The Stereocentre That Isn't: Designing Thiazolidinediones After the Glitazars

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***Abstract*—Proposing a new 2,4-thiazolidinedione as a dual PPAR-α/γ agent means entering the most comprehensively failed therapeutic class in metabolic medicine: ragaglitazar, tesaglitazar, muraglitazar, MK-0767 and aleglitazar were all halted in or around phase III, for rodent carcinogenicity, irreversible rises in serum creatinine, excess cardiovascular events and, in the 7228-patient ALECARDIO trial, futility together with increased heart failure, gastrointestinal haemorrhage and fractures. The common feature of the failures was full PPARγ agonism, not an idiosyncratic off-target effect, which is why the word modulator in a project title is not a synonym for agonist but a different design brief. This review sets out what that brief requires, starting from the chemistry. Two facts about the ring govern everything a student series can claim. First, the 5-arylidene compounds produced by Knoevenagel condensation — where most series stop — are planar Michael acceptors at the oxidation level above the glitazones, not glitazone analogues; reduction is a separate step. Second, once reduced, the C-5 stereocentre racemises rapidly in every aqueous medium tested, irrespective of pH, and in human plasma, so enantioselective synthesis and single-enantiomer docking are both wasted unless the centre is deliberately stabilised. The live pharmacological hypothesis is not transactivation potency but blockade of CDK5-mediated phosphorylation of PPARγ at Ser273, an activity that the weak agonist MRL24 delivers at a tenth the concentration rosiglitazone needs. Assays, docking criteria and in vivo endpoints are discussed on that basis.**

***Index Terms*—2,4-Thiazolidinedione; PPAR-α/γ; glitazar; selective PPARγ modulator; Ser273 phosphorylation; racemisation; Knoevenagel condensation**

I. INTRODUCTION

The thiazolidinedione ring reached the clinic quickly. Ciglitazone was described in 1982, rosiglitazone in 1994, and the mechanism was settled in 1995 when the acidic head group was shown to be a high-affinity ligand for the nuclear receptor PPARγ [1,22,23]. A crystal structure followed three years later, showing the thiazolidinedione carbonyls and N–H hydrogen bonded to His323, His449, Tyr473 and Ser289 at the activation function-2 surface, holding helix 12 in the active position [2]. It is a clean structure–activity story, and it is taught as one.

The receptor itself explains why the scaffold attracted so much effort. PPARγ is the master regulator of adipocyte differentiation and a determinant of whole-body insulin sensitivity; PPARα, its closest relative, drives fatty-acid oxidation and is the target of the fibrates. A single molecule engaging both would, on paper, correct the two halves of the metabolic syndrome at once [35,36]. That reasoning is sound, and it is why the idea has been pursued for thirty years.

What follows the structure is less often taught. Troglitazone was withdrawn for hepatotoxicity; rosiglitazone was restricted for five years after a meta-analysis of myocardial infarction; pioglitazone carries a bladder-cancer signal; and the whole class produces fluid retention, weight gain, heart failure and fractures [3–9]. Then came the glitazars, designed to add PPARα agonism and so treat the dyslipidaemia alongside the hyperglycaemia. Every one of them was stopped (Table 1).

A project proposing novel thiazolidinediones as dual PPAR-α/γ agents is therefore proposing to re-enter

that space, and the title's own word for what it intends — modulator rather than agonist — is the right one. This review takes it seriously. Sections 2 and 3 set out what the clinical record and the ring chemistry actually constrain; sections 4 to 6 set out what a modulator is, how it is detected, and which assays and docking criteria distinguish it from the agonists that failed. The central claim is that two chemical facts about this scaffold — the oxidation state of C-5 and the lability of the stereocentre there — decide in advance what a new series is entitled to claim, and both are routinely overlooked.

