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The Development Of Saroglitazar An Understanding Of The Procedures And Development Of A New Chemical Entity (Nce) At Zydus Lifesciences Limited

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I. Introduction

Zydus Lifesciences Limited is an Indian multinational pharmaceutical company based in Ahmedabad, India. Previously known as Cadila Healthcare Limited, it was founded in 1952 by Mr. Ramanbhai Patel. It is India's fourth-largest pharmaceutical company with more than 30 manufacturing plants worldwide and business operations in 55 countries including, India, the US, France, Spain, Brazil, Mexico, and South Africa. As one of the key players among pharmaceutical manufacturing companies, Zydus Lifesciences has manufacturing capabilities across the entire pharmaceutical value chain including formulations, APIs, vaccines, biosimilars, complex products, animal health products, and wellness products. Traditionally known for manufacturing generic drugs, it is one of India's first companies which discovered, developed, and marketed its own chemical entities. Zydus Lifesciences ZyCoV-D vaccine was the world's first plasmid DNA vaccine to be approved for human use and is currently approved in India as a two-dose regimen for adults and children aged 12 years and above. Similarly, it worked to produce Saroglitazar, a drug used to battle diabetic dyslipidemia.

Background (Why Diabetic Dyslipidemia)

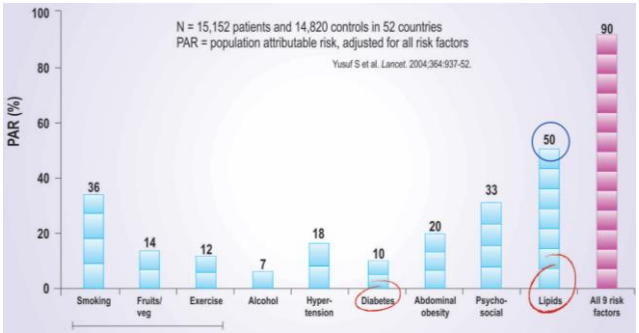
Dyslipidemia is a condition caused by the abnormal increase in one or more types of lipids (fats) in the human blood. The body has three types of lipids - high-density lipoproteins (HDLs), low-density lipoproteins (LDLs), and triglycerides. It can also be referred to as an increase in high serum cholesterol and high plasma triglyceride levels to be specific. The disease has no symptoms but can heavily impact your lifestyle in terms of increasing the chance of clogged arteries (atherosclerosis) and heart attacks, strokes, or other circulatory concerns. High cholesterol can be caused by an increase in production by the liver to help digest foods or an increase in the intake of cholesterol-heavy foods. Other causes include smoking, a lack of exercise, a sedentary lifestyle, and even hypertension as seen in Figure 1 (*Yusuf S et al, Lancet; 364:937-52, 2004).*

However, it is most probable to be caused by diabetes, specifically type II diabetes. Diabetes increases the insulin in the body during the first reaction. In a diabetic patient, the glucose doesn't enter the liver and muscle cells for use, it is instead stored as glucagon due to the release of insulin as a part of the negative feedback loop that occurs. The influence and requirement of drug therapy further increase the levels of insulin in a patient's system due to the increase in insulin-based medication as well, to help reduce blood sugar levels. The increase in insulin directly correlates to the level of plasma triglycerides, in this case causing it to increase as well. The disease is statistically seen to be more prominent in females.

- For every increase in TG level of 89 mg/dL, CVD risk increases by 32% in men and 76% in women

Meta-analysis of 17 studies (> 55,000 patients)

Zydus particularly targeted this disease due to the high risks that it poses. Increased lipid levels are one of the primary causes of myocardial infections (heart attacks), and thus the need to control lipid increases grew. Every 3 out of 4 diabetics suffer from dyslipidemia, if one has both they are more than 75% of the population that is suffering from dyslipidemia. Thus while controlling diabetes will further control the chances of dyslipidemia. Furthermore, the blood cholesterol



level will increase at least 2 fold for someone that's diabetic. A person with diabetic dyslipidemia has a higher chance of going into cardiac arrest which makes them more vulnerable to disabilities, as seen in figure 2 (*J Stamler et al, Diabetes Care February 1993:16:434-444*). Therefore, the company has underscored this particular disease to find a suitable medical solution to.

