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SYNTHESIS OF A BIOTIN CONJUGATE OF DARGLITAZONE, A NEW ANTIDIABETIC AGENT. A GENERAL PROTOCOL FOR THE REVERSIBLE BIOTINYLATION OF KETONES.

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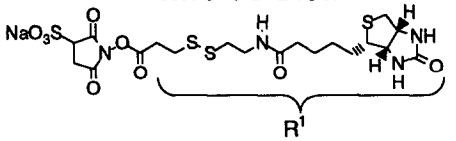
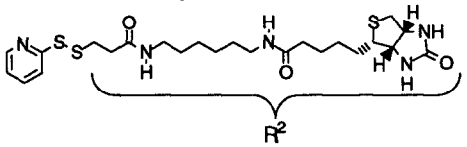
Abstract: Biotin conjugates of darglitazone (CP-86,325), a new antihyperglycemic agent, were prepared. The protocol used allows variation of the chain linking the two units and is applicable to other ligands containing a ketone function.

Affinity chromatography is a well-tested method used to isolate ligand receptors and enzymes[1]. One frequent problem with the procedure is the specific elution of the bound protein in high yield. Reversible biotinylation is a recent modification designed to facilitate the dissociation of the ligand-protein complex from the stationary phase[2]. The method takes advantage of the high affinity of biotin (K_d=10^{-15}M) to avidin, a 66 kDa glycoprotein which recognizes the bicyclic portion of the biotin molecule and which functions as the stationary phase. Several reagents are available (Pierce Chemical Co.) for the reversible attachment of biotin. Biotin-HPDP is a disulfide bond-forming reagent which can be reacted with a thiol, while NHS-SS-Biotin can introduce the biotin moiety via amide formation with an amine.

NHS-SS-Biotin

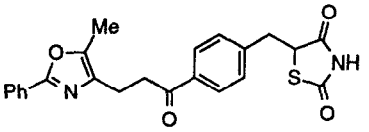


Biotin-HPDP



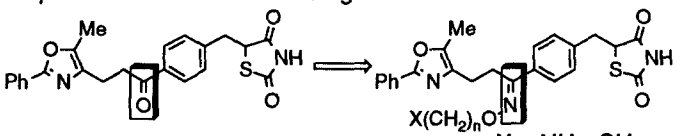
As part of our mechanistic studies with antihyperglycemic agents, we became interested in the characterization of a potential binding site for our new agent, darglitazone (CP-86,325, **1**)[3]. Darglitazone, an analogue of ciglitazone, is part of a family of antidiabetic

compounds with a unique mechanism of action. Unlike the commonly used sulfonylureas, the "glitazones" exert their hypoglycemic action without stimulating insulin secretion, but rather by increasing the sensitivity of the target tissues to insulin. We have synthesized biotinylated derivatives of darglitazone in order to identify and characterize its cellular receptor and provide insight into its mechanism of action.



Darglitazone (CP-86,325)

Our approach to the biotinylation of darglitazone consisted of replacing the ketone moiety by an isosteric group that would allow the attachment of a tether ending with the amine or thiol group required for coupling with NHS-SS-Biotin or Biotin-HPDP. A convenient ketone replacement allowing such functionalization is the iminoether group[4]. The length of the chain linking the iminoether to the amine or thiol can be varied at will so as to minimize any negative effects of the biotin portion on the overall binding.



