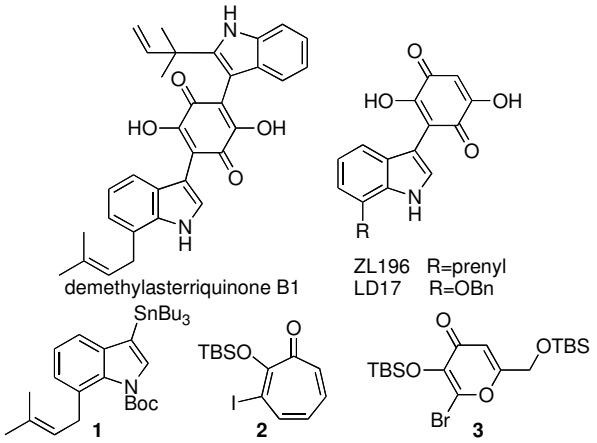
DOI: 10.1002/cbic.200((will be filled in by the editorial staff))

Small Molecule Insulin Mimic

Quinone Replacements for Small Molecule Insulin Mimics

Michael C. Pirrung,*[a] Liu Deng,[b] Bo Lin,[c] and Nicholas J. G. Webster[c]

The 1999 discovery that a natural product from *Aspergillus terreus* and *Pseudomassaria* fungi, demethylasterriquinone B1 (DAQ B1; Figure 1), is an insulin mimic with oral activity in mouse models of diabetes[1] represented a major breakthrough. Not only might millions of diabetics treated via insulin injections anticipate taking a pill instead,[2] but also this discovery represents one solution to a grand challenge of medicinal chemistry: a small molecule that mimics the action of a protein. Biotechnology currently provides the main route to therapeutic proteins. DAQ B1 is thought to act on the intracellular kinase domain of the insulin receptor (IR), whose dimerization leads to autophosphorylation; it has a low micromolar EC_{50} in cell-based assays.



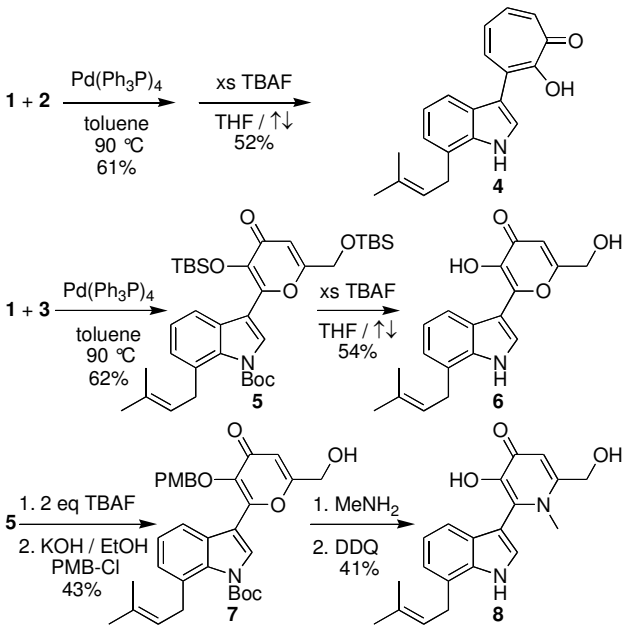
Scheme 1. Orally active insulin mimics and building block molecules.

[a] *Prof. Dr. M. Pirrung,*
Department of Chemistry
University of California
Riverside, CA 92521 USA
Fax: (+1)951-827-2749
E-mail: michael.pirrung@ucr.edu
[b] *Dr. L. Deng*
Department of Chemistry
Duke University
Durham, NC, 27708 USA
[c] *Dr. B. Lin, Prof. Dr. N. J. G. Webster*
Veterans Medical Research Foundation and the VASDHS
San Diego, CA, 92161 USA)

Supporting information for this article is available on the WWW under http://www.chembiochem.org or from the author.

A second-generation, DAQ B1-related quinone was reported that mimics insulin in rodents and whose pharmacokinetics were studied in primates,[3] and a library of asterriquinone analogs revealed novel structural patterns that give insulin receptor activators.[4] Despite these promising early results, molecules of this family have not entered clinical development. Likely a major concern is the safety of candidate pharmaceuticals containing the potentially problematic quinone substructure.[5] While quinones are certainly present in some currently marketed drugs, these agents are used primarily in acute therapy, such as anti-infectives. A drug that treats a metabolic disease like diabetes must be used chronically.

Extending early structure-activity relationship studies,[6] past work in our laboratory identified DAQ B1's major pharmacophore as the quinone and 7-prenylindole.[7] Compound ZL196 comprising just these portions is an orally active insulin mimic in mouse models of diabetes, as is analog LD17 that replaces the 7-prenyl group with a 7-benzyloxy group,[8] though both include the offending quinone. Replacements for quinones are not common in medicinal chemistry, and it is unclear what portions of DAQ B1's quinone are needed for activity. Hypotheses were developed concerning its key feature(s), such as the conjugated α-hydroxycarbonyl group or the quinone tautomeric form. The former might be mimicked in a tropolone, while the latter might be mimicked by substituting a heteroatom for the quinone carbonyl, creating a pyrone or pyridone that lacks redox chemistry. These designs include some simple replacements that have been used in the past in medicinal chemistry.[9] This work reports the preparation of three compounds that exchange the quinone of ZL196 for other cyclic compounds and the activation of IR in cells by one that includes the fungal natural product kojic acid.