II. THE RECORD THE TITLE INHERITS

2.1 The glitazones

Pioglitazone and rosiglitazone (Fig. 1a, b) remain the reference compounds. Both are full PPARγ agonists, both lower HbA1c durably — rosiglitazone outperformed metformin and glyburide on glycaemic durability in ADOPT — and both carry the mechanism-linked adverse effects of PPARγ activation [3,9]. ADOPT also surfaced the excess of fractures in women that is now accepted as a class effect. Pioglitazone showed a benefit on the main secondary endpoint of PROactive and has a more favourable lipid profile than rosiglitazone, which is usually attributed to weak PPARα activity [7]; its bladder-cancer association emerged later [8]. Rosiglitazone's cardiovascular controversy, opened by a 2007 meta-analysis and addressed by the RECORD trial, led to restrictions that were subsequently relaxed [5,6].

The point for a designer is that these are not scattered toxicities. Fluid retention, weight gain, heart failure risk and bone loss follow from PPARγ activation in the kidney, adipose tissue and bone marrow respectively. They are the pharmacology, not a contaminant of it [13,34].

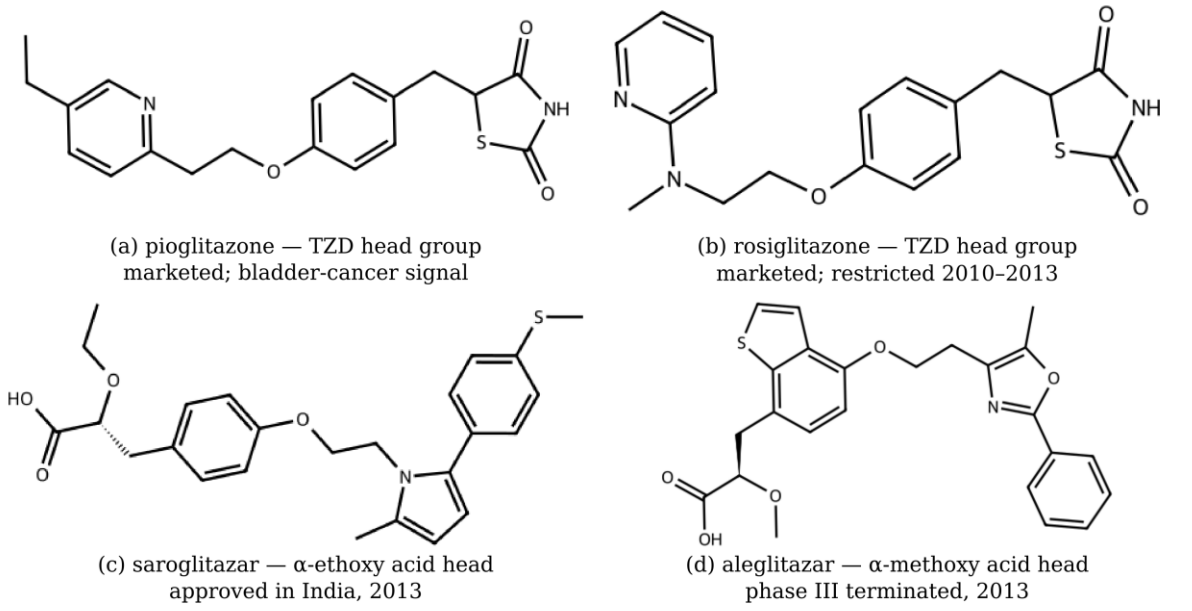


Fig. 1. The clinical landscape a new series enters. (a, b) The two marketed glitazones, both full PPARγ agonists with a thiazolidinedione head group and a stereocentre at C-5. (c, d) Two α-alkoxy carboxylic acid dual agonists with contrasting outcomes: saroglitazar, predominantly PPARα, approved in India; aleglitazar, balanced α/γ, terminated in phase III.

2.2 The glitazars

Adding PPARα agonism was a rational idea with a consistent outcome (Table 1). Ragaglitazar was stopped for urothelial tumours in rodents; tesaglitazar for irreversible increases in serum creatinine and falling glomerular filtration rate; muraglitazar after an independent analysis found an excess of death, myocardial infarction, stroke and heart failure against placebo and pioglitazone; MK-0767 for haemangiosarcoma in rodents [10,12–14]. Aleglitazar

was the most thoroughly tested: ALECARDIO randomised 7228 patients with type 2 diabetes after an acute coronary syndrome to 150 µg daily or placebo, and was stopped at a median of 104 weeks for futility together with increased heart failure, gastrointestinal haemorrhage, renal dysfunction, hypoglycaemia and fractures (Fig. 2a) [11].