	Men with Diabetes			Men without Diabetes				
	MEN (N)	CVD DEATHS (N)	Rate (Per 10,000 Person-YR)	MEN (N)	CVD DEATHS (N)	Rate (Per 10,000 Person-YR)	RR For Diabetic / Nondiabetic	Absolute Excess Risk For Diabetic Minus Nondiabetic / (Per 10,000 Person - Yr)
SERUM CHOLESTEROL Level (MG/DL)								
< 180	1105	96	**61.72**	62,448	859	**13.84**	4.46	47.88
180-199	972	101	**76.67**	64,363	1223	**17.27**	4.44	59.40
200-219	1038	128	**84.79**	75,122	1750	**20.19**	4.20	64.60
220-239	823	96	**80.94**	60,386	1767	**24.53**	3.30	56.41
240-259	529	68	**91.99**	40,090	1411	**29.02**	3.17	62.97
260-279	323	58	**139.34**	22,802	983	**35.53**	3.92	103.81
≥ 280	353	56	**130.43**	17,604	972	**46.12**	2.83	84.31
Coefficient*		0.0030 ± 0.0007			0.0061 ± 0.0002			

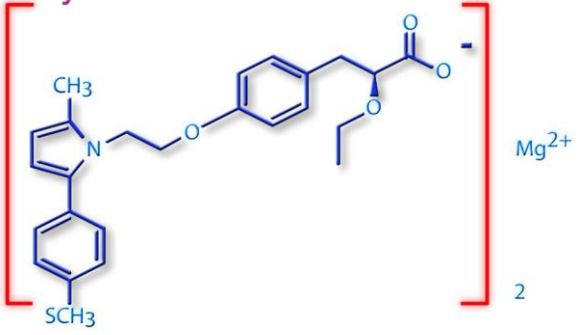
Furthermore, the previous management of Diabeties a in the market was working for some patients but not on the scale that was necessary. 75% of those diagnosed were still suffering. The drug created by Zydus started being researched in 2010 when over 55 million people in India had been diagnosed. Those patients are still not receiving complete therapy that can cure them or keep their disease in check, thus emphasizing the need for the drug. Previous and current treatments and management included lifestyle changes and the treatment of secondary causes such as diabetes, hypothyroidism, obesity, etc, along with pharmacologic therapy. This included lipid management through drugs such as Statins, Fibrate, Niacin, and omega-3 fatty acids. Antidiabetic therapy and combination therapy were also other ways to combat the disease. However, even after giving these drugs along with a placebo (A placebo is a medical or pharmacological intervention that has no therapeutic effect on the condition it is meant to treat. It is often used in clinical trials and medical research as a control to compare the effects of an active treatment (e.g., a new drug) against those of a non-active substance, such as a sugar pill, saline solution, or inactive substance) there's only a slight decrease in the risk of CVD by 20-35%. Studies have also proved that statins and other current drugs are not sufficient. Residual CVD risk still remains in patients with diabetes who have been treated with statins. Statins can in fact cause new-onset diabetes in recent reports.

Basics of the drug

The drug created by Zydus was named “Saroglitazar”. Saro in Gujarati means ‘good’ while Glitazar is the class. It belongs to a class of drugs known as dual peroxisome proliferator-activated receptor (PPAR) agonists. PPARs are nuclear receptors that play a role in regulating various metabolic processes in the body, including lipid (fat) metabolism and glucose homeostasis. It is a small molecule with the name of the formulation of the drug/ brand name ‘Lipaglyn. Lipglyn is the name of the formulation of the drug/ this is the brand name. (the brand is always a formulation, that can be in different forms such as an injectible or ingestible, etc..)

Diabetes increases blood sugar and blood lipids such as triglycerides and glycerol. A diabetic person wouldn't only have high sugar but also high lipids (this is known as dyslipidemia). Until now dyslipidemia was found to be a disease of the rich and of the West, thus a drug hadn't been invented due to it not being needed for ‘lifestyle diseases’. It s is broadly a Cardiovascular disease that included dyslipidemia and it’s made its way into the higher percentile of the low-middle-income countries as one of the most severe diseases. Simply put, the drug works by regulating lipid and glucose metabolism, potentially reducing lipid levels in the blood and improving insulin sensitivity.