Scheme 2. Preparations of candidates for quinone replacements.

A versatile synthon for the 7-prenylindole portion of DAQ B1 is stannane **1**.[10] It permits a variety of "head pieces" to be easily introduced via Stille coupling. Halides **2** and **3** were used here as coupling partners (Scheme 2). Compounds **4** and **6** are produced following removal of the *O*-silyl and *N*-Boc groups with excess fluoride ion. Retention of the *N*-Boc group can be achieved by controlled deprotection of **5** with fluoride ion. Selective protection of the enol gives **7**, which on treatment with methylamine undergoes pyrone-to-pyridone exchange, and which also removes the *N*-Boc group. Final deprotection of the PMB group gives **8**.

These three compounds were examined by immunoblotting for their ability to activate human IR in a cell-based receptor phosphorylation assay. A CHO cell line engineered to over-express this receptor was used.[4] Data for receptor activation by **6** (Figure 1) show a maximum at 1 µM. This is expected behavior for molecules acting via receptor dimerization.[11] Using a binding model for dimerization,[11] the 1 µM maximum for **6** is estimated to be its EC_{50}. Activation of the IR by **6** in this assay is comparable to the known orally active insulin mimic ZL196. We earlier reported[7] (in a different cell line) an EC_{50} for ZL196 of ca. 100 µM, whereas the current studies gave ~30 µM. Tropolone analog **4** has little positive effect on IR phosphorylation and appears cytotoxic, while **8** is an also an activator with an EC_{50} of ~1 µM.

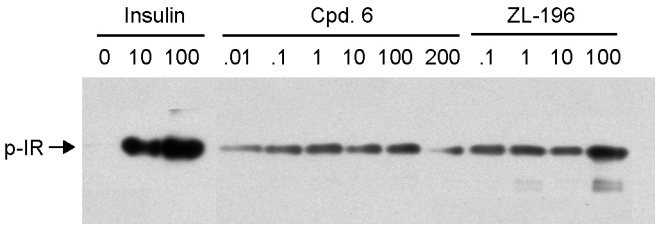


Figure 1. **Activation of the insulin receptor in cells.** Serum-starved CHO-IR cells were treated with insulin (10 or 100 ng/mL) or compounds (0.01-100 µM) for 10 min at 37 °C. Insulin receptor phosphorylation was detected by immunoblotting with anti-phospho-IR (Y1162/63) antibodies and chemiluminescence. The dose-response relationship for **6** is not conventional, which may be due to the self-inhibition known for dimerizing molecules.[11]

Compound **6** represents a new chemical entity whose possible off-target activities must be considered. A significant question is its ability to cause QT prolongation by acting on the cardiac potassium channel (hERG). A cell-based primary screening assay (patch clamp, performed by a contract research laboratory) was used to evaluate such safety concerns with **6**. It produces a 8.2% inhibition of the hERG tail current at 1 µM. Based on a reported hERG ranking system,[12] **6** has low potency as a hERG channel blocker. Compound **6** was also examined at 10 µM in over 40 *in vitro* assays against many human enzymes (cyclooxygenases, phosphodiesterases, and all four classes of proteases), and showed <20% inhibition.

An active analog of a small molecule natural product insulin mimic has been prepared in which kojic acid replaces the original quinone, a structure thought to present intrinsic safety risks.[13] An achievement such as this was essential for the field of small molecule insulin replacements to advance beyond the original 1999 discovery. Many structural variations on **6** and **8** will be needed to delineate structure-activity and toxicity relationships to eventually reach compounds with true therapeutic potential. More efficient routes for their chemical synthesis are under development (Pirrung, M. C.; Xiong, X., unpublished). The tantalizing possibility also exists, given recent access to all of the biosynthetic genes of *Aspergillus*, and specifically those required to generate DAQ B1,[14] to create an engineered biosynthetic route to **6**. As kojic acid is produced by *Aspergillus flavus*[15] and the 7-prenylindole of DAQ B1 is produced by *Aspergillus terreus*, active structure **6** can be viewed as an *Aspergillus* natural product hybrid.

Abbreviations: CHO, Chinese hamster ovary; DDQ, dichlorodicyanoquinone; IR, insulin receptor; PMB, *p*-methoxybenzyl; TBAF, tetra-*n*-butylammonium fluoride; TBS, *tert*-butyldimethylsilyl; THP, tetrahydropyranyl.

Acknowledgements

Financial support from the NIH (DK-60532, AG-023191), the Paul G. Garrett Foundation and the American Diabetes Association is acknowledged.

