Journal Pre-proofs

Synthesis of paramagnetic ligands that target the C-terminal binding site of Hsp90

Vishal C. Birar, Ioannis Gelis, Ang Zuo, Brian S.J. Blagg

PII: S0960-894X(20)30414-5
DOI: https://doi.org/10.1016/j.bmcl.2020.127303
Reference: BMCL 127303

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 9 April 2020
Revised Date: 28 May 2020
Accepted Date: 31 May 2020

Please cite this article as: Birar, V.C., Gelis, I., Zuo, A., Blagg, B.S.J., Synthesis of paramagnetic ligands that target the C-terminal binding site of Hsp90, *Bioorganic & Medicinal Chemistry Letters* (2020), doi: https://doi.org/10.1016/j.bmcl.2020.127303

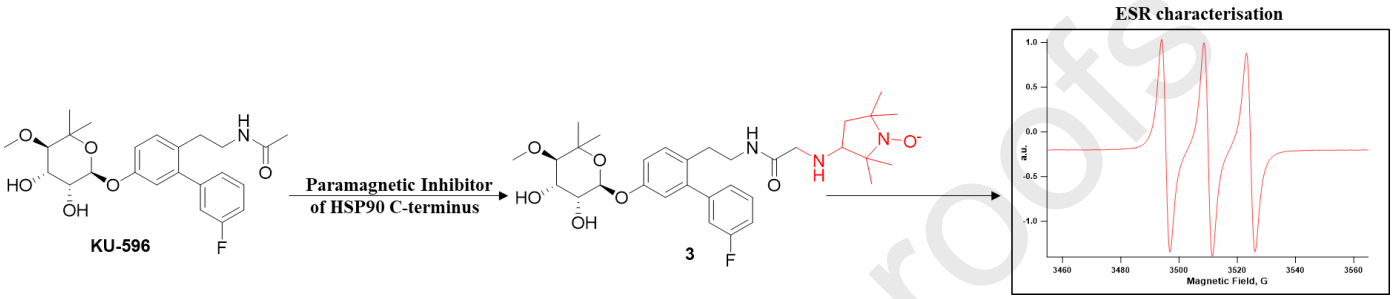
Graphical Abstract

To create your abstract, type over the instructions in the template box below.

Synthesis of Paramagnetic ligands that target the C-terminal binding site of HSP-90

Vishal C Birar, Ioannis Gelis, Ang Zuo and Brian SJ Blagg.

Leave this area blank for abstract info.



Fonts or abstract dimensions should not be changed or altered.

ELSEVIER

Bioorganic & Medicinal Chemistry Letters
journal homepage: www.elsevier.com

Synthesis of paramagnetic ligands that target the C-terminal binding site of Hsp90

Vishal C. Birar[a], Ioannis Gelis[b], Ang Zuo[a] and Brian S. J. Blagg[*a]

[a] Department of chemistry and biochemistry, University of Notre Dame, Indiana, USA
[b] Department of Chemistry, University of South Florida, Tampa, FL 33620

ARTICLE INFO

Article history:
Received
Revised
Accepted
Available online

Keywords:
Paramagnetic ligand
HSP90
C-terminal
KU-596
ESR

ABSTRACT

Identification of the ligand binding site represents the starting point for a structure-based drug development program. Lack of a binding site hampers the development of improved ligands that modulate the protein of interest. In this letter, we describe the development of chemical tools that will allow for elucidation of the Hsp90 C-terminal ligand binding site. Our strategy is based on the preparation of paramagnetic analogs of KU-596, an investigational new drug that is currently undergoing clinical trials for the treatment of neuropathy and interacts with the Hsp90 C-terminal domain. In particular, we report the design and synthesis of three novel paramagnetic analogs of KU-596, which will be used to obtain long range distances for NMR structural studies of Hsp90 in complex with C-terminal ligands.

Heat shock protein 90 (Hsp90) is an ATP-dependent molecular chaperone that modulates cell signalling processes through the folding of nascent polypeptides and the rematuration of denatured proteins[1]. Hsp90 is responsible for the activation and maturation of more than 300 client protein substrates, many of which are directly linked to the ten hallmarks of cancer[2]. In fact, Hsp90 inhibition results in the simultaneous degradation of client proteins that serve as oncogenic signals, and as a result, Hsp90 is considered a promising target for the development of new anti-cancer agents[3].

