Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 19, pp. 2491-2496, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)10017-8

SOLID-PHASE SYNTHESIS OF HYBRID THIAZOLIDINEDIONE-FATTY ACID PPARγ LIGANDS

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Abstract: A library of thiazolidinedione-fatty acid hybrid molecules was designed to probe the relationship between natural and synthetic PPAR ligands. Solid-phase synthesis of the library led to the identification of several high affinity PPARγ ligands. © 1997 Elsevier Science Ltd.

The Peroxisome Proliferator-Activated Receptors (PPARs) are nuclear hormone receptors whose natural ligands appear to be fatty acids (FAs).[1-3] Recently, several synthetic ligands for the PPARs have been discovered that demonstrate potent effects on lipid metabolism and glucose homeostasis.[4] Most notably, the thiazolidinedione (TZD, **1**) exerts its antidiabetic activity through binding and activation of the subtype PPARγ.[5,6] We were intrigued by the possibility that synthetic ligands such as **1** may bind to the PPARs as mimics of naturally occurring polyunsaturated FAs (e.g., **2**, Figure 1). Preliminary NMR analysis of **1** and **2** demonstrated that both ligands adopted turn structures in solution.[7] This observation led us to explore the synthesis of TZD-FA hybrid molecules which combined some of the topological features of **1** and **2** (Figure 1). Herein, we report the solid phase synthesis of a library of novel TZD-FA hybrid molecules (**3**,**4**), several of which were found to be high affinity PPARγ ligands.

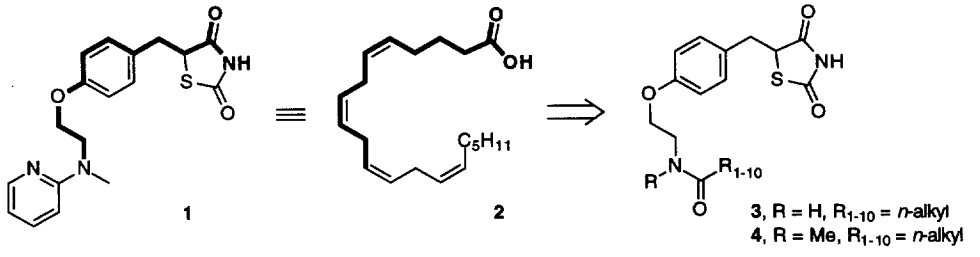

Figure 1. Design of TZD-FA hybrid molecules.

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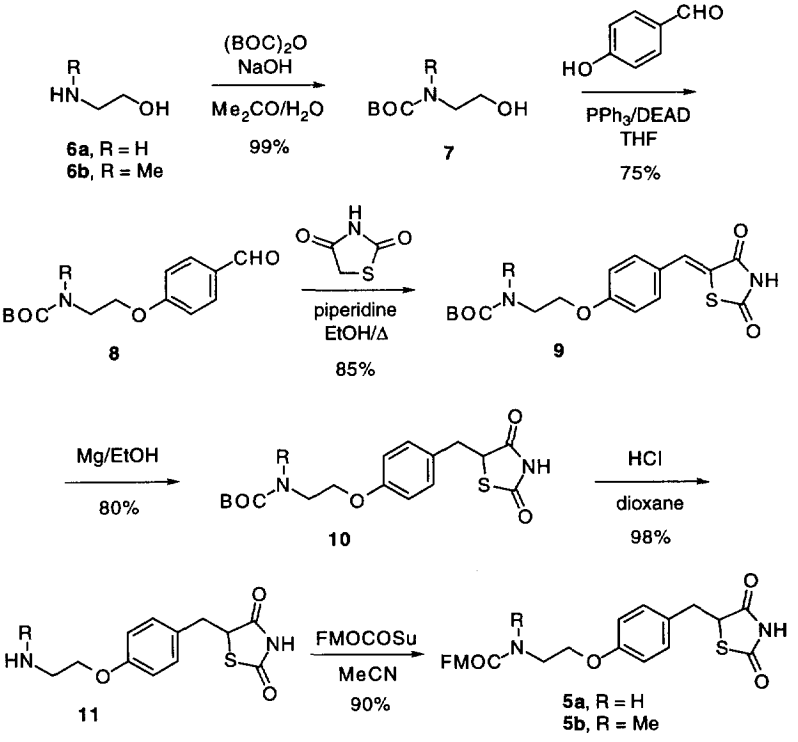
At the outset of our studies we had two primary goals: (a) to establish the potential bioisosteric relationship between the antidiabetic TZDs and naturally occurring polyunsaturated FAs, and (b) to develop a solid-phase synthesis of TZDs that would facilitate the production of libraries of novel analogs. To simplify the synthetic task we chose to prepare, by conventional solution synthesis, two functionalized amino-substituted TZDs (**5**) for loading onto the solid support (Scheme 1).