Two readings of that record are available. The pessimistic one is that simultaneous PPARα and PPARγ activation is intrinsically unsafe. The more useful one, and the one supported by the compounds themselves, is that the failed agents were without exception full PPARγ agonists with higher affinity for γ than for α, so that the dose required for the lipid effect delivered a supra-maximal γ effect [13,14]. Saroglitazar (Fig. 1c), approved in India in 2013, is the partial exception: it is predominantly a PPARα agonist with moderate γ activity, and its reported profile shows triglyceride lowering with less weight gain [38,39]. Whether that distinction holds in a cardiovascular outcome trial is still unknown, and a review should say so.

A second partial exception points the same way. Balaglitazone is a PPARγ partial agonist that reached phase III, where it lowered HbA1c comparably to pioglitazone with less oedema and less weight gain [37]. Neither saroglitazar nor balaglitazone is a triumph, and neither has the outcome data that would settle the question. But both are agents whose γ activity was deliberately held below maximal, and both avoided the failure mode that stopped the balanced full agonists. That is the pattern a new series should be designed to extend, and it is the reason the word in this project's title is the right word.

Table 1. Dual PPAR-α/γ agents taken into late-phase development. Every balanced or γ-dominant agent was halted; the one approved agent is α-dominant.

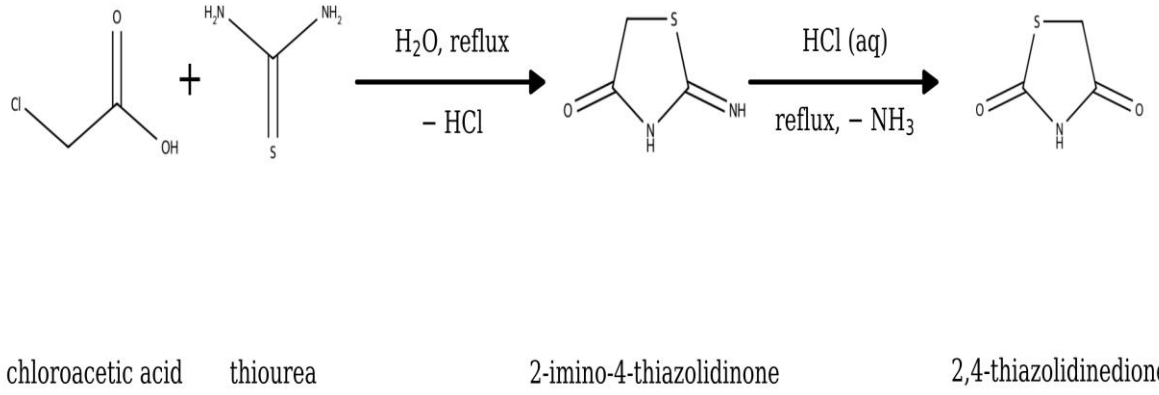
Agent	Sponsor	Stage reached	Reason development stopped	Ref.
Ragaglitazar	Novo Nordisk	Phase III	Urothelial (bladder) tumours in rodents; oedema, anaemia, weight gain, leucopenia	[12–14]
MK-0767	Merck / Kyorin	Phase III	Haemangiosarcoma in rodents; developed as the racemate because the C-5 epimers interconvert too fast to separate	[13,14]
Tesaglitazar	AstraZeneca	Phase III	Irreversible rise in serum creatinine with falling glomerular filtration rate; weight gain, anaemia	[12–14]
Muraglitazar	Bristol-Myers Squibb	NDA filed	Excess of death, myocardial infarction, stroke and heart failure versus placebo and pioglitazone	[10,14]
Aleglitazar	Roche	Phase III (ALECARDIO, n = 7228)	Stopped at 104 weeks for futility plus heart failure, GI haemorrhage, renal dysfunction and fractures	[11]
Saroglitazar	Zydus	Approved (India, 2013)	Marketed; predominantly PPARα with moderate γ activity; no cardiovascular outcome trial reported	[38,39]

III. WHAT THE RING CHEMISTRY PERMITS

3.1 Building the ring

Almost every student series begins the same way. Chloroacetic acid and thiourea in hot water give 2-imino-4-thiazolidinone, which is hydrolysed in acid to 2,4-thiazolidinedione (Scheme 1) [24,25]. The sequence is cheap, high-yielding and well documented, and there is nothing to criticise in it. The ring that results has an acidic N–H and, more importantly, an acidic C-5 methylene flanked by a carbonyl and a sulfur.

