer and contains a C-terminus, a middle domain, and an N-terminal ATP-binding site.³ In stressed cells, Hsp90 exists as a heteroprotein complex bound to various co-chaperones and partner proteins, and Hsp90 inhibitors exhibit 200-fold higher affinity for the heteroprotein complex versus the

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homodimer that resides in non-stressed cells.^{14–20} Inhibition of Hsp90 by traditional inhibitors that bind the N-terminal ATP-binding site results in induction of the heat shock response (HSR) and the overexpression of Hsp27, Hsp40, Hsp70, Hsp90.^{21–23} Since N-terminal inhibitors induce this HSR, challenges associated with dosing and scheduling have arisen in the clinic.^{9, 13, 24, 25} As a result, new inhibitors that do not induce the heat shock response are actively sought.^{26, 27}

The natural product, novobiocin, was the first Hsp90 C-terminal inhibitor identified,^{28, 29} and preliminary SAR studies resulted in the identification of KU-32, a C-terminal inhibitor that contains an acetamide in lieu of the benzamide side chain and the acetamide on noviose sugar.^{23, 30} Interestingly, KU-32 induces the HSR, but at significantly lower concentrations than that needed to induce the degradation of client proteins.^{31, 32} Since KU-32 was able to induce the pro-survival HSR, it was investigated for the treatment of neurodegenerative diseases. Studies in mice demonstrated that KU-32 was able to protect against neuronal glucotoxicity and reverse the clinical endpoints of diabetic peripheral neuropathy.³³ A second generation of noviosylated analogs contained a biphenyl scaffold in lieu of the coumarin ring, and allowed the preparation of additional analogs. KU-596, the lead compound resulting from this latter generation, was found to exhibit comparable biological activity to KU-32.^{34, 35} Since the biphenyl scaffold resulted in improved neuroprotection, compounds containing this new core were investigated for the treatment of cancer. An initial study found that the biphenyl scaffold in KU-596 could be modified to append a biaryl side chain in lieu of the acetamide as well as the replacement of the noviose sugar with N-methyl piperidine to produce compounds that manifested anti-cancer activity without induction of the HSR.³⁵ Since modifications on the central biaryl ring have not been investigated, analogs were prepared to determine whether such modifications could improve the anti-cancer activity of this class of compounds, Figure 1.

Molecular modeling studies were performed with a homology model of the C-terminal binding site to identify potential interactions with the biaryl core. Two hydrogen-bonding interactions appeared attainable by inclusion of a phenol onto the central biphenyl ring as suggested in Figure 2. Molecular modeling studies suggested that compounds containing substituents pointing toward amino acids Glu537 and Thr540 could also form hydrogen-bonding interactions, and consequently may be more efficacious. In silico studies suggested that the attachment of a methyl group onto the biphenyl core could produce hydrophobic interactions within the binding site and increase affinity. These interactions might be similar to previously studied Hsp90 C-terminal inhibitors that contain a coumarin core. Additionally, incorporation of a methyl group may reinstate interactions lost upon transitioning from the coumarin core to the biaryl ring system.^{36–38} Consequently, analogs were sought to include such modifications.

Derivatives of the central ring were selected for initial investigation, as illustrated in Scheme 1A. Synthesis of each derivative began via a different core. Synthesis of the derivative containing a methyl substituent (**19**) began by reduction of 2,4-dihydroxybenzaldehyde, **12**, with sodium cyanoborohydride in acidic tetrahydrofuran to yield **13** (Scheme 1B).⁴⁰ Silver triflate and dichloro-methyl methyl ether were then used to formylate **13**, which produced aldehyde **14**.⁴¹ The resulting methylbenzaldehyde, **14**, was then benzylated to give **15**,

which was then transformed into the trifluoromethanesulfonate derivative under microwave conditions.⁴² Subsequent Suzuki coupling of **16** with 3-fluorophenyl boronic acid generated the biaryl core of **17**. Henry olefination conditions were used to homologate the aldehyde into the α,β -unsaturated nitro compound **18**, which was subsequently reduced to provide amine **19**.

The remaining analogs required alternative routes for their preparation, however installation of the triflate and subsequent steps to yield the free amine were identical to the conditions described for **19**, as outlined in Scheme 1B. Synthesis of the methoxy and phenol derivatives began by formylation of phloroglucinol, followed by selective benzyl ether formation to give **20** (Scheme 2A and B). Aldehyde **20** was then treated with dimethyl sulfide in water and acetone to yield **21** (Scheme 2A).⁴³ Intermediate **21** was subjected to conditions previously outlined in Scheme 1B to produce amine **22**. In order to obtain the phenol, derivative **20** was treated with chloromethyl methyl ether to produce **23** (Scheme 2B),^{44, 45} which was then subjected to conditions described in Scheme 1B to attain amine **24**. Synthesis of amine **27** began by selective benzylation of 2,3,4-trihydroxybenzaldehyde, which was subsequently reacted with chloromethyl methyl ether and diisopropylamine to yield aldehyde **26** (Scheme 2C). Compound **26** was then subjected to the conditions described in scheme 1B to afford amine **27**. Amine **30**, was attained via selective benzylation of 2,4-dihydroxy-3-methyl benzaldehyde to yield ether **29** (Scheme 2D), which was subjected to previously described methods to produce amine **30**. Final derivative **33** was synthesized by subjecting 2-nitrobenzene-1,3-diphenol to Duff reaction conditions to yield **31** (Scheme 2E). Aldehyde **31** then underwent selective benzylation to produce the key intermediate, **32**, which was subsequently modified to give free amine **33**.