On a molecular level, Hsp90 is a homodimer that consists of three domains; the N-terminal domain (N-Hsp90), the middle domain (M-Hsp90) and the C-terminus (C-Hsp90). Each region is well characterized, and their role elucidated. For example, the N-terminal domain binds and hydrolyzes ATP to afford the energy necessary for the protein folding process, whereas the middle region interacts with client and partner proteins, and the C-terminal domain is responsible for formation of the Hsp90 homodimer[4]. Studies to inhibit Hsp90 function have identified numerous small molecules, including the natural products geldanamycin and radicicol, which have led to the generation of clinically evaluated inhibitors[5]. Mechanistically, all of these ligands inhibit Hsp90 by bindin

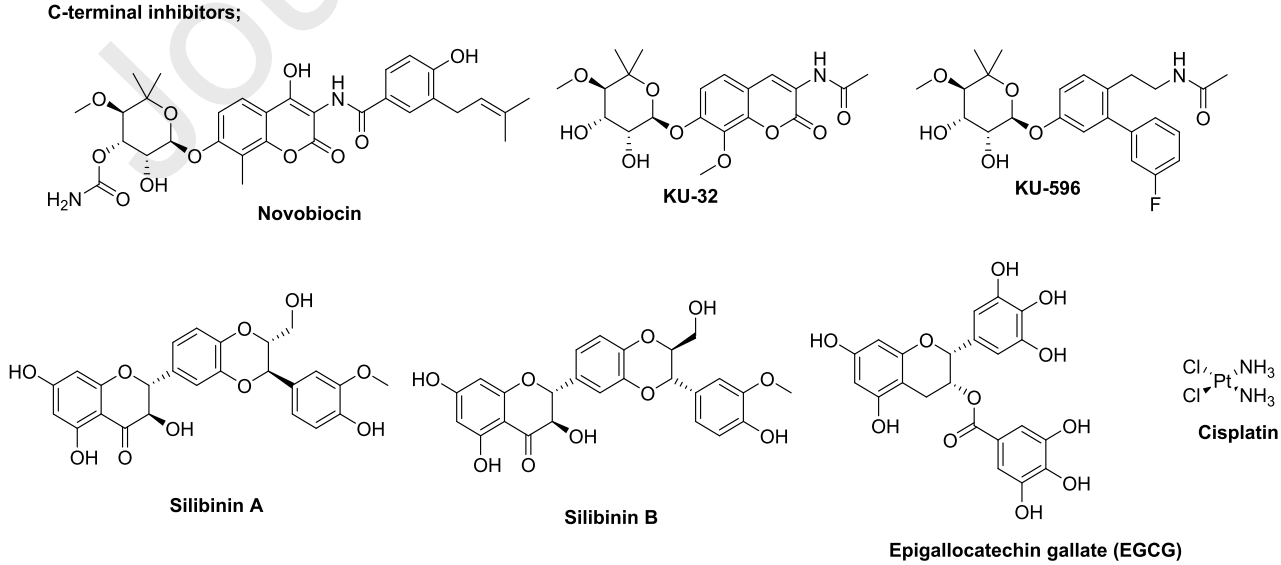


Figure 1. Hsp90 C-terminal inhibitors

ATP[5]. As a result, the protein folding cycle is disrupted and consequently, the clients are degraded via the ubiquitin-proteasome pathway[5].

Hsp90 contains a nucleotide binding site within its N- and C-terminal domains. Hsp90 inhibitors that bind and displace ATP from the N-terminus have been evaluated in clinical trials for the treatment of cancer[5]. However, these N-terminal inhibitors also induce the pro-survival heat shock response[7], resulting in increased levels of Hsp90 that require an escalation in dose and frequency and ultimately, pushes the patient towards the maximum tolerated dose.

In 2000, Neckers and co-workers reported that novobiocin binds the C-terminus to inhibit Hsp90 function in SKBr3 cells with an EC50 of approximately 700 μM.[8,9] Instead of binding to the N-terminal ATPase domain, novobiocin was found to bind a previously unrecognized C-terminal domain, which also disrupted cancer cell growth similar to N-terminal inhibitors[10,11]. However, subsequent studies revealed that novobiocin does not induce the HSR, and thus represents an alternative to Hsp90 N-terminal inhibition. The C-terminal binding pocket to which novobiocin binds was localized to amino acids 542-738, and subsequent experiments revealed amino acids 657-677 to be essential, as removal of these residues significantly diminished affinity for novobiocin.[8,9] Furthermore, these residues are also required for Hsp90 dimerization and co-chaperone binding.[12,13] As a result, derivatives of novobiocin have been pursued to increase efficacy, establish SAR, and to elucidate the location of the C-terminal binding pocket[14,15]. In addition to novobiocin and novobiocin analogs, epilgallocatechi-3-gallate (EGCG), silybin, and cisplatin were also found to bind the Hsp90 C-terminus and to inhibit chaperone function in cells.[16-20] Although several C-terminal inhibitory scaffolds have been reported in the literature, the exact location to which these ligands bind Hsp90 has not been elucidated. Consequently, clarity regarding the location and the mode by which these ligands bind Hsp90 remains unknown and significantly hinders the development of improved analogs.