Both acidities matter pharmacologically. The imide N–H has a pK_a near 6.8, so at physiological pH the head group is substantially ionised, and it is that anion which makes the thiazolidinedione a carboxylate bioisostere and lets it occupy the same polar sub-pocket as the α-alkoxy acid head of the glitazars [2,35]. The C-5 acidity is what makes the Knoevenagel condensation proceed under mild base, and — as section 3.3 argues — it is also what destroys any stereochemical information installed there. One structural feature therefore delivers the binding, enables the chemistry and defeats the stereocontrol.



Scheme 1. The standard two-step preparation of the 2,4-thiazolidinedione ring from chloroacetic acid and thiourea. The product has an acidic N–H and an acidic C-5 methylene; the latter governs everything that follows.

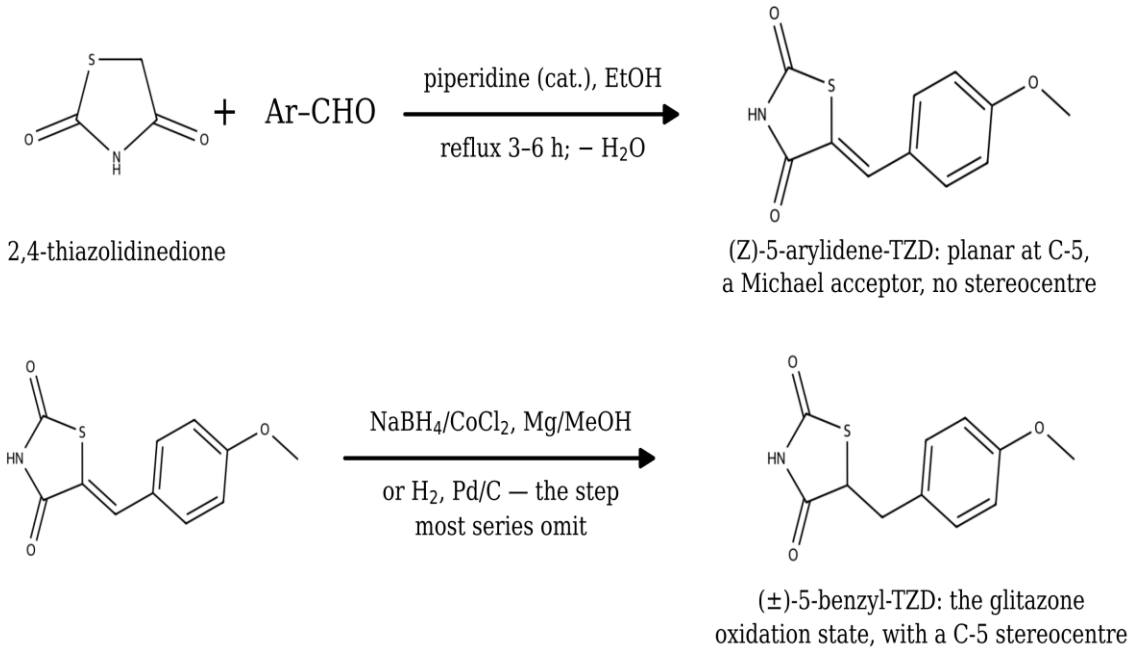
3.2 An arylidene is not a glitazone

That acidic methylene is what makes the next step easy: Knoevenagel condensation with an aromatic aldehyde under piperidine catalysis gives the 5-arylidene compound in high yield (Scheme 2, upper) [24]. A very large fraction of published thiazolidinedione series stops here, and describes the products as glitazone analogues. They are not.