Figure 4: Chemical structure of Saroglitazar



II. The development procedure/ method of development

How drugs are discovered and made

The following procedure is the standard drug formulation procedure, that has been followed through for the creation of Saroglitazar: The researchers at Zydus first identify the target. In a regular drug, this target could be a gene or a protein involved in the disease in our body. In this case, the condition is diabetic dyslipidemia where there's a high amount of lipids in a person who's also hyperglycemic. The drug was made to target the PPAR gene that existed. The compound could attack the gene in two ways: by either increasing or decreasing the activity of the gene. This sort of alternation in the functioning of the gene bases itself off of the structure of the gene - that's made up of DNA and nucleic acids or a protein - that's made up of amino acids. These can be seen as lock and key models, where chemists have to find which compound would fit the gene to change it. This works on a trial-and-error basis until the model fits. The first fitting model is known as the HIT structure - this is seen once putting the compound and protein in a testing tube and seeing the change occurring in the protein.

Furthermore, to get the compound to fit the gene or protein, it has to identify the location that the gene or protein is targets. For instance, diabetic dyslipidemia targets the liver and thus the drug should be able to make its way to the liver. This is known as lead identification. However, the HIT compound might not meet all the criteria to make it a LEAD compound, and similarly, the LEAD compound might not have all the necessary components to make it a suitable drug. Therefore, you keep optimizing the compounds found until you get a few that work. Once a primary staged few drugs are found, the formal preclinical testing begins.

Pre-clinical testing

This section explores pre-clinical testing in generic drugs and its application when creating Saroglitazar. Testing starts off by taking place in animals - rats, mice, dogs, and monkeys. The disease is inhibited and modeled in these animals simply by interesting the diseased gene into them. The different formulations are then tested. The results of this is known as the preclinical model. Animal testing required stages of authorization and permission from the authorities present. During the analysis of the results and throughout the process, the side effects are meant to be observed in a variety of body parts to record their optimal usage and whole effect. These body parts include the liver, kidneys, testes, ovaries, brain, and heart primarily; however, all standard vitals should be measured. Once the animal studies have been conducted, assessments have to be undergone before clinical testing, ie. human testing begins to take place. A margin between the most toxic dose and the most effective dose has to be calculated. If the toxic dose, for instance, is tenfold more than the effective dose, it depends upon the authorities to advise changes made to the drug to adjust the toxicity levels to make it safe enough to be tested on humans. There are also 2 types of animal testing: Pharmacokinetics - PK, in which the scientists measure the amount of drug needed in the blood and test if its purpose is being achieved of reducing triglycerides solely, and the second Pharmakodynamics which studies the effect of the drug throughout the body.

Saroglitazars pre-clinical models:

- In vivo Efficacy Studies
- Pharmacodynamic effects in db/db mice
- Pharmacodynamic effects in Zucker fa/fa rats
- Pharmacodynamic effects in ob/ob mice
- Pharmacodynamic effects in Swiss albino mice
- Pharmacodynamic effects in Golden Syrian hamsters fed on HF-HC diet
- Pharmacodynamic effects in Sprague Dawley rats fed on high cholesterol (HC) diet
- Pharmacodynamic Effects in Primates (Marmosets)

Preclinical results:

Table 1: Preclinical result observed in testing subjects

Testing subject	Observation
Zucker fatty fa/fa Rats: Animal model for Insulin-resistance	85% reduction in serum insulin; Up to 52 % reduction in AUC_{Glu} in OGTT + Up to 86 % TG reduction + Improvement in Glucose infusion rate in hyperinsulinemic-euglycemic clamp study
Swiss Albino Mice - Non-diabetic model	Up to a 68% improvement in lipid clearance + Up to 76 % TG reduction + Potential for Postprandial hyperlipidemia
Human ApoB100-CETP trangenic mice models	82 % reduction in LDL-C with ED50 of 0.44 mg/kg & 67% reduction in total cholesterol in mice, which has human-like lipid metabolism