Studies have reported various binding modes for Hsp90 C-terminal inhibitors, but none are consistent with all of the acquired biochemical data[8-9,14]. Prior studies utilized computational approaches, biochemical analyses, and/or affinity labelling to identify putative binding sites. For example, mutational analysis suggested that the novobiocin binding site is contained within amino acids 538-728.[8] Moreover, a related severely compromised novobiocin binding, and a synthetic peptide that mimicked amino acids 663-676 was found to compete for novobiocin binding to Hsp90[8,14]. Other studies utilized photoaffinity analogs of novobicin that interacted with the Hsp90 C-terminal domain[21], whereas another approach by Sogba and co-workers[22] used homology modelling and molecular dynamics simulation to identify cavities on the surface that could bind a nucleotide or novobiocin. Ultimately, the data suggests that ATP interacts with amino acids 609-632, 676-681, and/or 497-501 and supports the existence of three potential binding sites within the Hsp90 C-terminus[22].

More recently, saturation transfer difference (STD) NMR spectroscopy was used to probe for molecular insights into the mode by which **KU-32** and **KU-596** bind Hsp90[23]. These researchers reported the primary binding epitope for both ligands is localized to the central core (coumarin or biphenyl), and suggested specific locations on **KU-596** that can be modified to increase interactions with Hsp90. In addition, methyl-TROSY NMR data were obtained and provided insight into the mechanism by which these ligands bind and modulate Hsp90 function[23]. Similar to other C-terminal inhibitors, **KU-32** and **KU-596** were found to elicit long range structural rearrangements upon binding Hsp90 that are propagated to the N-terminus[23,24].

In this communication, the design and synthesis of **KU-596** paramagnetic ligands are reported, which can be used to identify the exact location to which these ligands bind Hsp90. In particular, carboxy TEMPO (2,2,6,6-tetramethylpiperidine1-oxyl) and tetramethylpyrroline-1-oxyl appendages were chosen to be incorporated into the **KU-596** scaffold, which is undergoing clinical evaluation for neuropathy.

Studies with **KU-596** using STD experiments provided evidence that the noviose sugar exhibits the lowest STD effect and thus provides minimal interaction with Hsp90, highlighting an opportunity to install additional moieties. Therefore, we designed a paramagnetic ligand at this location in an effort to reveal nearby residues upon binding to Hsp90. Paramagnetic ligands such as 2,2,6,6-tetramethyl-piperidine-1-oxyl (TEMPO) and 2,2,5,5-tetramethylpyrroline-1-oxyl are widely used in structural studies by NMR. The presence of the paramagnetic center on a small ligand or other protein partner causes a dramatic increase in the *R2* relaxation rates of protons in a radius of 28 Å. In turn, this is manifested as a significant signal attenuation, which can be very accurately converted into a large set of long-range distance restraints for structure calculation protocols[25]. Therefore, we sought to incorporate

