Solution and Solid Phase Synthesis of Peptide-Substituted Thiazolidinediones as Potential PPAR Ligands

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Abstract Solution and solid-phase methods for the preparation of peptide-substituted thiazolidinediones have been developed as an approach towards the preparation of library of these compounds as potential ligands for the peroxisome proliferator-activated receptors (PPARs).

Perturbation of the balance between energy intake and expenditure in humans can result in a number of unhealthy conditions including hypertension, type II diabetes, and obesity. At the molecular level, the peroxisome proliferator activated receptors (PPARs) are thought to play a role in each of these metabolic disorders and thus have been the focus of much intense investigation.1 As nuclear hormone receptors, the PPAR isoforms (α, γ, and β/δ) differentially respond to peroxisome proliferators (such as clofibrate),2,3 thiazolidinediones (such as BRL49653, 1, Figure 1),4 and long chain fatty acids.5-7 The finding that the latter compounds and their derivatives are agonists for PPARs represents the novel concept of fatty acids behaving as hormones controlling their own regulation,8 since the genes regulated by PPARs include ones encoding enzymes involved in fatty acid synthesis, storage, and metabolism (including β- and ω-oxidation, transport, and intracellular binding).9

To examine the functional and structural features which facilitate the binding of small molecules to PPARs and to find new activators

Figure 1. Thiazolidinedione PPAR Ligands
and/or inhibitors, we have begun work towards synthesizing libraries of thiazolidinedione-peptide hybrids. These targets allow utilization of the business end of the thiazolidinedione antidiabetic agents along with the structural diversity of commercially available amino acids. Methods for the synthesis of peptides are well-documented, and the solid phase approach is particularly effective for library production. The use of peptide libraries to find new biologically active leads has proven successful for serotonin reuptake agents along with the structural diversity of diastereomers; however, the yields were unacceptably low. While this result precluded the application of this chemistry to the solid phase, it nevertheless provided an encouraging precedent.

Because the difficulties with these and other attempted peptide coupling methods were most likely due to competing side reactions resulting from the acidity of thiazolidinedione proton (pKₐ = 6 - 8), it was logical to protect the ring nitrogen. Unfortunately, none of a variety of protecting groups (for example, TBDMS, Bn) and conditions resulted in the selective reaction of the thiazolidinedione nitrogen over the carboxylic acid moiety in 4. Therefore, an alternate approach was adopted (Scheme 2), in which the nitrogen was functionalyzed prior to introduction of the carboxylic acid moiety. Thus, commercially available thiazolidinedione (9) was doubly deprotonated and mono-alkylated with allyl bromide to give 10, in which the nitrogen was subsequently protected with either the trimethylsilyl ethyl or the fluorenylmethyl group under Mitsunobu conditions, followed by ozonolysis of the alkene to give 13 or 14. Oxidation of the aldehyde 13 to the acid 15 was accomplished in 56% yield by NaClO₂ (t-BuOH, isobutene in buffered aqueous THF) gave the carboxylic acid 12. With KMnO₄, a competing process gives a 37% yield of the overoxidized product 17, which could be exploited to furnish an additional diversity element. Attempts to employ an oxidative workup for the ozonolysis to provide 15 acid directly from 11 led only to the complete destruction of the material. Jones oxidation of fluorenylmethyl-protected compound 14 produced a 94% yield of acid 16, giving a 55% overall yield from 9.

Gratifyingly, amide bond formation (Scheme 3) between the N-protected thiazolidinedione acid 15 and Phe-Gly-OMe (5) gave a much improved yield of the desired compound 18.

Encouraged by these preliminary indications of success, we then turned our attention to the solid phase synthesis of a small library of these compounds. The Fmoc protection strategy and a 2-chlorotrityl functionalized polystryene resin were employed because of the mild conditions available for amino group deprotection and removal of the target from the resin. Additionally, any excess reagents used in cleaving the compound from the solid phase would be easily removed by evaporation, allowing evaluation of the final

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**Scheme 1**. Initial synthesis thiazolidinedione-substituted dipeptides.

**Scheme 2**. Synthesis of protected thiazolidinedione acid

**Scheme 3**. Between the N-protected thiazolidinedione acid 15 and Phe-Gly-OMe (5) gave a much improved yield of the desired compound 18.

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library without purification. Thus, standard solid-phase peptide synthetic methods were applied using Fmoc-protected glycine, leucine, or phenylalanine to produce (in parallel) nine resin bound dipeptides of the general formula 19 with unprotected N-termini. (Scheme 4). These were subsequently treated with the carboxylic acid 16 in the presence of dicyclohexylcarbodiimide (DCC), hydroxybenzotriazole (HOBt), and triethylamine, followed by piperidine-induced deprotection of the thiazolidinedione nitrogen. Trifluoroacetic acid (TFA) caused release of the two diastereomers of each of the nine desired compounds from the solid support, as determined by \(^1\)H NMR and mass spectrometry. Interestingly, in contrast to the solution phase studies, the unprotected thiazolidinedione acid 4 could also be employed for reactions on the solid support with no apparent decrease in yield or purity, presumably because it is used in excess under these latter conditions.

In summary, we have developed both solution and solid phase approaches to the synthesis of peptide-substituted thiazolidinediones as the first steps in the preparation of libraries of these compounds. Through the synthesis of novel, selective PPAR ligands, it is anticipated that new signal pathways for regulating lipid production and metabolism may be identified to ultimately provide new therapeutic agents for treating type II diabetes, obesity, atherosclerosis, and/or hypertension.