The difference is an oxidation state. In pioglitazone and rosiglitazone C-5 is sp^3, bearing a hydrogen and a benzyl group, and the ring is free to adopt the puckered geometry seen in the PPARγ complex [2]. In a 5-arylidene compound C-5 is sp^2 and conjugated through an exocyclic alkene to the aryl ring; the molecule is planar, extended, and electronically quite different. Reduction with sodium borohydride and cobalt chloride, magnesium in methanol, or hydrogen over palladium gives the saturated compound (Scheme 2, lower), and it is a separate operation with its own yield and its own purification.

There is a second consequence. The exocyclic alkene is conjugated to two carbonyls, which makes a 5-arylidene thiazolidinedione a Michael acceptor and a candidate thiol-trapping agent. Such compounds react with cysteine residues and with glutathione, give reproducible but target-unrelated readouts in many assay formats, and are flagged by standard interference-substructure filters [26–28]. This does not make them uninteresting — covalent chemistry is a legitimate strategy — but it does mean that activity observed for an arylidene series needs a thiol-competition control before it is attributed to receptor binding, and that the series should not be presented as an analogue of a non-covalent glitazone.

The geometry of the double bond is worth one sentence, because it is almost always assigned without comment. Knoevenagel condensation of 2,4-thiazolidinedione with an aromatic aldehyde gives the Z-isomer essentially exclusively, since that arrangement places the bulky aryl group away from the adjacent C-4 carbonyl, and the vinyl proton appears as a singlet near δ 7.7 in dimethyl sulfoxide. A series reporting the E-isomer, or reporting no assignment at all, has either made an unusual observation worth documenting or has not looked. Stating the evidence costs one line.



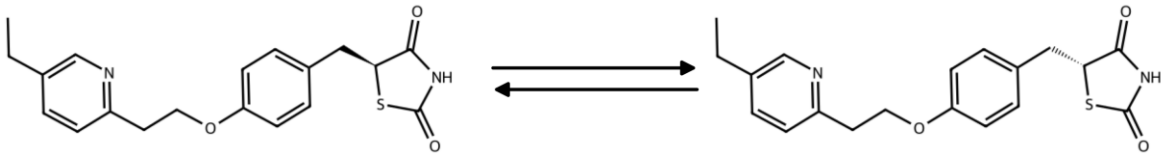
Scheme 2. Knoevenagel condensation gives the 5-arylidene compound (upper), which is planar at C-5 and is a Michael acceptor. Reduction (lower) is a separate step and is what produces a compound at the glitazone oxidation state — together with a stereocentre.

3.3 The stereocentre that erases itself

Reduce the arylidene and a stereocentre appears at C-5. The temptation is to treat it as a design variable: to attempt an asymmetric synthesis, to resolve the enantiomers, or at least to dock one of them. All three are misconceived for this ring, and the reason is the same proton that made the Knoevenagel condensation easy.

The C-5 hydrogen sits between a carbonyl and a sulfur. Its removal gives a stabilised carbanion, and reprotonation occurs from either face (Scheme 3). A dedicated study of a stereolabile 5-aryl-thiazolidinedione found rapid racemisation in most solvents, including every aqueous system examined and irrespective of pH, and confirmed it in dog and human plasma by chiral chromatography with mass detection [19]. Chiral separations of the marketed drugs show the same thing, with racemisation accelerating as pH rises [20]; a reversible sulfur oxidation has been proposed as an additional pathway [21]. MK-0767 was developed deliberately as the racemate because its enantiomers interconvert too quickly in biological samples to do otherwise.

The pharmacological consequence is sharp, and it is not that the enantiomers are equivalent. (S)-Rosiglitazone was identified as the more potent PPARγ agonist; it simply could not be studied, because it equilibrated to a 1:1 mixture faster than it could be dosed. More recently, stabilising the centre by deuterium substitution has allowed the two pioglitazone enantiomers to be separated pharmacologically, with the (R) form showing little PPARγ activity while the (S) form carries the agonism and the weight gain. So the stereochemistry matters a great deal and is simply unavailable unless it is engineered to be. For a dissertation series the practical rules are: synthesise and report the racemate, do not claim enantiopurity, dock both enantiomers or state plainly that the modelled configuration is arbitrary, and do not build an argument on a chiral HPLC trace recorded at one time point.