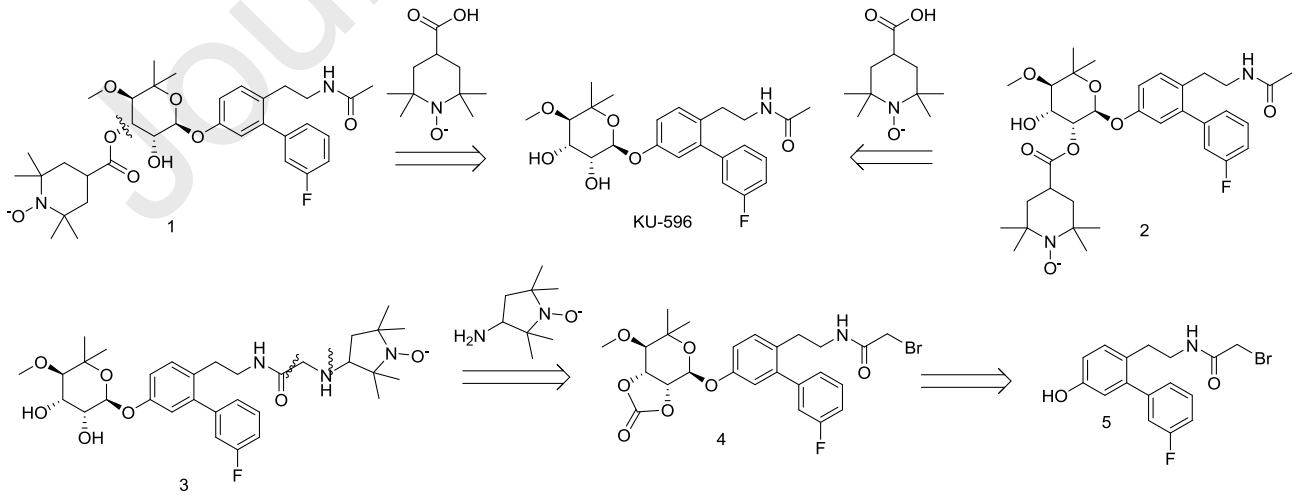
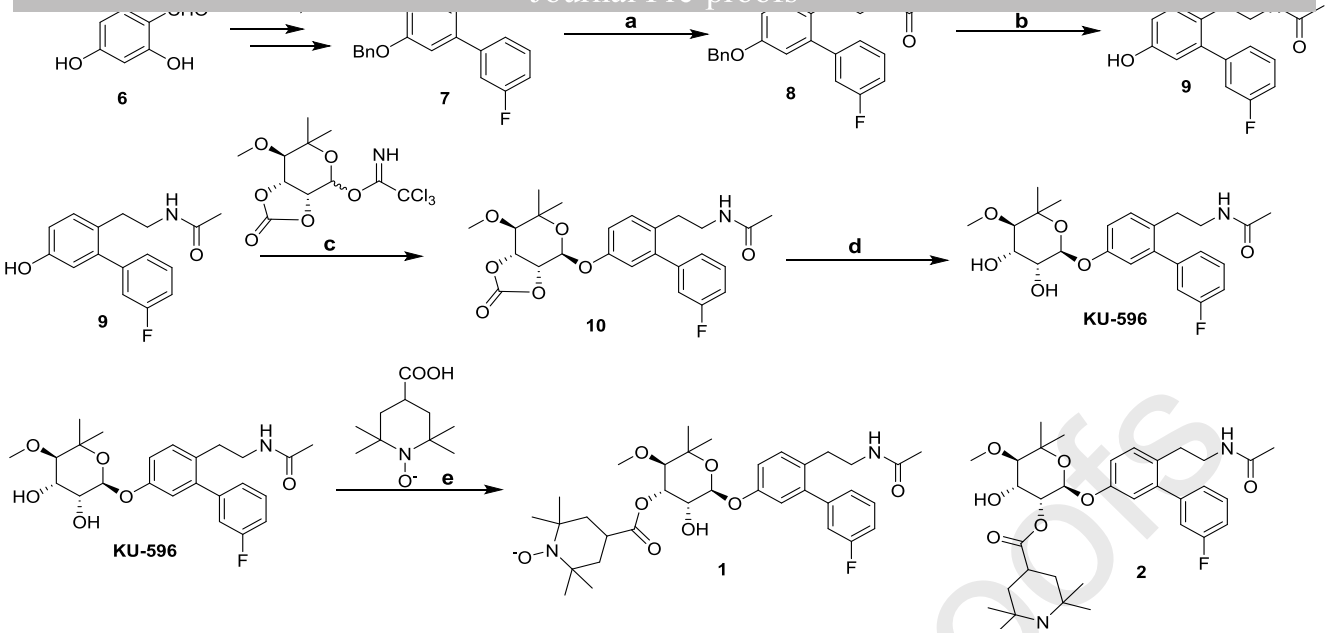


Figure 2. Retrosynthetic analyses of KU-596 paramagnetic ligands.