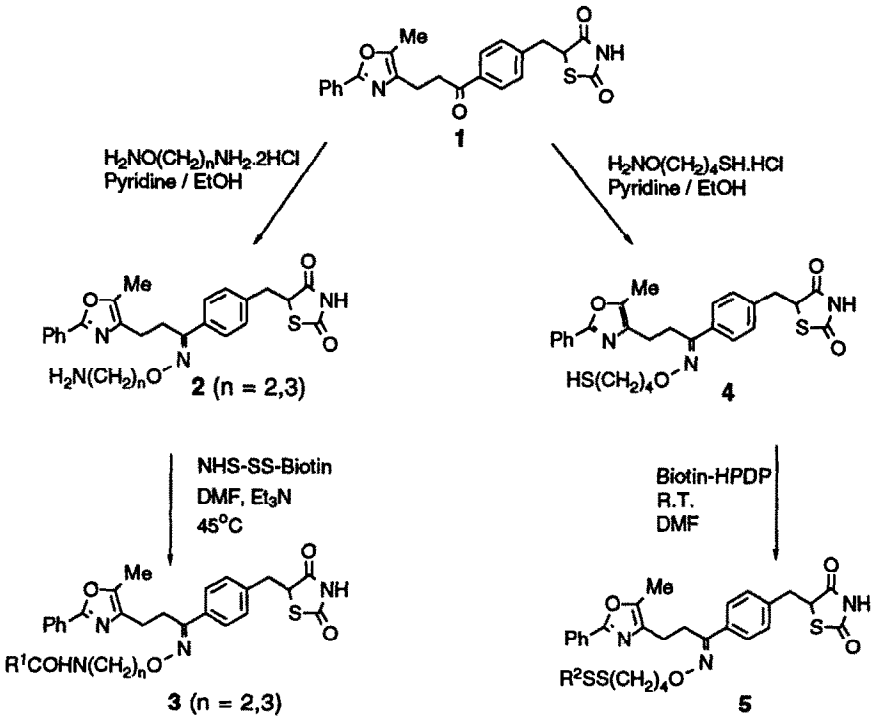
X = NH_2, SH

The synthesis of the biotinylated derivatives of darglitazone is shown in Scheme I. Treatment of darglitazone (**1**) with the easily accessible $NH_2O(CH_2)_nNH_2 \cdot 2HCl$[5] and pyridine in ethanol afforded a single isomer of the oxime **2**, presumably the *E* isomer[6]. Coupling this oxime-amine with NHS-SS-Biotin at 45-50 °C in anhydrous DMF provided the desired biotinylated darglitazone conjugate **3**[7]. Similarly, treatment of **1** with the known reagent $NH_2O(CH_2)_nSH \cdot HCl$[8] and pyridine in ethanol afforded an oxime thiol **4**[9], which was tethered to Biotin HPDP through a disulfide linkage to give **5**[10].

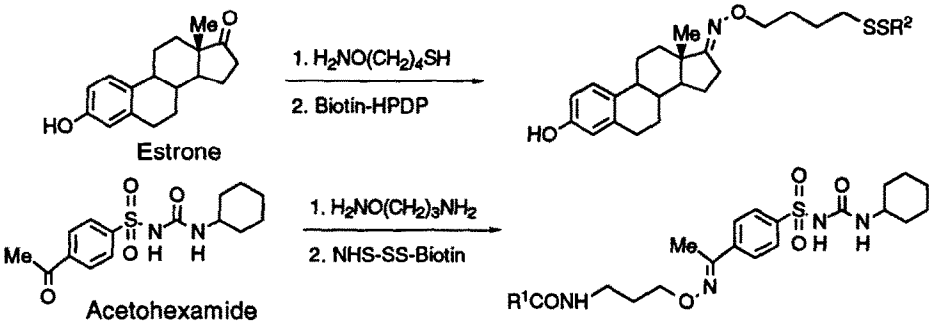
The use of such derivatized compounds for receptor characterization is of course dependent on their ability to bind to the receptor. In this respect, preliminary binding studies using [^{3}H]-darglitazone show that both biotinylated derivatives **2**(n=3) and **4** displace the radioligand with affinities similar to darglitazone itself[11]. Further studies directed at the identification of the cellular receptor are in progress.

The generality of this procedure for the derivatization of drugs or receptor ligands equipped with the appropriate handle, i.e. a ketone function, is exemplified by the synthesis of biotinylated derivatives of estrone and acetohexamide, which were obtained by similar procedures (Scheme II).