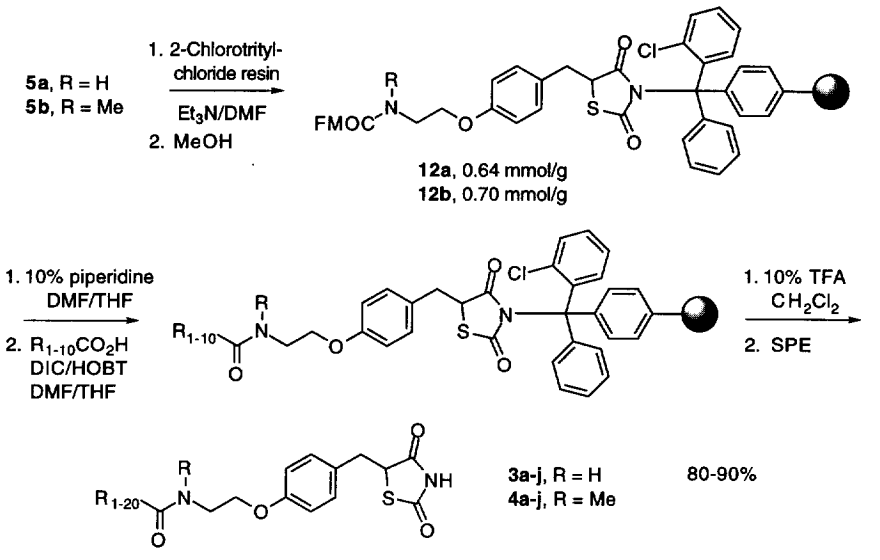


Scheme 1

Protection of the amino alcohols (**6**) as their BOC derivatives (**7**) followed by Mitsunobu reaction with 4-hydroxybenzaldehyde gave the alkoxyaldehydes (**8**) in 75% yield. Knoevenagel condensation with 2,4-thiazolidinedione and subsequent conjugate reduction with magnesium in ethanol gave the BOC-protected TZDs (**10**), which were converted into their FMOC derivatives (**5**) under standard conditions. All reactions proceeded in high yield to provide multigram quantities of the FMOC-protected amino-substituted TZDs (**5**).

Several solid supports were surveyed in order to identify one which gave high loading of the resin-bound TZDs (**12**) without requiring a large excess of **5**. The most suitable resin proved to be 2-chlorotritylchloride polystyrene resin (Novabiochem), which was available at initial loading of 1.3 mmol/g (Scheme 2).[8] Reaction of 1 molar equivalent of TZD (**5a**) or TZD (**5b**) with the resin, followed by capping of

the unreacted sites with MeOH generated the resin-bound TZDs (**12**). Analysis of the resins showed that resin (**12a**) had a loading of 0.64 mmol/g and resin (**12b**) had a loading of 0.70 mmol/g.



Scheme 2

In two independent series of reactions, the resins (**12**) were each deprotected with 10% piperidine in a co-mixture of 1:1 DMF/THF (Scheme 2). Use of the DMF/THF solvent mixture was critical in achieving adequate deprotection, presumably due to greater swelling of the polystyrene resin. The resulting free amines were coupled with a series of ten straight-chain alkyl and alkenyl carboxylic acids which were chosen to probe the effects of chain length and the addition of unsaturation (Table 1). Cleavage of the resins with 10% TFA in CH_2Cl_2 followed by purification of the products by solid phase extraction (SPE) gave a total of twenty TZD-FA hybrid molecules (**3**,**4**) in yields ranging from 80–90%. HPLC and ^1H NMR analysis indicated that all of the products (**3**,**4**) were >90% pure.

The TZD-FA hybrid molecules (**3**,**4**) were assayed for their binding affinity to human PPARγ (Table 1). In this assay TZD (**1**) displayed a K_i of 49 nM and FA (**2**) had a K_i of 1.5 μM. Among the TZDs (**3a–j**), where R = H, only compounds derived from the long-chain saturated FAs caprylic acid (**3h**), nonanoic acid (**3i**), and capric acid (**3j**) showed a binding affinity less than 1 μM. TZDs derived from shorter chain FAs (**3a**, **3e**) showed weaker binding and addition of unsaturation (**3b–3d**) did not lead to a measurable increase in affinity. The TZDs derived from heptanoic acid (**3f**) and heptadienoic acid (**3g**) showed moderate binding affinity. In all cases the TZDs (**4a–j**), where R = Me, demonstrated higher affinity binding than the corresponding desmethyl analogs (**3a–j**). In particular, the TZDs derived from heptanoic acid (**4f**),