(5S)-pioglitazone (5R)-pioglitazone

fast in every aqueous medium tested, irrespective of pH, and in dog and human plasma

Scheme 3. Why that stereocentre cannot be used. The C-5 proton is flanked by a carbonyl and sulfur; its loss gives a planar carbanion that is reprotonated from either face, racemising the compound faster than it can be dosed.

IV. AGONIST, PARTIAL AGONIST, MODULATOR

4.1 Two separable activities at one receptor

The most important result for anyone designing in this space is that the antidiabetic effect of PPARγ ligands is largely separable from their agonism. Obesity activates CDK5 in adipose tissue, which phosphorylates PPARγ at Ser273 (Ser245 in the γ1 isoform). That modification does not change adipogenic capacity, but it represses a specific set of insulin-sensitising genes including adiponectin, and its reversal tracks the clinical antidiabetic response [15].

Rosiglitazone blocks that phosphorylation. So does MRL24, a non-TZD ligand of the benzoyl 2-methylindole class, with high affinity for PPARγ but poor agonist activity [18]: 30 nM MRL24 blocked the modification as completely as 300 nM rosiglitazone, at concentrations where MRL24 had essentially undetectable transcriptional activity (Fig. 2b) [15]. The follow-up study produced SR1664, a ligand that binds tightly, blocks Ser273 phosphorylation, improves insulin sensitivity in mice, and is devoid of classical agonism and of adipogenic activity [16].

This is what the word modulator means, and why it is not interchangeable with agonist. A compound optimised for transactivation potency is being optimised for the activity that produced the oedema, the weight gain and the heart failure. A compound optimised for Ser273 blockade is being optimised for the activity that lowers glucose.

4.2 How partial agonists bind, and why it matters for docking

The structural basis is well characterised and has a direct consequence for any modelling section. Full agonists such as rosiglitazone place their acidic head group in the AF-2 pocket, hydrogen bonding to Tyr473 on helix 12, His449, His323 and Ser289, which locks helix 12 and recruits coactivator [2,31]. Partial agonists and non-agonists occupy a different sub-pocket, between helix 3, the Ω-loop and the β-sheet, typically hydrogen bonding to Ser342 and stabilising the β-sheet region that carries Ser273, without engaging helix 12 at all [32,33]. Table 2 sets the two modes side by side.

A docking study that reports a strong hydrogen bond from the thiazolidinedione head group to Tyr473 has therefore not supported the project's stated aim; it has predicted full agonism. The informative result for a modulator programme is the opposite one: occupancy of the β-sheet sub-pocket, contact with Ser342, and a pose that leaves helix 12 unengaged. Reporting which of the two modes a compound adopts is more useful than reporting a binding energy, and it is a prediction that an experiment can check.

Two practical points follow for setting up the calculation. The PPARγ ligand-binding pocket is unusually large, roughly 1300 Å^3, and an alternate binding site outside the canonical pocket has been characterised, so a docking box drawn tightly around a co-crystallised rosiglitazone will exclude in advance the sub-pocket the project cares about [33]. The box should cover the whole cavity. And because PPARγ functions as a heterodimer with RXRα whose

two halves are conformationally asymmetric, the choice of crystal structure is not neutral [31]: a structure solved with a full agonist has helix 12 already locked, which biases any pose toward the agonist mode. Stating which Protein Data Bank entry was used, and why, is part of reporting the result.