Two different amide side chains were investigated with these analogs, the original prenylated side chain present in novobiocin and the optimized biaryl side chain found in **4**.

Additionally, compounds were made to contain either the noviose sugar or the noviose surrogate, N-methyl piperidine.⁴⁶ Installation of the biaryl side chain began by an acid chloride coupling of previously synthesized **34**³⁶ with the free amine of each central ring derivative (Scheme 3A). Once the coupled product was obtained (**35–40**), hydrogenolysis was employed to produce the free phenols, **41–46**. The free phenol was then coupled with the activated noviose sugar (**47**),⁴⁷ using Schmidt conditions.⁴⁸ The carbonate was then removed under solvolysis conditions to procure the corresponding noviose products, **6–10**.⁴⁹ Simultaneously, free phenols **41–46** were subjected to Mitsunobu conditions with 1-methylpiperidin-4-ol (**48**) to yield the amine-containing products, **6a–11a** (Scheme 3A).

Analogues that contained the prenylated side chain were synthesized by an acid chloride coupling reaction between **51**³⁷ and the free amine of each analogue (Scheme 3B). Coupled products **52–55** were subjected to Pd(OAc)₂, Et₃SiH, Et₃N, to give the silyl protected phenols. Upon cleavage of the silyl ether with TBAF at 0°C, **56–59** were obtained in good yields (Scheme 3B). The resulting phenols, **56–59**, were then subjected to a Mitsunobu reaction with **48**, to give the amino products, **6b–9b**, respectively.

Upon preparation of these new analogs, an MTS⁵⁰ anti-proliferation assay was conducted against two breast cancer cell lines, MCF-7 (estrogen receptor positive cells) and SkBr3

(estrogen receptor negative HER2 overexpressing), and two prostate cancer cell lines, LNCap-LN3 (androgen receptor sensitive prostate cancer cells) and PC3-MM2 (androgen receptor insensitive prostate cancer cells). These cell lines were selected because the oncogenic drivers of these cancers are Hsp90-dependent client proteins: estrogen receptor, HER-2, and the androgen receptor.⁵⁰ All new analogs exhibited moderate anti-proliferative activity against the 4 cancer cell lines as detailed in Table 1. Compounds containing an N-methyl piperidine exhibited greater inhibitory activity than those containing noviose. Additionally, compounds with a prenylated amide side chain were found to be more efficacious than compounds that contain the biaryl amide side chain.

Interestingly, the synthesized analogs followed the same trend in activity independent of the sugar surrogate or amide side chain. The methyl derivatives were more active than the parent compound; the methoxy derivatives were more active than the methyl derivatives, and the phenol derivatives more active than both the methoxy and the methyl derivatives. Analogues that contained the sugar surrogate, N-methyl piperidine, were more active than the analogs containing the noviose sugar. Additionally, analogs that contained the prenylated side chain were more active than analogs containing the biaryl side chain. Analogue **6** was similarly active to the parent compound, **5**. However analogs **6a** and **6b**, which contained N-methyl piperidine in lieu of noviose, were more active than **6**. In addition, analogs containing moieties that could participate in hydrogen bonding interactions manifested improved inhibitory activity as compared to **5**.

After determination of IC₅₀ values, compounds **8a**, **9**, and **10** were further evaluated by western blot analysis of MCF7 cell lysates. Compound **10** exhibited significant Hsp90-dependent client protein degradation at concentrations that mirrored its anti-proliferative activity, clearly linking Hsp90 inhibition to cell viability, as illustrated in Figure 3. In fact, Hsp90 dependent clients Her-2, Cyclin D1, and c-Raf were degraded upon treatment with **10** for 24 hours. **8b** and **9** also demonstrated client protein degradation, but to a lesser extent than **10**. Treatment with analog **8b** resulted in HER2 degradation at the lowest concentrations, which may be due to mechanisms of cellular resistance.^{51, 52} Importantly, no increase in Hsp90 levels was observed with these C-terminal inhibitors, which is an important attribute manifested by C-terminal inhibitors that exhibit anti-cancer activity.

In summary, a series of Hsp90 C-terminal inhibitors with modifications to the central ring of the biphenyl scaffold were designed, synthesized, and biologically evaluated. Modifications to the central ring produced compounds that exhibited increased biological activity. Structure-activity relationship studies revealed that modifications that include a hydrogen bond donor was most beneficial. Finally, western blot analysis confirmed these compounds to manifest their anti-proliferative activity via Hsp90 inhibition, as client protein degradation was observed at concentrations that mirrored anti-proliferative activity.