High fat-High cholesterol-fed Hamsters - Non-diabetic model	Up to 90 % TG reduction
db/db mice	Up to 55% TG reduction
Primates	Up to 61% reduction in serum triglycerides

Conclusions of Acute & Repeated Dose Safety Toxicity Studies

In acute dose studies, the maximum tolerated dose (MTD) in Swiss albino mice was 500 mg/kg, and in Wistar rat, it was 1200 mg/kg. Furthermore, In repeat dose toxicity studies, Saroglitazar was shown to have an acceptable safety profile at doses several-fold higher than the approved human doses. At high doses, the toxic effects observed were mainly the exaggerated pharmacological effects mediated by PPAR mechanisms. Overall, no hepatotoxicity, myotoxicity, nephrotoxicity, or cardiotoxicity at doses equivalent to or higher than efficacy doses were observed. The toxic effects observed at very high doses were mainly the exaggerated pharmacological effects mediated by PPAR mechanisms. Non-genotoxic & Non-teratogenic Passed 2-year carcinogenicity study (confirmed by a mechanistic study using non-human primates employing molecular biomarkers). Overall Saroglitazar was safe and well tolerated in the pre-clinical stage.

Table 2: The outline of the drug's pathway and its general effect.

ADME	Findings
Absorption	The drug was rapidly absorbed with good oral bioavailability. T1/2 of about 3-4 hours.
Distribution	Plasma protein binding of about 96% was present in rodents along with no drug levels being detectable in any tissues 24 hours after the last dose was taken, in a 12-month repeated dose toxicology study in Beagle dogs.
Metabolism	The drug was stable in liver microsomes and did not show any CYP interaction. There was no potential for CYP-mediated drug-drug interactions.
Excretion	The drug was eliminated by non-renal routes and unchanged Saroglitazar was not detectable in urine. It was mainly eliminated by the hepatic-biliary route.

Clinical studies

Post the preclinical studies the Phase 1 clinical study begins. A selective number of patients are gathered as control groups with no presence of the disease recorded. Researchers continuously give the doses at different time periods and might even increase their weight until a certain level of toxicity is recorded in the human body. This creates a threshold level, apprising the chemists of the maximum dosage required. Moving forward, phase 2 begins to test patients with the disease. The success rate is then compared with the concentration of lipids in normal patients to determine the effectiveness of the drug.

Clinical testing results: (quotes from studies conducted)

- No Serious Adverse Events (SAEs) were reported during the study.
- Saroglitazar up to 128 mg once orally was well tolerated.
- Saroglitazar single and multiple doses were safe and well tolerated.
- Pharmacokinetics (Cmax, AUC) was dose-dependent and linear.

Overall Conclusions of Preclinical Safety & Toxicity Studies:

Saroglitazar demonstrated: Reduction in Triglyceride levels LDL Total cholesterol Fasting plasma glucose Insulin C-reactive protein including an increase in HDL levels. During stage 2 of the clinical trials, tests on different body parts were also conducted to evaluate their stability. The results of these tests are as follows:

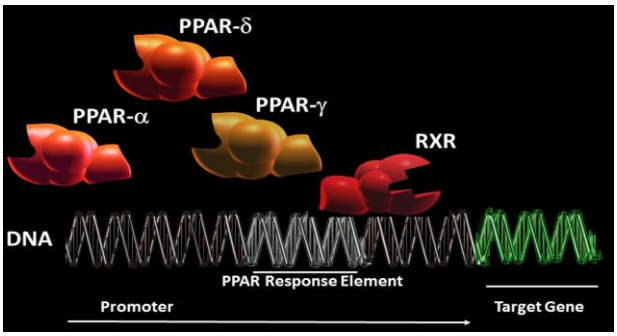
- Liver function test: No potential for drug-induced liver injury
- Renal function test: No potential for kidney toxicity
- Musculoskeletal effect: No report of myolitis (CPK>10UNL).
- ECG abnormality/cardiotoxicity: No abnormality reported

How the drug works in the human body

Saroglitazar is a novel drug that has gained significant attention for its potential in treating dyslipidemia and diabetes. As a dual peroxisome proliferator-activated receptor (PPAR) agonist, Saroglitazar exerts its therapeutic effects by modulating gene expression and lipid metabolism. This section aims to elucidate the

molecular mechanism of action of Saroglitazar in the human body, focusing on its interactions with PPARα and PPARγ receptors and the subsequent downstream effects on lipid and glucose homeostasis. Understanding the mechanisms underlying saroglitazar's action will provide valuable insights for future drug development and clinical use.