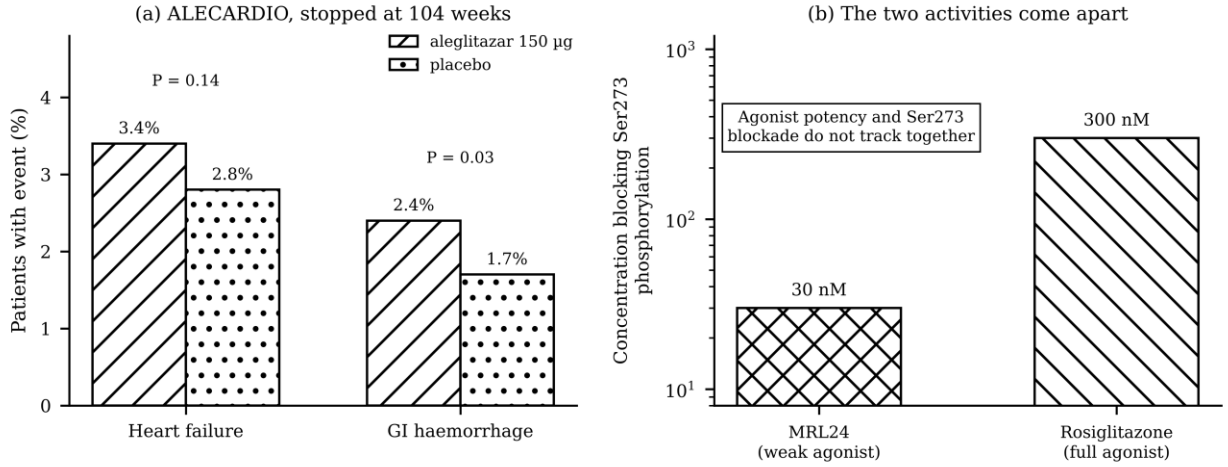


Fig. 2. (a) Safety endpoints from ALECARDIO, which randomised 7228 patients and was stopped at a median of 104 weeks for futility and safety [11]. (b) Concentrations at which the weak agonist MRL24 and the full agonist rosiglitazone block CDK5-mediated phosphorylation of PPARγ at Ser273; MRL24 achieves it at a tenth the concentration while showing essentially no transcriptional activity [15].

Table 2. The two binding modes at PPARγ and the observations that separate them. A project aiming at a modulator is aiming at the right column [2,15,16,31–33].

Property	Full agonist (e.g. rosiglitazone)	Partial agonist / non-agonist modulator
Binding sub-pocket	AF-2 pocket around helix 12	Between helix 3, the Ω-loop and the β-sheet
Key hydrogen bonds	Tyr473, His449, His323, Ser289	Ser342 and the β-sheet backbone; helix 12 not engaged
Transactivation in a reporter assay	Maximal	Weak to undetectable
Blockade of CDK5 phosphorylation at Ser273	Yes, at around 300 nM for rosiglitazone	Yes, at around 30 nM for MRL24
Adipogenesis in 3T3-L1 cells	Strongly induced	Little or none
Expected liabilities	Fluid retention, weight gain, heart failure, fracture	Reduced in rodent studies; not yet tested in a large outcome trial

V. CHOOSING ENDPOINTS THAT CAN DISTINGUISH THE TWO

Table 3 sets out the questions a dual-modulator series has to answer and the assay that answers each. Three points deserve emphasis.

A transactivation reporter assay measures agonism, which for this project is the activity to be limited rather than maximised. It remains worth running, because the ratio of PPARα to PPARγ transactivation is the quantity that distinguishes a saroglitazar-like profile from an aleglitazar-like one, but it should not be the primary screen, and reporting an EC_{50} at PPARγ as evidence of promise inverts the argument.

An adipogenesis assay in 3T3-L1 cells is the cheapest available proxy for the liability. A compound that drives lipid accumulation as strongly as rosiglitazone is behaving as a full agonist whatever else is claimed for it; one that does not, while still lowering glucose in vivo, is the profile being sought [16,17].

In vivo, the high-fat-fed, low-dose streptozotocin rat reproduces the insulin-resistant phenotype these compounds are intended for and is widely used for

screening [41]. The endpoints that matter are fasting glucose and insulin with a calculated HOMA-IR, an oral glucose tolerance test, the lipid panel for the antihyperlipidaemic claim, and — critically for this class — body weight, food intake, haematocrit and any oedema, recorded and reported rather than omitted [40]. The adverse effects that ended the glitazars are measurable in a rodent study, and a series that does not measure them has not tested its own hypothesis.