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50. Cells were maintained in Advanced DMEM/F12 (Gibco) media and grown in a humidified atmosphere (37° C, 5% CO₂). The cells were grown to confluence, seeded (3000cells/well, 100 μL) in a 96-well plate, and placed in the incubator for 24 hours. Compound or vehicle at varying concentrations in DMSO (1% DMSO final concentration) were administered and then incubated for 72 hours. At 72 h, the % of viable cells was determined using the MTS/PMS cell proliferation kit (Promega) per the manufacturer's instructions. Cells incubated with vehicle were normalized to 100% proliferation, and values were adjusted accordingly.
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Figure 1.
Rationale for the development of new analogs.

Figure 2.
Molecular modeling of compounds **9** and **10** docked into the Hsp90 C-terminal homology model.³⁹

Figure 3.

Western Blot analysis from MCF-7 cells. The high concentration is five times the IC_{50} value and the low concentration is half the IC_{50} value. Concentrations for each analog tested were as follows: **8b** (high: 29.45 μ M, low: 2.945 μ M), **9** (high: 68 μ M, low: 6.8 μ M), and **10** (high: 44.95 μ M, low: 4.49 μ M). The positive control was GDA (500nM) and DMSO was used as the negative control.

Scheme 1.

A. Proposed analogs with substitutions off of the central ring. B. Synthesis of free amine, **19**.

a). NaBH_3CN , HCl , THF, 90%; b). AgOTf , Cl_2CHOMe , DCM, 40%; c). NaHCO_3 , BnBr , MeCN, 70%; d). N-PhTf_2 , K_2CO_3 , THF, 120 °C, microwave, 95%; e). $\text{FC}_6\text{H}_4\text{B(OH)}_2$, Pd(dppf)Cl_2 , K_2CO_3 , DMF, 75%; f). NH_4OAc , CH_3NO_2 , 90%; g). LAH, THF, 60%.

Scheme 2.

Synthesis of free amines **22**, **24**, **27**, **30**, and **33**. Conditions: a). Me_2SO_4 , K_2CO_3 , H_2O /Acetone (1/1), 55%; b). MOMCl, DIPA, DCM, 65%; c). NaHCO₃, BnBr, MeCN, 45%; d). MOMCl, DIPEA, DCM, 70%; e). NaHCO₃, BnBr, KI, MeCN, 75%.

Scheme 3.

A. Synthesis of final products **6–10** and **6a–11a**. B. Synthesis of final products **6b–9b**.

Conditions: a). DMAP, Pyridine, DCM, 60%; b). H₂ (g), Pd/C, EtOAc, 70%; c). BF₃ OEt, DCM, 40%; d). Et₃N, MeOH, 85%; e). Ph₃P, DIAD, THF, 30%; f). DMAP, Et₃N, DCM, 35%; g). Pd(OAc)₂, Et₃Si, Et₃N, DCM, 75%; h). TBAF, THF, 0 °C, 15 min, 60%; i). Ph₃P, DIAD, THF, 30%; j). K₂CO₃, MeOH, 60%.

Table 1

Anti-proliferative values of new analogs

Compound	X	Y	Z	R	R ¹	MCF7 ^a (μM)	SKBr3 ^a (μM)	LNCap-LN3 (μM)	PC3-MM2 (μM)
3	H	H	H	A	-	>500	>500	>500	>500
5	H	H	H	A	A	>50	>50	15.9	>50
5a	H	H	H	A	B	15.4±1.75	23.7±0.04	22.5	5.49
6	CH ₃	H	H	A	A	>50	43.9±4.4	>100	>100
6a	CH ₃	H	H	A	B	9.14±2.72	7.81±1.1	20.8	13.9
6b	CH ₃	H	H	B	B	35.6±3.03	15.2±1.67	>100	>100
7	H	OCH ₃	H	A	A	44.7±4.15	49.4±2.03	8.09	10.6
7a	H	OCH ₃	H	A	B	8.71±1.20	5.72±0.89	>100	32.1
7b	H	OCH ₃	H	B	B	7.86±0.20	8.16±0.29	>100	1.84
8	H	OH	H	A	A	23.8±0.47	23.5±0.44	>50	>50
8a	H	OH	H	A	B	12.2±0.89	15.7±1.5	44.8	>50
8b	H	OH	H	B	B	5.89±0.67	7.59±0.58	22.8	1.32
9	H	H	OH	A	A	13.6±2.01	16.7±0.93	>100	9.37
9a	H	H	OH	A	B	11.3±1.62	9.29±1.07	22.4	8.61
9b	H	H	OH	B	B	1.45±0.87	3.25±1.2	23.1	42.3
10	H	H	CH ₃	A	A	8.99±0.28	43.5±1.03	25.1	>50
10a	H	H	CH ₃	A	B	17.8±1.48	21.3±2.82	>50	>50
11a	H	H	NH ₂	A	B	11.3±1.3	15.3±2.3	22.4	18.9

^aValues represent mean ± standard deviation for at least three separate experiments performed in triplicate.