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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 a 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**, Fig. 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]

Despite the significant sequence homology seen for all PPAR isoforms,[3,7,10] these enzymes do not bind or respond equally to small molecule ligands. For example, PPARα agonism is induced by medium- and long-chain fatty acids, leukotriene B_4, and, unexpectedly, KRP-297 (**2**),[11,12] the only thiazolidinedione reported to activate PPARα. The primary target for such molecules, including the insulin sensitizer BRL49653 (**1**), appears to be the γ form.[4]

Interestingly, **1** exhibits a PPARγ binding affinity 10 times that of the thiazolidinedione MCC-555 (**3**), yet the antidiabetic potency of **3** in reducing plasma glucose is greater.[13] This apparent anomaly might be explained by differing modes of binding,[14] although this has not been determined. Thus, despite much work, the biological activity of the thiazolidinediones still remains poorly understood. Additionally, unlike PPARs α and γ, the β (or δ) isoform has received little attention. Few of its biological functions have been elucidated, most probably because no highly selective β agonist or antagonist has been discovered.[15,16]

To examine the functional and structural features that facilitate the binding of small molecules to PPARs and to find new activators 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,[17] 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 inhibitors[18] and HIV protease inhibitors.[19] To date, the few libraries of thiazolidinediones substituted only at the 5-position have been highly directed,[20] based on the *para*-alkoxybenzyl moiety in BRL49653.[21]

The feasibility of the synthetic approach was demonstrated by initial studies focused on the solution-phase synthesis of two thiazolidinedione–peptide molecules, **7** and **8** (Scheme 1). Thus, employing standard peptide

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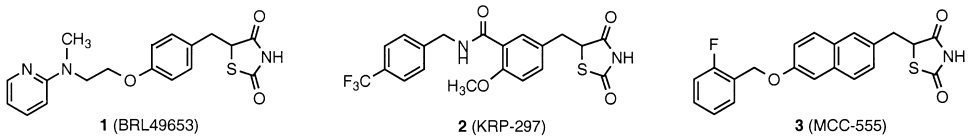


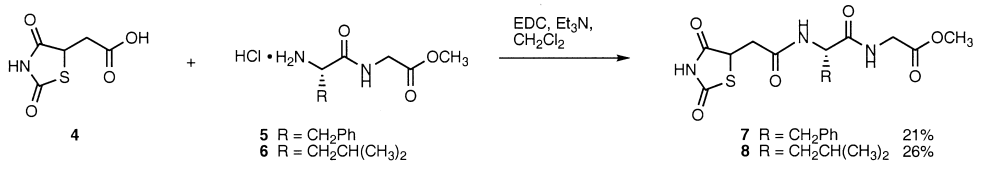
Figure 1. Thiazolidinedione PPAR ligands.

bond formation conditions, thiazolidinedione acid **4** (available in two steps from maleic anhydride and thiourea)[22] was treated with dipeptide **5** or **6** to furnish each of the target compounds as a mixture of diastereomers;[23] 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.

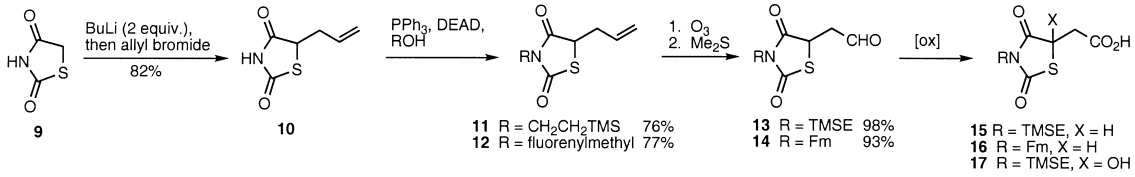
Because the difficulties with these and other attempted peptide coupling methods were most likely due to competing side reactions resulting from the acidity of the thiazolidinedione proton ($pK_a \approx 6$–8[24]), it was logical to protect the ring nitrogen. Unfortunately, none of a variety of protecting groups (for example, TBDMS, Bn) and conditions resulted in the selective reaction of the thiazolidine-dione nitrogen over the carboxylic acid moiety in **4**. Therefore, an alternate approach was adopted (Scheme 2), in which the nitrogen was functionalized prior to introduction of the carboxylic acid moiety. Thus, commercially available thiazolidinedione (**9**) was doubly deprotonated and mono-alkylated[25] with allyl bromide to give **10**, in which the nitrogen was subsequently protected with either the trimethylsilylethyl 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 75% yield by $NaClO_2$ (*t*-BuOH, isobutene in buffered aqueous THF) to give the carboxylic acid **12**. With $KMnO_4$, 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 work up 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 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 unprotected dipeptides of the general formula **19** (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 ^1H 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.