heptadienoic acid (**4g**), caprylic acid (**4h**), nonanoic acid (**4i**), and capric acid (**4j**) showed binding affinities comparable to **1**. Although the origin of this increased affinity remains unclear, one intriguing possibility is that the N-methyl group of the amide causes the conformation of the side-chain to more closely resemble the shape of the natural unsaturated FAs. As in the desmethyl series, the TZD derived from the shorter chain pentanoic acid (**4a**) showed weaker binding, and addition of unsaturation did not lead to a significant increase in affinity (**4b–4d**). It should be noted that, of the unsaturated analogs, only compounds (**3c**) and (**4c**) contained significant amounts of *cis* configured double bonds that are found in polyunsaturated FAs. Thus, the fact that some of the hybrid molecules containing unsaturated FAs did not show an increase in binding affinity may be due to the *trans* configuration of their double bonds. Alternatively, this region of the receptor ligand binding pocket may be able to accommodate molecules with unsaturated *cis* and *trans* double bonds as well as saturated side-chains.

The TZD-FA hybrid molecules (**4f–4j**), which had the highest binding affinities, were assayed for their activity in a PPARγ dependent cell-based reporter gene assay. Activity in this assay has previously been shown to correlate with the in vivo potency of a series of antidiabetic TZDs.[6] Interestingly, despite their comparable binding affinity, the TZD-FA hybrid molecules (**4f–4j**) were found to be 5- to 16-fold less potent than **1** in the cell-based assay. Since, all the compounds gave the same maximal stimulation of reporter gene activity (data not shown), differences in functional efficacy do not explain the differences in potency between **1** and **4**. A likely explanation for the relative lack of potency of the TZD-FA hybrid molecules (**4f–4j**) is that the observed functional response is a reflection of their effective intracellular concentrations. Notably, FA (**2**) also showed lower potency in the cell-based assay when compared to its affinity for PPARγ in the in vitro binding assay. It has been reported that long-chain FAs such as **2** cross cell membranes by a specific saturable mechanism[9] and may not be able to freely diffuse into the cytosol or nucleus. Thus, the TZD-FA hybrid molecules (**4f–4j**), which have physical properties similar to FA (**2**), may also be less effective at entering cells by passive diffusion. In addition, the presence of intracellular lipid binding proteins as well as serum binding proteins may result in lower concentrations of the free ligands in the cell-based assay.

In summary, several novel TZD-FA hybrid molecules were identified that were high affinity ligands for human PPARγ. Although this does not definitively prove our hypothesis that TZDs may be synthetic mimics of naturally occurring unsaturated FAs, it is encouraging to note that incorporation of lipophilic long-chain FAs into these hybrid molecules resulted in the identification of PPARγ ligands with receptor affinity greater or equal to one of the most potent TZDs (**1**) reported to-date.[10] Finally, an efficient solid phase synthesis was developed that has the potential to be used in the production of libraries of novel TZDs. Combined with the ability to rapidly screen the resulting molecules using in vitro binding assays, these synthetic techniques may allow the rapid discovery of new and improved antidiabetic agents.

Table 1. Biological activity of TZD-FA hybrid molecules.

Compound[a]	R	$R_{1\text{-}10}CO_2H$	K_i (nM)[b]	EC_{50} (nM)[c]
1	—	—	49	50
2	—	—	1500	30000
3a	H	$CH_3(CH_2)_3CO_2H$	>10000	
3b	H	$CH_3CH_2CH{=}CHCO_2H$[d]	>10000	
3c	H	$CH_3CH{=}CHCH_2CO_2H$[e]	>10000	
3d	H	$CH_2{=}CH(CH_2)_2CO_2H$	>10000	
3e	H	$CH_3(CH_2)_4CO_2H$	10000	
3f	H	$CH_3(CH_2)_5CO_2H$	1400	
3g	H	$CH_2{=}CH(CH_2)_2CH{=}CHCO_2H$[d]	6800	
3h	H	$CH_3(CH_2)_6CO_2H$	290	
3i	H	$CH_3(CH_2)_7CO_2H$	270	
3j	H	$CH_3(CH_2)_8CO_2H$	130	
4a	Me	$CH_3(CH_2)_3CO_2H$	1100	
4b	Me	$CH_3CH_2CH{=}CHCO_2H$[d]	1200	
4c	Me	$CH_3CH{=}CHCH_2CO_2H$[e]	1200	
4d	Me	$CH_2{=}CH(CH_2)_2CO_2H$	600	
4e	Me	$CH_3(CH_2)_4CO_2H$	180	
4f	Me	$CH_3(CH_2)_5CO_2H$	61	810
4g	Me	$CH_2{=}CH(CH_2)_2CH{=}CHCO_2H$[d]	18	520
4h	Me	$CH_3(CH_2)_6CO_2H$	48	280
4i	Me	$CH_3(CH_2)_7CO_2H$	18	280
4j	Me	$CH_3(CH_2)_8CO_2H$	20	290

