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Quinone Replacements for Small Molecule Insulin Mimics

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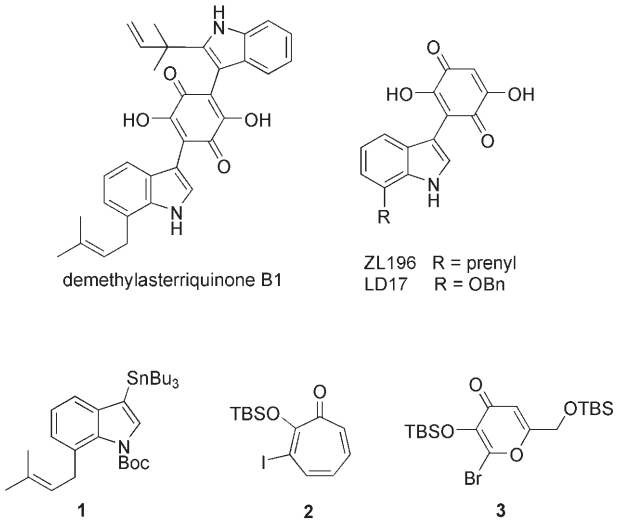
The 1999 discovery that a natural product from *Aspergillus terreus* and *Pseudomassaria* fungi, demethylasterriquinone B1 (DAQ B1; Scheme 1), is an insulin mimic with oral activity in mouse models of diabetes[1] represented a major breakthrough. Not only might millions of diabetics treated with insulin 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), the dimerization of which 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 second-generation, DAQ B1-related quinone was reported that mimics insulin in rodents and its pharmacokinetics were studied in primates;[3] also, a library of asterriquinone analogues revealed novel structural patterns that act as 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 that contain 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, which comprises just these portions, is an orally active insulin mimic in mouse models of diabetes, as is analogue LD17, in which the 7-prenyl group is replaced 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 to create 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.

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** were produced following removal of the *O*-silyl and *N*-Boc groups with excess fluoride ion. Retention of the *N*-Boc group was achieved by controlled deprotection of **5** with fluoride ion. Selective protection of the enol gave **7**, which on treatment with methylamine resulted in pyrone-to-pyridone exchange, and which also removed the *N*-Boc group. Final deprotection of the PMB group gave **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 (CHO-IR) 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 that act through receptor dimerization.[11] By using a binding model for dimerization,[11] the 1 μM maximum for **6** was 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. Earlier we reported[7] (in a different cell line) an EC_{50} for ZL196 of about 100 μM, whereas the current studies gave ~30 μM. Tropolone analogue **4** had little positive

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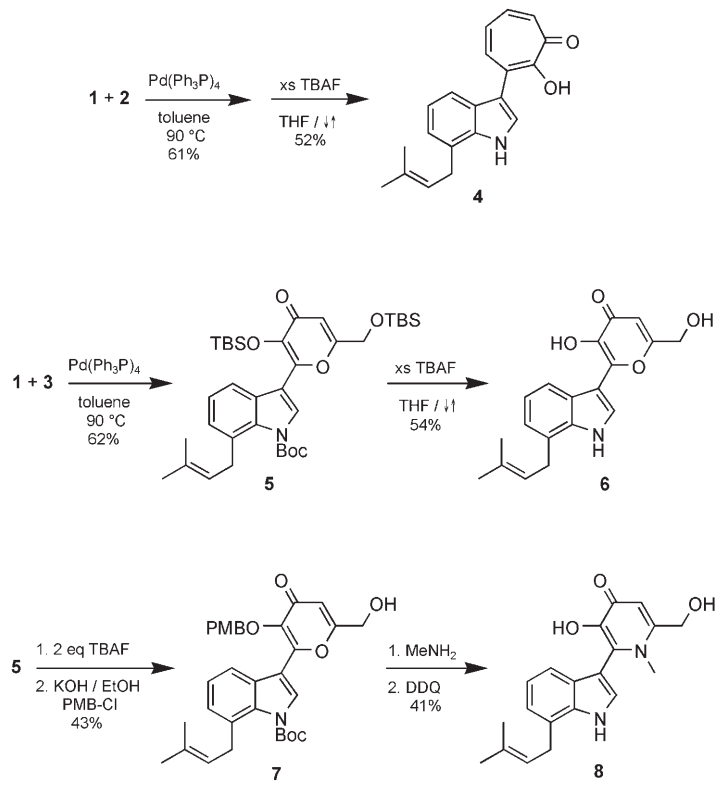

Scheme 2. Preparation of candidates for quinone replacements.

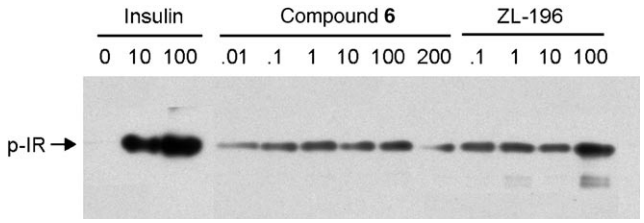

Figure 1. Activation of the insulin receptor in cells. Serum-starved CHO-IR cells were treated with insulin (10 or 100 ng mL^{-1}) or compounds (0.01–100 μM) for 10 min at 37 °C. Insulin receptor phosphorylation was detected by immunoblotting with anti-phospho-IR (Y1162/63) antibodies and chemiluminescence.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

effect on IR phosphorylation and appeared to be cytotoxic, while **8** was also an activator with an EC_{50} of ~1 μM.

Compound **6** represents a new chemical entity the possible off-target activities of which 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—CEREP, Seattle, USA) was used to evaluate such safety concerns with **6**. It produced 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 analogue of a small molecule natural-product insulin mimic has been prepared in which kojic acid was used to replace the original quinine—a structure thought to present intrinsic safety risks.[13] An achievement such as this is 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 (M.C.P., X. Xiong, unpublished results). 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.

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