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15. Even L165041, the most selective PPARδ binding ligand reported (Kᵢ = 6 nm vs. 730 nm for the γ isoform), showed only tenfold greater activation of PPARδ/GAL4 than the PPARγ chimera. 16


21. (300 MHz, CDCl3): (270 MHz, CD3OD) δ 7.452 (m, 4H), 7.268–7.324 (m, 2H), 4.483 (dd, J = 11.6, 3.2, 1H), 4.400 (t, J = 6.9, 1H), 3.987–4.030 (m, 2H), 3.532 (dd, J = 19.4, 3.3, 1H), 3.038 (dd, J = 19.5, 10.2, 1H). 15: (270 MHz, CDCl3) δ 4.39 (dd, J = 8.9, 3.7, 1H), 3.60–3.66 (Abm, 2H), 3.30 (dd, J = 18.0, 3.5, 1H), 3.00 (dd, J = 18.1, 8.9, 1H), 0.88–0.95 (Abm, 2H), 0.05 (s, 9H).

16: (300 MHz, CDCl3) δ 7.755 (d, J = 7.2, 2H), 7.375–7.446 (m, 4H), 7.308 (dd, J = 6.9, 2.4, 2H), 4.508 (dd, J = 9.3, 3.2, 1H), 4.384 (t, J = 6.9, 1H), 3.950–4.025 (m, 2H), 3.308 (dd, J = 19.4, 3.2, 1H), 2.970 (dd, J = 19.4, 9.3, 1H).

17: (270 MHz, CDCl3) δ 3.63–3.69 (Abm, 2H), 3.41 (d, J = 17.3, 1H), 3.14 (d, J = 17.6, 1H), 0.89–0.96 (Abm, 2H), 0.06 (s, 9H).

18: (270 MHz, CDCl3) δ 7.18–7.27 (m, 5H), 6.72–6.81 (2d + t, 1.5H), 6.59 (t, 0.5H), 4.72–4.82 (m, 1H), 4.38 (ddd, J = 25.9, 10.3, 3.8, 1H), 3.85–4.05 (m, 2H), 3.70 (s, 3H), 3.57–3.63 (Abm, 2H), 3.14 (dd, J = 16.2, 3.8, 1H), 2.98–3.06 (m, 2H), 2.63–2.78 (m, 1H), 0.87–0.93 (Abm, 2H), 0.03 (s, 9H).

21: (400 MHz, CDOD) δ 7.204–7.277 (m, 5H), 4.650–4.690 (m, 1H), 4.565 (dd, J = 9.6, 4.0, 0.5H), 4.464 (dd, J = 9.2, 4.4, 0.5H), 3.901–3.936 (m, 2H), 3.109–3.258 (m, 2H), 2.678–3.033 (m, 2H).

22: (400 MHz, CDOD) δ 4.593–4.668 (m, 1H), 4.420–4.446 (m, 1H), 3.873–3.926 (m, 2H), 3.084–3.185 (m, 1H), 2.837–3.012 (m, 1H).

23: (400 MHz, CDOD) δ 7.108–7.293 (m, 5H), 4.601–4.670 (m, 1H), 4.519 (dd, J = 9.6, 4.0, 0.5H), 4.440 (dd, J = 9.6, 4.0, 0.5H), 3.170–3.265 (m, 1H), 3.077–3.133 (m, 1H), 2.937–3.066 (m, 1H), 2.647–2.842 (m, 1H).

25: (400 MHz, CDOD) δ 7.170–7.290 (m, 5H), 4.568–4.663 (m, 1.5H), 4.360–4.430 (m, 1H), 4.170–4.280 (m, 0.5H), 2.975–3.264 (m, 3H), 2.784–2.911 (m, 1H), 1.580–1.670 (m, 1H), 1.458–1.558 (m, 2H), 0.877–0.974 (m, 6H).

26: (400 MHz, CDOD) δ 4.570–4.694 (m, 1H), 4.460–4.509 (m, 1H), 4.126–4.273 (m, 2H), 3.340–3.440 (m, 1H), 2.717–2.817 (m, 1H), 1.641–1.728 (m, 3H), 0.969 (d, J = 4.0, 3.0H), 0.927 (d, J = 4.0, 3.0H).

27: (400 MHz, CDOD) δ 7.197–7.270 (m, 5H), 4.673 (dd, J = 9.2, 5.2, 1H), 4.449–4.565 (m, 2H), 3.201 (t, J = 5.4, 0.5H), 3.166 (t, J = 5.4, 0.5H), 3.110 (dd, J = 16.2, 3.8, 0.5H), 3.150 (dd, J = 16.2, 3.8, 0.5H), 2.816–2.897 (m, 1H), 2.737 (dd, J = 16.4, 5.2, 1H), 1.679–1.726 (m, 1H), 1.630–1.652 (m, 2H), 0.958 (d, J = 6.2, 3.0H), 0.921 (d, J = 6.2, 3.0H).

28: (400 MHz, CDOD) δ 4.592–4.643 (m, 1H), 4.409–4.462 (m, 2H), 3.093–3.163 (m, 1H), 2.851–2.944 (dd overlapping dd, J = 11.8, 9.0 and 12.2, 8.8, 1.0H), 1.548–1.728 (m, 6H), 0.895–0.974 (m, 12H).

24. The pH of 2,4-thiazolidinedione has been reported as 6.74 at 25 °C: Kanolt, C. W. J. Am. Chem. Soc. 1907, 29, 1402–1416.