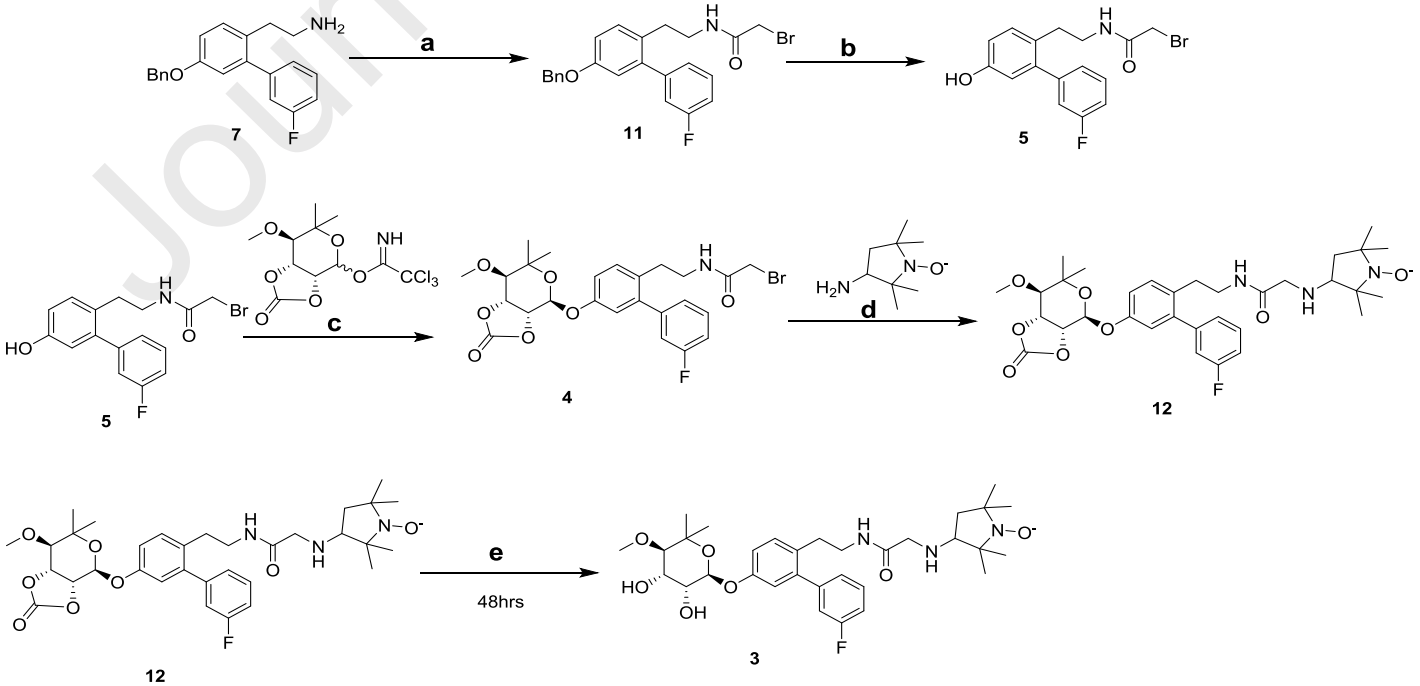
Scheme 1. Reagents and conditions: a) Acetyl chloride, Et_3N, 80%; (b) H_2, Pd/C, MeOH, 72%; (c) $BF_3.OEt_2$ solution, CH_2Cl_2, rt; (d) Et_3N, MeOH, rt, overnight, 45% e) DCC, DMAP, CH_2Cl_2, rt.

rporate paramagnetic handles onto the 2'- and 3'-hydroxyls of noviose as well as onto the amide side chain of **KU-596**. Retrosynthetic analysis of the molecules (**1** and **2, Figure 2**) suggested that they could be prepared from KU-596, following modification of the reported synthetic procedure[26]. In regards to analog **3**, the N-acetyl bromide intermediate **4** was envisioned as a key intermediate that could undergo nucleophilic displacement to give the desired analog, **3**.

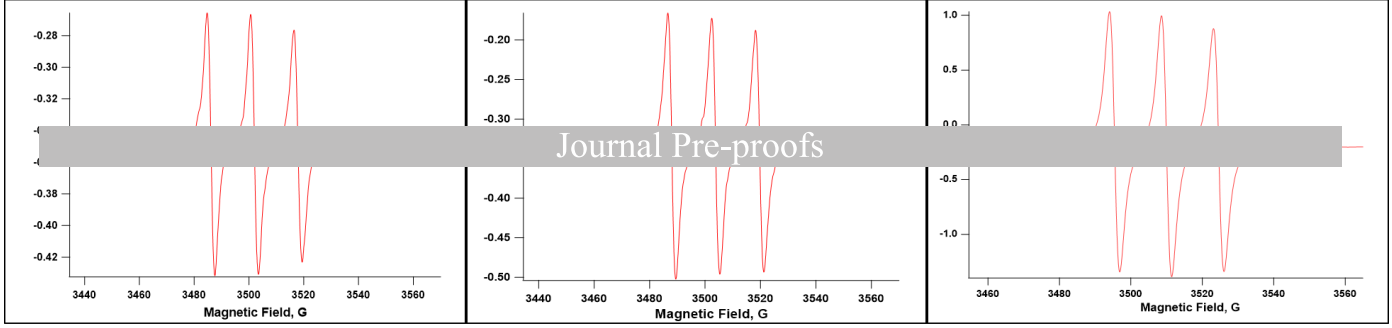
Preparation of the paramagnetic ligands is described in **Schemes 1** and **2**. As mentioned previously, analogs **1** and **2** were synthesized from **KU-596** [26]. The KU-596 diol was coupled with an equimolar amount of 4-carboxy-2,2,6,6-tetramethylpiperidine 1-oxyl (4-carboxy TEMPO) using N,N′-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) to give a 3:1 mixture of regioisomers that upon careful analysis was found to represent the corresponding 3'- and 2'-esters, respectively [26,27].