[a] All test compounds were >90% pure by ^1H NMR and HPLC analysis.

[b] Binding affinity determined by displacement of 20 nM [^3H]-1 from 4 nM biotinylated human PPARγ ligand binding domain. Receptor-bound radioactivity was determined by scintillation proximity assay; K_i, inhibition constants for the test ligands determined from least squares fit of concentration response curves to a model of competitive inhibition; data from a representative experiment are shown, less than 2-fold variation in the K_i was observed in replicate experiments.

[c] Transactivation of human PPARγ-GAL4 chimera in CV-1 cells (ref. 5); EC_{50}, concentration of test ligand that resulted in half maximal activation of reporter gene; assay performed in triplicate, n = 2, ±15%.

[d] Test compound contained a *trans* double bond.

[e] Test compound contained a 1:1 mixture of *cis* and *trans* double bonds.

Acknowledgment: The authors wish to thank Tom Consler (Department of Molecular Sciences) for supplying biotinylated human PPARγ ligand binding domain.

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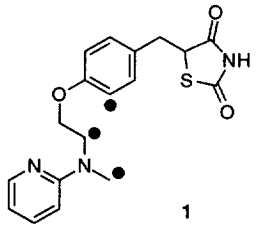
7. The ^{1}H NOESY and ROESY spectra (500 MHz) of **1** and **2** in DMSO-d_6 suggested that both ligands adopted turn structures in solution. For TZD (**1**) NOEs were detected between a proton on the phenoxy ring and the protons adjacent to the side-chain nitrogen, and for FA (**2**) NOEs were detected between the allylic methylene protons adjacent to the terminal double bonds of the polyene (see below, NOEs observed between protons marked with the closed circles).



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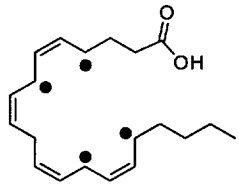

2

8. Solid-phase synthesis of TZD-FA hybrid molecules (**4**):

   2-Chlorotritylchloride resin (Novabiochem, 1.3 mmol/g, 3.8 g) was swelled with DMF (8 mL). TZD (**5b**) (2.5 g, 5.0 mmol) in DMF (8 mL) was added followed by Et_3N (1.6 mL, 12.4 mmol). The resulting suspension was shaken for 4 h. MeOH (5 mL) was added. After 1 h, the resin was drained and washed with DMF, CH_2Cl_2, THF, MeOH, DMF, CH_2Cl_2, THF, MeOH, and CH_2Cl_2. The resin (**12b**) was dried under vacuum. Analysis showed a loading of 0.7 mmol/g.

   Resin (**12b**) (35 mg, 0.7 mmol/g) was suspended in THF (0.25 mL) and treated with 20% piperidine in DMF (0.25 mL) for 12 h. The solution was drained and the resin was washed with THF, DMF, CH_2Cl_2, MeOH, CH_2Cl_2, DMF, and THF. The resulting resin was resuspended in THF (0.25 mL) and a solution of a carboxylic acid (1M in DMF, 0.25 mL), HOBT (1M in DMF, 0.25 mL), and DIC (0.04 mL) were added. The mixture was shaken at room temperature for 14 h. The solution was drained and the resin washed with THF, DMF, CH_2Cl_2, MeOH, THF, DMF, and CH_2Cl_2. The resulting resin was suspended in 10% TFA in CH_2Cl_2 (1 mL) for 30 min. The solution was filtered and the filtrate evaporated. The crude product was dissolved in a small volume of CH_2Cl_2 and loaded onto an SPE column (Bakerbond, SiOH). The SPE column was washed with two column volumes of CH_2Cl_2, one volume of $CHCl_3$, one volume of Et_2O, and two volumes of EtOAc. The EtOAc fraction was evaporated to yield 4–7 mg (80–90%) of TZD-FA hybrid molecules (**4**).
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(*Received in USA* 11 *August* 1997; *accepted* 2 *September* 1997)