Scheme I. Biotinylation of Darglitazone



Scheme II. Biotinylation of Other Ketones



References and Notes

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2. (a) Shimkus, M.; Levy, J.; Herman, T. *Proc Natl. Acad. Sci. USA* , **1985**, *82*, 2593. (b) Seyer, R. ; Aumelas, A.; Marie, J.; Bonnafous, J.-C.; Jard, S.; Castro, B. *Helv. Chim. Acta* , **1989**, *72*, 678. (c) Kozikowski, A.; Tuckmantel, W. *Tetrahedron Lett.*, **1989**, *35*, 4613.
3. Hulin, B.; Clark, D. A.; Goldstein, S. W.; McDermott, R. E.; Dambek, P. J.; Kappeler, W. H.; Lamphere, C. H.; Lewis, D. M.; Rizzi, J. P. *J. Med. Chem.* **1992**, *35*, 1853.
4. For the use of the iminoether function as a replacement for the ketone,see for example: (a) Gasc, J.-C.; Gouin d'Ambrieres, S.; Lutz, A.; Chantot, J.-F. *J. Antibiotics* **1991**, *44*, 313. (b) ref. 3. (c) For the use of isoxazoles as ketone isosteres see Lipinski, C. A. *Ann. Rep. Med. Chem.* **1986**, *21*, 283 and references therein.
5 Pankaskie, M. C.; Scholtz, S. A. *Syn. Commun.* **1989**, *19*, 339.
6. The oxime **2** (n = 3) was isolated as a white solid in 93% yield, mp 193-197 °C.
7 NHS-SS-Biotin-CP-86,325 conjugate **3** (n=3) was isolated as a white solid in a 43% yield after purification by silica gel chromatography using 10:1 CH_2Cl_2/MeOH as the eluent. ^1H NMR (DMSO-d_6, 500 MHz) δ 8.00 (m, 2 H), 7.8 (m, 2 H), 7.5 (d, J = 8 Hz, 2 H), 7.49 (m, 3 H), 7.25 (d, J = 8 Hz, 2 H), 6.43 (s, 1 H), 6.37 (s, 1 H), 4.91 (dd, J = 4 Hz, 9 Hz, 1 H), 4.29 (m, 1 H), 4.14 (m, 3 H), 3.17 (m, 4 H), 3.02 (t, J = 7 Hz, 2 H), 2.89 (t, J = 7 Hz, 2 H), 2.83-2.74 (m, 4 H), 2.67 (t, J = 8 Hz, 2 H), 2.57 (d, J = 12 Hz, 2 H), 2.47 (t, J = 7 Hz, 2 H), 2.19 (s, 3 H), 2.06 (t, J = 7 Hz, 2 H), 1.79 (m, J = 7 Hz, 2 H), 1.60-1.27 (m, 6 H); MS (FAB) m/z 882 (M^+). 3 (n=2) was isolated similarly. ^1H NMR (DMSO-d_6, 300 MHz) δ 8.05 (br, 1 H), 7.95 (br, 1 H), 7.86 (m, 2 H), 7.55 (d, J = 8 Hz, 2 H), 7.48 (m, 3 H), 7.22 (d, J = 8 Hz, 2 H), 6.39 (br, 1 H), 6.30 (br, 1 H), 4.85 (dd, J = 4 Hz, 9 Hz, 1 H), 4.25 (m, 1 H), 4.10 (m, 3 H), 3.1-2.9 (m, 4 H), 2.89 (t, J = 7 Hz, 2 H), 2.83-2.74 (m, 4 H), 2.75-2.4 (m, 6 H), 2.20 (s, 3 H), 2.03 (t, J = 7 Hz, 2 H), 1.79 (m, J = 7 Hz, 2 H), 1.60-1.20 (m, 6 H); MS (FAB) m/z 868 (M^+).
8. Webb II, R. R.; Kaneko, T. *Bioconjugate Chemistry*, **1990**, *2*, 96.
9. The oxime **4** was isolated in 11% yield as white crystals, mp 165-169 °C, after silica gel chromatography (EtOAc/Hexanes, 3:7).
10. The conjugate **5** was isolated in 89% yield: ^1H NMR (DMSO-d_6, 500 MHz) δ 7.90 (m, 3 H), 7.73 (m, 1 H), 7.57 (d, J = 8 Hz, 1 H), 7.49 (m, 3 H), 7.25 (d, J = 8 Hz, 1 H), 6.42 (s, 1 H), 6.36 (s, 1 H), 4.88 (dd, J = 4 Hz, 9 Hz, 1 H), 4.30 (m, 1 H), 4.13 (m, 2 H), 3.40 (m, 1 H), 3.09 (m, 1 H), 3.01 (m, 5 H), 2.89-2.80 (m, 4 H), 2.74-2.64 (m, 4 H), 2.58 (d, J = 12 Hz, 1 H), 2.44 (q, J = 6.9 Hz, 2 H), 2.16 (s, 3 H), 2.04 (m, 2 H), 1.71-1.58 (m, 4 H), 1.52-1.44 (m, 4 H), 1.36-1.23 (m, 10 H); MS (FAB) m/z 952 (M^+).
11. Binding data to be reported later.