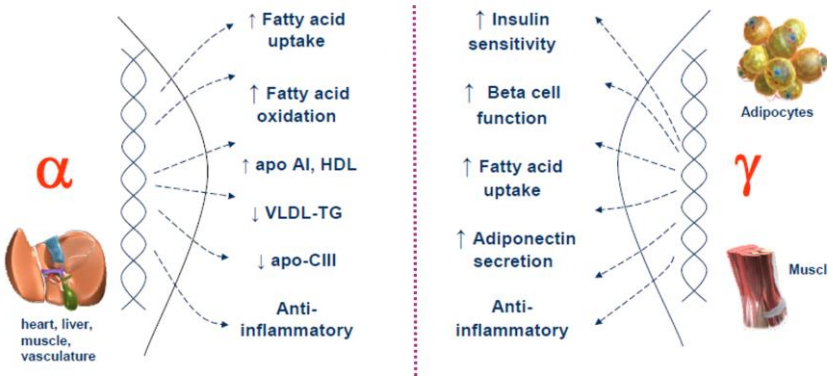
Primarily, understanding PPAR receptors and their roles is crucial. PPARs are Nuclear Receptors (lipid-activated transcription factors) that regulate the expression of various genes involved in the control of lipid and lipoprotein metabolism, glucose homeostasis, and inflammatory processes. When this gene is activated it changes the function of the gene that focuses on reducing the lipids in the body; thus wanting to increase the activity of this gene. There are 3 types of PPAR genes/ receptors: alpha, gamma, and delta. RX receptor also exists, as seen in Figure 5 (*CURRENT SCIENCE, VOL. 79, NO. 10, 25 NOVEMBER 2000)*. If the PPARS want to work they need RXR as well. Heterodimer (a combination of both) activates the target genes to make the proteins/ enzymes that control glucose levels, lipids, and inflammation.



(1) Peroxisome Proliferator-Activated Receptor Alpha (PPARα) PPARα is predominantly expressed in the liver, heart, and skeletal muscle. Its activation plays a crucial role in the regulation of lipid metabolism, specifically in fatty acid oxidation, lipoprotein metabolism, and triglyceride homeostasis. (2) Peroxisome Proliferator-Activated Receptor Gamma (PPARγ) PPARγ is mainly found in adipose tissue and plays a pivotal role in adipogenesis, glucose metabolism, and insulin sensitivity. Saroglitazar exhibits dual PPAR agonism, simultaneously activating both PPARα and PPARγ receptors. This unique feature distinguishes Saroglitazar from other PPAR agonists and contributes to its therapeutic effects on dyslipidemia and diabetes.

The mechanism of action of the drug works through the activation of PPARα - to improve the plasma lipid profile and PPARγ - to improve insulin sensitivity. (1) Upon administration, Saroglitazar binds to and activates PPARα receptors in the liver, heart, and skeletal muscle. The activated receptor heterodimerizes with retinoid X receptor (RXR) and binds to specific PPAR response elements (PPREs) in the promoter region of target genes involved in lipid metabolism. This binding stimulates the transcription of genes encoding enzymes responsible for fatty acid uptake, β-oxidation, and lipoprotein metabolism. Consequently, Saroglitazar reduces plasma triglyceride levels and enhances high-density lipoprotein (HDL) cholesterol levels, thereby ameliorating dyslipidemia. (2) Simultaneously, Saroglitazar also activates PPARγ receptors in adipose tissue. PPARγ activation leads to enhanced insulin sensitivity, improved glucose uptake by adipocytes, and reduced adipose tissue inflammation. These effects contribute to better glycemic control, making Saroglitazar beneficial in managing diabetes.