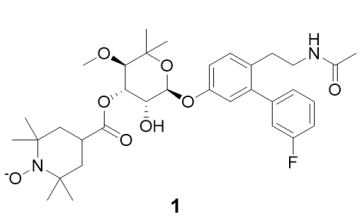
Paramagnetic ligand **3** was prepared from biphenyl ethyl amine **7**, the latter of which was synthesized following our previously reported method.[26] Acetylation of biphenylamine **7** with bromoacetyl bromide gave the α-bromo intermediate **11**, which underwent debenzylation to give the corresponding phenol **5** after treatment with a solution of boron trichloride. The free phenol of **5** was subsequently coupled with activated noviose carbonate [26,27] in the presence of catalytic boron trifluoride etherate to give the corresponding noviosylated product, **4**. Displacement of the bromide upon refluxing with 3-amino-pyrrolidine-1-oxyl led to formation of paramagnetic ligand **12**, which underwent solvolysis to cleave the carbonate and afford the desired product, **3**.

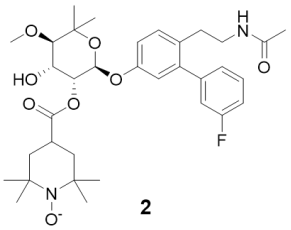
The synthesized paramagnetic ligands were characterized by NMR, mass spectroscopy, and ESR spectroscopy. However, NMR data for the final compounds were not conclusive due to presence of the paramagnetic spin label. In fact, the NMR signals exhibited broad peaks due to inclusion of the nitroxide free radical in addition to missing signals from the protons on the

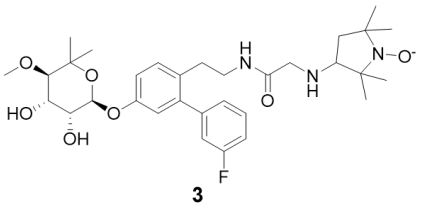


Scheme 2. Reagents and conditions: a) Bromoacetyl bromide, TEA, DCM, 2Hrs, 40%; b) BCl_3SMe_2, dry DCM, -20°C, 55%; c)) INt, $BF_3.OEt_2$, CH_2Cl_2, rt 6 hrs, 60% d) Radicle, Toluene, reflux overnight, 85% e)) Et_3N, MeOH, rt, overnight, 58%.




1


2


3

nitroxide ring,

both of which are consistent with prior reports[28-30]. Therefore, incorporation of the paramagnetic residue onto the **KU-596** scaffold was confirmed via electron spin resonance (EPR) spectroscopy and mass spectrometry. The triplet observed in the ESR spectra was due to the nitroxide signal, which produced characteristic constants of a = 15.55 G and g = 2.00585. Figure 3 shows the continuous-wave (CW) EPR spectra of spin-labelled molecules **1-3**.

Figure 3. Continuous wave (CW) electron paramagnetic resonance spectra (EPR) of paramagnetic ligands 1, 2 and 3 in DCM.

After the discovery of novobicin as an Hsp90 inhibitor, numerous analogs were prepared that eventually led to the establishment of preliminary SAR. However, the exact location of the C-terminal binding site remains unknown, despite the efforts of several research groups. In an effort to circumvent these concerns, efforts have shifted from attempting to identify the binding site via solution of a co-crystal structure, to the use of NMR to obtain a solution structure of the binding site. Initial studies demonstrated the key binding motifs for both **KU-596** and **KU-32** were the central cores, allowing the attachment of additional moieties onto the sugar and amide side chains. Thus, paramagnetic ligands were designed and synthesized in this study, which can now be used to interrogate the location of the Hsp90 C-terminal binding site, which can then be used to develop more efficacious analogs for the treatment of cancer and/or neurodegenerative diseases. The results from such studies will be reported in due course.