The antihyperlipidaemic half of the title deserves its own endpoints rather than a line in a table. PPARα activation lowers triglycerides by inducing lipoprotein lipase and suppressing apolipoprotein C-III, and raises HDL cholesterol by inducing apolipoproteins A-I and A-II [12,36]. A series claiming PPARα engagement can therefore be tested on something more specific than a total-cholesterol number: the triglyceride fall relative to the HDL rise, and, where quantitative PCR is available, expression of a PPARα target gene such as acyl-CoA oxidase in liver. That is a modest addition to a dissertation protocol and it converts a general lipid-lowering observation into evidence about the stated mechanism. It also guards against the commonest confound in this literature, which is that an animal eating less because it feels unwell will show an improved lipid panel for reasons that have nothing to do with the receptor.

Table 3. Questions a dual PPAR modulator series must answer, and the experiment that answers each [15,16,29,30,40,41].

Question	Assay	What a positive result means	Common substitute that does not answer it
Does it bind PPARγ?	Competitive binding or thermal-shift assay	Affinity, independent of what the ligand then does	A docking score
Does it activate PPARγ?	Transactivation reporter in transfected cells	Agonism — the activity to be limited, not maximised	Treating a low EC_{50} as the project's success criterion
Is it α-selective enough?	Parallel PPARα and PPARγ reporter assays	A ratio; α-dominance is the profile of the one approved agent	Two EC_{50} values reported separately and never compared
Does it avoid the liability?	3T3-L1 adipogenesis; body weight, oedema and haematocrit in vivo	Separation of glucose lowering from adipogenic and fluid effects	Omitting weight and haematocrit from the results
Does it act by the modulator mechanism?	Immunoblot for PPARγ phospho-Ser273 in treated adipocytes	Engagement of the activity that tracks the antidiabetic effect	Asserting the mechanism from a docking pose
Does it work in vivo?	High-fat-fed, low-dose streptozotocin rat; OGTT, HOMA-IR, lipid panel	Antidiabetic and antihyperlipidaemic efficacy in a relevant phenotype	Acute oral glucose tolerance in normal animals only

VI. A CHECKLIST FOR THIS PROJECT

For the chemistry: state explicitly whether the compounds are 5-arylidene or reduced 5-benzyl thiazolidinediones, and if the series stops at the arylidene stage, describe them as arylidene derivatives rather than glitazone analogues; report the C-5 proton in the ^{1}H spectrum, near δ 7.7 for the arylidene vinyl proton and near δ 4.9 as a doublet of doublets for the saturated compound, which distinguishes them unambiguously; describe reduced compounds as racemic; run a thiol-competition control on any arylidene series before attributing activity to receptor binding.

For the pharmacology: present PPARα and PPARγ transactivation as a ratio rather than as two isolated EC_{50} values; include a 3T3-L1 adipogenesis assay as the liability counter-screen; and, where a collaborating laboratory makes it possible, assay Ser273 phosphorylation, because that is the endpoint the design brief actually targets.

For the modelling: dock both C-5 epimers, or state that the configuration is arbitrary; report which sub-pocket the pose occupies and whether helix 12 is engaged, not only a score; and redock a co-crystallised ligand with an RMSD so the protocol is validated against a known answer [29,30].

For the in vivo work: report weight, oedema and haematocrit alongside glucose and lipids, and report them whichever way they come out.

VII. CONCLUSION

There is still a case for new chemistry on this scaffold, but it is a narrower and more interesting case than the one usually made. The broad case — that dual PPAR-α/γ agonism would treat hyperglycaemia and dyslipidaemia together — has been tested five times in late-phase development and has failed each time, for reasons traceable to full PPARγ agonism rather than to the individual molecules. The narrower case is that the antidiabetic signal and the liability signal come from different activities at the same receptor, that ligands exist which deliver the first with little of the second, and that nobody has yet done this from a thiazolidinedione head group with a PPARα-active tail.

Pursuing that case changes what is made and what is measured rather than requiring new equipment. It means reducing the arylidene instead of stopping at it; reporting the racemate honestly instead of claiming a stereochemistry the ring will not hold; docking for the β-sheet sub-pocket instead of for Tyr473; and running an adipogenesis assay alongside the glucose data. A series handled that way can produce a negative result that is worth reading, which is more than most of this literature manages.

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