Figure 6: Summary of the effects of PPARa and PPARy (*Diabetes. 2005 Aug;54(8):2460-70)*



Testing whether this drug actually worked in the human body follows its own process. The chemical in the drug is to bind to the targetted protein. A cell culture is also present - it proved all the machinery to the gene that is required for it to function the same way it would in the body. This cell culture is maintained in a watery solution also known as the aqueous buffer where the drug is kept. The PPAR gene would then be creating what it normally would in the body. The gene produces the protein that's not otherwise available in the cell, this is tested

through an indicator, and when there's light and fluorescence produced it means the drug worked. Such experiments can be conducted in-vitro (IV - in the body) and ex-vitro (EV - outside of the body)

Saroglitazar's unique mechanism of action as a dual PPAR agonist offers a promising therapeutic approach for managing dyslipidemia and diabetes simultaneously. Its ability to regulate lipid metabolism and glucose homeostasis presents a novel and effective treatment option for patients with coexisting dyslipidemia and diabetes.

Additional Benefits of Saroglitazar Compared to failed Glitazars

Structurally different Glitazars failed due to different types of safety concerns such as CKD (), cancer, a CV event, or even lead to a bone fracture. Hence, they can not be labeled as a class effect of the Glitazar family. The majority of failed glitazar had major action on PPAR gamma activity along with PPAR alpha activity. The Mevastatin failure specifically, in 1972, laid the foundation stone for the discovery of many statins of today considered as life-saving. Further on, Phenformin failed but laid the foundation stone for the only lifesaver today in T2DM, i.e. metformin. Overall, there were multiple failures in the individual molecule, but not the concept, which led them to improve on and create the final drug.

Moreover, other benefits have been observed from the selected molecule. Apart from its dual PPAR agonistic activity, Saroglitazar has been reported to exert anti-inflammatory and antioxidant effects, which further contribute to its therapeutic potential in mitigating cardiovascular complications associated with dyslipidemia and diabetes. Furthermore, several clinical trials have demonstrated the efficacy and safety of Saroglitazar in patients with dyslipidemia and diabetes. The drug has shown significant improvements in lipid and glycemic parameters without severe adverse effects.

III. Conclusion

In retrospect and summary, Saroglitazar is a New Molecular Entity (NME) designed & developed by Zydus Cadila Belonging to α-alkoxy-aryl propionic acid class Saroglitazar is a potent & predominant PPARα agonist with moderate PPAR activity. The development of the drug took over 12 years with the final product profile launching in 2013, with a suggested dosage of 4mg (tablet) once daily through oral dosing, making it the first approved glitazar class drug. Saroglitazar has shown promise as a dual peroxisome proliferator-activated receptor (PPAR) agonist, providing potential benefits in the management of dyslipidemia and diabetes mellitus. Through its action on lipid and glucose metabolism, Saroglitazar has the potential to improve lipid profiles, reduce triglycerides, increase HDL cholesterol, and aid in glycemic control. Moreover, it may offer hepatic benefits by addressing non-alcoholic fatty liver disease (NAFLD). These effects make Saroglitazar a valuable therapeutic option for patients with combined dyslipidemia and diabetes, simplifying treatment regimens and potentially reducing the risk of cardiovascular complications.

Further investigations can also be conducted, for instance, exploring long-term safety and efficacy: As with any new medication, continuous research and post-marketing surveillance are essential to assess the long-term safety and efficacy of Saroglitazar in diverse patient populations. Comparative studies can also be conducted with trials pitting Saroglitazar against other established medications used for dyslipidemia and diabetes could provide valuable insights into its relative effectiveness and safety profile. Expanded indications and investigating the potential use of Saroglitazar in other metabolic disorders and associated complications, such as obesity, metabolic syndrome, and cardiovascular diseases, may broaden its clinical applications. Furthermore, exploring combination therapies and exploring the use of Saroglitazar in combination with other medications could unveil synergistic effects, potentially enhancing overall treatment outcomes.

In conclusion, while Saroglitazar holds promise as a valuable therapeutic option for dyslipidemia and diabetes, ongoing research and future investigations are necessary to fully understand its potential, optimize its use, and explore its applications in other metabolic disorders. With a commitment to rigorous scientific exploration, Saroglitazar could contribute significantly to improving the health and well-being of patients in the future.

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