ACKNOWLEDGMENT

The authors gratefully acknowledge the funding support of this project by NIH CA203175 (B.S.J.B.) and GM115854 (I.G.).

AUTHORS INFORMATION

Vishal C Birar -v.birar@nd.edu, & Ang Zuo- azuo@nd.edu,

Brian SJ Blagg **bblagg@nd.edu,** Department of Chemistry, Notre Dame University, USA.

Ioannis Gelis-igelis@usf.edu, Department of Chemistry, University of South Florida, Tampa, FL 33620

SUPPLEMENTRY INFORATION

Attached here.

REFERENCES

1. Saibil H. Chaperone machines for protein folding, unfolding and disaggregation. Nat Rev Mol Cell Biol. 2013;14(10):630–642. doi:10.1038/nrm3658.

2. A) Li J, Soroka J, Buchner J. The Hsp90 chaperone machinery: conformational dynamics and regulation by co-chaperones. Biochim Biophys Acta. 2012;1823(3):624–635.

Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–674. doi:10.1016/j.cell.2011.02.013.

3. Li J, Buchner J. Structure, function and regulation of the hsp90 machinery. Biomed J. 2013;36(3):106–117. doi:10.4103/2319-4170.113230. B) Byrd KM, Blagg BSJ. Chaperone substrate provides missing link for cancer drug discovery. J Biol Chem. 2018;293(7):2381–2382. doi:10.1074/jbc.H118.001591.

4. A) Prodromou C, Pearl LH. Structure and functional relationships of Hsp90. Curr Cancer Drug Targets. 2003;3(5):301–323. doi:10.2174/1568009033481877. B) Schopf FH, Biebl MM, Buchner J. The HSP90 chaperone machinery. Nat Rev Mol Cell Biol. 2017;18(6):345–360. doi:10.1038/nrm.2017.20. C) Ali MM, Roe SM, Vaughan CK, et al. Crystal structure of an Hsp90-nucleotide-p23/Sba1 closed chaperone complex. Nature. 2006;440(7087):1013–1017. doi:10.1038/nature04716.

5. A) Chatterjee S, Burns TF. Targeting Heat Shock Proteins in Cancer: A Promising Therapeutic Approach. Int J Mol Sci. 2017;18(9):1978. doi:10.3390/ijms18091978. B) Zuehlke AD, Moses MA, Neckers L. Heat shock protein 90: its inhibition and function. Philos Trans R Soc Lond B Biol Sci. 2018;373(1738), doi:10.1098/rstb.2016.0527

6. Schulte TW, Akinaga S, Soga S, et al. Antibiotic radicicol binds to the N-terminal domain of Hsp90 and shares important biologic activities with geldanamycin. Cell Stress Chaperones. 1998;3(2):100–108. doi:10.1379/14661268(1998)003<0100:arbttn>2.3.co;2.

7. A) Seo YH. Small Molecule Inhibitors to Disrupt Protein-protein Interactions of Heat Shock Protein 90 Chaperone Machinery. J Cancer Prev. 2015;20(1):5–11. doi: 10.15430 /JCP. 2015.20.1.5. B) Neckers L, Workman P. Hsp90 molecular chaperone inhibitors: are we there yet?. Clin Cancer Res. 2012;18(1):64–76. doi:10.1158/1078-0432.CCR-11-1000. C) Workman P. Overview: translating Hsp90 biology into Hsp90 drugs. Curr Cancer Drug Targets. 2003;3(5):297–300. doi:10.2174/1568009033481868.D) Li L, Wang L, You QD, Xu XL. Heat Shock Protein 90 Inhibitors: An Update on Achievements, Challenges, and Future Directions. J Med Chem. 2020;63(5):1798–1822. doi:10.1021/acs.jmedchem.9b00940

8. Marcu MG, Chadli A, Bouhouche I, Catelli M, Neckers LM. The heat shock protein 90 antagonist novobiocin interacts with a previously unrecognized ATP-binding domain in the carboxyl terminus of the chaperone. J Biol Chem. 2000;275(47):37181–37186. doi:10.1074/jbc.M003701200

9. Bickel D, Gohlke H. C-terminal modulators of heat shock protein of 90 kDa (HSP90): State of development and modes of action. Bioorg Med Chem. 2019;27(21):115080. doi:10.1016/j.bmc.2019.115080.