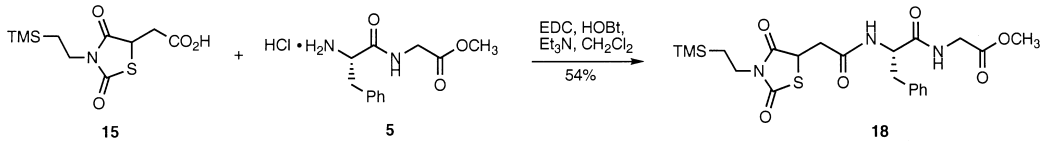
In summary, we have developed both solution- and solid-phase approaches to the synthesis of peptide-substituted thiazolidinediones as the first steps in the preparation of libraries of these compounds. Through the synthesis of



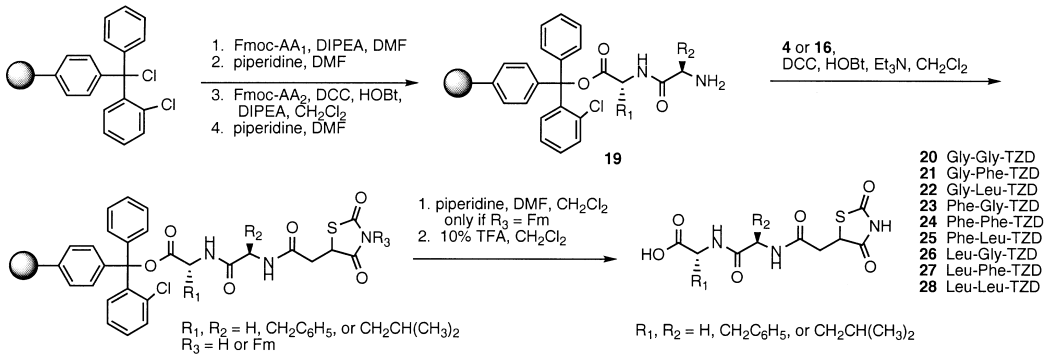
Scheme 1. Initial synthesis of thiazolidinedione-substituted dipeptides.



Scheme 2. Synthesis of protected thiazolidinedione acid.



Scheme 3. Coupling of *N*-protected thiazolidinedione acid to a dipeptide.



Scheme 4. Solid-phase synthesis of a small library of peptide-substituted thiazolidinediones.

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.

Acknowledgements

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23. All new compounds gave satisfactory spectral and analytical data, including ^{1}H NMR: **7**: (270 MHz, CD_3OD) δ 7.91 (s, 1H), 7.13–7.33 (m, 5H), 4.66–4.69 (m, 1H), 4.50 (ddd, $J = 28.9$, 10.1, 4.7, 1H), 3.93–3.99 (s, 2H), 3.71 (s, 3H), 3.19 (dd, $J = 15.3$, 7.7, 1H), 2.81–2.99 (m, 2H), 2.72 (dd, $J = 16.2$, 9.5, 1H). **10**: (300 MHz, $CDCl_3$) δ 8.466 (br s, 1H), 5.746–5.882 (m, 1H), 5.208–5.259 (m, 2H), 4.368 (dd, $J = 9.0$, 3.8), 2.901–2.984 (m, 1H), 2.619–2.726 (m, 1H). **11**: (270 MHz, $CDCl_3$) δ 5.69–5.84 (m, 1H), 5.21 (d, $J = 5.5$, 1H), 5.16 (s, 1H), 4.21 (dd, $J = 8.7$, 4.0, 1H), 3.56–3.64 (ABm, 2H), 2.87–2.98 (m, 1H), 2.52–2.65 (m, 1H), 0.84–0.93 (ABm, 2H), 0.06 (s, 9H). **12**:

(300 MHz, $CDCl_3$) δ 7.755 (d, $J=7.5$, 2H), 7.434 (dd, $J=7.4$, 0.9, 2H), 7.384 (d, $J=7.5$, 2H), 7.305 (dd, $J=7.7$, 0.9, 2H), 5.725–5.807 (m, 1H), 5.253 (dd, $J=6.6$, 1.2, 1H), 5.206 (d, $J=1.2$, 1H), 4.384 (t, $J=7.5$, 1H), 4.329 (dd, $J=9.0$, 4.2, 1H), 3.913–4.041 (m, 2H), 2.917–2.985 (m, 1H), 2.559–2.662 (m, 1H). **13**: (270 MHz, $CDCl_3$) δ 9.79 (s, 1H), 4.38 (dd, $J=9.9$, 3.4, 1H), 3.61–3.67 (ABm, 2H), 3.53 (dd, $J=19.4$, 3.2, 1H), 3.05 (dd, $J=19.4$, 9.9, 1H), 0.90–0.96 (ABm, 2H), 0.05 (s, 9H). **14**: (300 MHz, $CDCl_3$) δ 9.812 (s, 1H), 7.758 (d, $J=7.8$, 2H), 7.370–7.452 (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, $CDCl_3$) 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, $CDCl_3$) 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, $CDCl_3$) 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, $CDCl_3$) 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, CD_3OD) 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, CD_3OD) 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, CD_3OD) 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). **24**: (400 MHz, CD_3OD) δ 7.216–7.471 (m, 10H), 4.599–4.679 (m, 2H), 4.080–4.250 (m, 1H), 2.930–3.260 (m overlapping t at 3.138, $J=7.2$, 6H). **25**: (400 MHz, CD_3OD) 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, CD_3OD) 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, $J=4.0$, 3H), 0.927 (d, $J=4.0$, 3H). **27**: (400 MHz, CD_3OD) 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, $J=6.2$, 3H), 0.921 (d, $J=6.2$, 3H). **28**: (400 MHz, CD_3OD) 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, 1H), 1.548–1.728 (m, 6H), 0.895–0.974 (m, 12H).

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