Keywords: kojic acid · peptide mimics · pyridone · pyrone

[1] B. Zhang, et al. *Science* **1999**, *284*, 974.
[2] a) T. Gura, *Science* **1999**, *284*, 886. b) J.S. Flier, *Nat. Med.* **1999**, *5*, 614.
[3] K. Liu, L. Xu, D. Szalkowski, Z. Li, V. Ding, G. Kwei, S. Huskey, D.E. Moller, J.V. Heck, B.B. Zhang, A.B. Jones, *J. Med. Chem.* **2000**, *43*, 3487.
[4] M.C. Pirrung, Z. Li, Y. Liu, E. Hensley, A. Tanksale, B. Lin, A. Pai, N.J.G. Webster, *J. Comb. Chem.* **2007**, *9*, 844.
[5] J. Kazius, R. McGuire, R. Bursi, *J. Med. Chem.* **2005**, *48*, 312.
[6] H.B. Wood, R. Black, G. Salituro, D. Szalkowski, Z. Li, Y. Zhang, D.E. Moller, B. Zhang, A.B. Jones, *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1189.
[7] M.C. Pirrung, Y. Liu, L. Deng, D.K. Halstead, Z. Li, J.F. May, M. Wedel, D.A. Austin, N.J.G. Webster, *J. Am. Chem. Soc.* **2005**, *127*, 4609.
[8] B. Lin, Z. Li, K. Park, L. Deng, A. Pai, L. Zhong, M.C. Pirrung, N.J.G. Webster, *J. Pharm. Exp. Ther.* **2007**, *323*, 0000.
[9] R. P. Sheridan, *J. Chem. Inf. Comput. Sci.* **2002**, *42*, 103-8. N. T. Southall, Ajay, *J. Med. Chem.* **2006**, *49*, 2103-9. D. Y. Haubertin, P. Bruneau, *J. Chem. Inf. Model.* **2007**, *47*, 1294-302.
[10] M.C. Pirrung, Z. Li, K. Park, J. Zhu, *J. Org. Chem.* **2002**, *67*, 7919.
[11] a) G. Fuh, B. C. Cunningham, R. Fukunaga, S. Nagata, D. V. Goeddel, J. A. Wells, *Science* **1992**, *256*, 1677–1680. b) M. M. Ilonda, A. B. Damholt, B. A. Cunningham, J. A. Wells, P. De Meyts, R. M. Shymko, *Endocrinology* **1994**, *134*, 2397–2403. c) E. Ishizaka-Ikeda, R. Fukunaga, W. I. Wood, D. V. Goeddel, S. Nagata, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 123–127. A. d) Whitty, N. Raskin, D.L. Olson, C.W. Borysenko, C.M. Ambrose, C.D. Benjamin, L.C. Burkly, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 13165-70.
[12] O. Roche, G. Trube, J. Zuegge, P. Pflimlin, A. Alanine, G. Schneider, *Chembiochem.* **2002**, *3*, 455-9.
[13] Whereas kojic acid is found in foods and cosmetics: P. Bentley, *Nat. Prod. Rep.* **2006**, *23*, 1046.
[14] P. Schneider, M. Weber, K. Rosenberger, D. Hoffmeister, *Chem. Biol.* **2007**, *14*, 635.
[15] P. Bajpai, P.K. Agrawala, L. Vishwanathan, *Can. J. Microbiol.* **1982**, *28*, 1340.

Received: ((will be filled in by the editorial staff))
Published online: ((will be filled in by the editorial staff))

Entry for the Table of Contents (Please choose one layout)
While I realize I was supposed to use only one, I had technical difficulties with this template - I could not get the landscape graphic window and text window to work simultaneously, so I put one in each.
Layout 1:

COMMUNICATIONS

A 3-stannylindole was coupled with a 2-bromokojic acid to give an (indolyl)kojic acid that activates the insulin receptor in cell-based assays. This structure represents a hybrid between two *Aspergillus* natural products, demethylasterriquinone B1 and kojic acid.

((Insert TOC Graphic here))

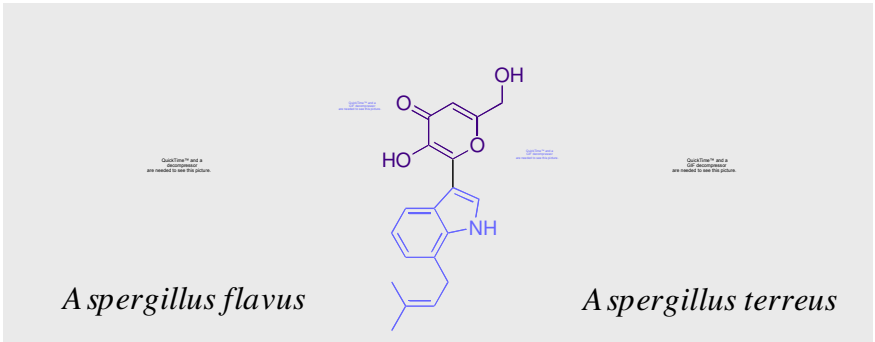
Michael C. Pirrung, Liu Deng, Bo Lin, and Nicholas J. G. Webster*

Page No. – Page No.

((Title))

Layout 2:

COMMUNICATIONS



Michael C. Pirrung, Liu Deng, Bo Lin, and Nicholas J. G. Webster*

Page No. – Page No.

((Title))