10. Schnur RC, Corman ML, Gallaschun RJ, et al. erbB-2 oncogene inhibition by geldanamycin derivatives: synthesis, mechanism of action, and structure-activity relationships. J Med Chem. 1995;38(19):3813–3820. doi:10.1021/jm00019a011

11. Schnur RC, Corman ML, Gallaschun RJ, et al. Inhibition of the oncogene product p185erbB-2 in vitro and in vivo by geldanamycin and dihydrogeldanamycin derivatives. J Med Chem. 1995;38(19):3806–3812. doi:10.1021/jm00019a010

12. Söti C, Rácz A, Csermely P. A Nucleotide-dependent molecular switch controls ATP binding at the C-terminal domain of Hsp90. N-terminal nucleotide binding unmasks a C-terminal binding pocket. J Biol Chem. 2002;277(9):7066–7075. doi:10.1074/jbc.M105568200

13. Ratzke C, Mickler M, Hellenkamp B, Buchner J, Hugel T. Dynamics of heat shock protein 90 C-terminal dimerization is an important part of its conformational cycle. Proc Natl Acad Sci U S A. 2010;107(37):16101–16106. doi:10.1073/pnas.1000916107

14. Yu XM, Shen G, Neckers L, et al. Hsp90 inhibitors identified from a library of novobiocin analogues. J Am Chem Soc. 2005;127(37):12778–12779. doi:10.1021/ja0535864. B) Burlison JA, Neckers L, Smith AB, Maxwell A, Blagg BS. Novobiocin: redesigning a DNA gyrase inhibitor for selective inhibition of hsp90. Journal of the American Chemical Society. 2006;128(48):15529-36, doi:10.1021/ja065793p. C) Yu XM, Shen G, Neckers L, Blake H, Holzbeierlein J, Cronk B, Blagg BS. Hsp90 inhibitors identified from a library of novobiocin analogues. Journal of the American Chemical Society. 2005;127(37):12778-9, doi:10.1021/ja0535864.

28. Anankanbil S, Mose JH, Pérez B, Cheng W, Pedersen JN, Guo Z. Mapping the location of DATEM in multi-phase systems:

Syn
Food Chem. 2019;275:474–479. doi:10.1016/j.foodchem.2018.09.140.
29. Ionita G, Madalan AM, Ariciu AM, Medvedovici A, Ionita P. Synthesis of novel TEMPO stable free (poly) radical derivatives and their host–guest interaction with cucurbit [6] uril. New Journal of Chemistry. 2016;40(1):503-11, doi:10.1039/C5NJ01518A.
30. Guo-Ping Yan, Daniel Bischa, Steven E. Bottle, Synthesis and properties of novel porphyrin spin probes containing isoindoline nitroxides, Free Radical Biology and Medicine, 2007, 43, (1),2007:111-116, doi:10.1016/j.freeradbiomed.2007.03.032.

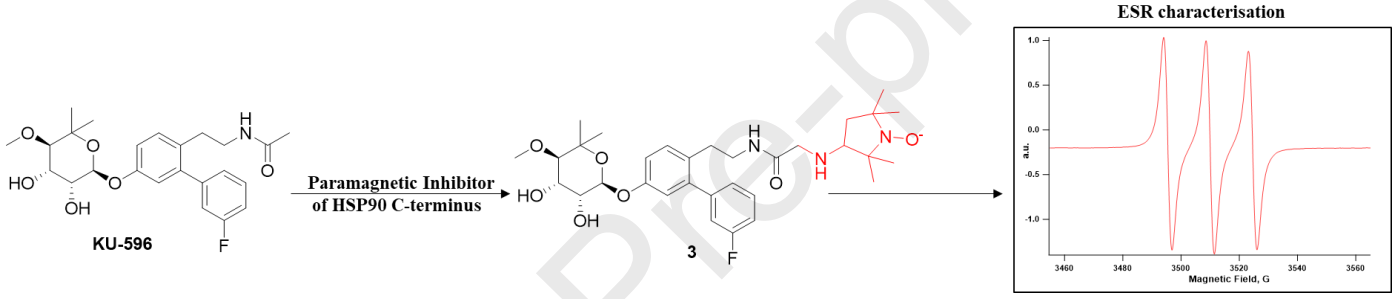
Graphical Abstract

To create your abstract, type over the instructions in the template box below.

Synthesis of Paramagnetic ligands that target the C-terminal binding site of HSP-90

Vishal C Birar, Ioannis Gelis, Ang Zuo and Brian SJ Blagg.

Leave this area blank for abstract info.



Fonts or abstract dimensions