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Synthesis and Biological Evaluation of Some Novel PPAR-γ agonists for the Management of Type II Diabetes

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Abstract

Diabetes mellitus (DM) is a progressive disease characterized by hyperglycemia due to insulin deficiency and insulin resistance or both. The fasting and postprandial blood glucose gets elevated, exposing the patient to acute and chronic complications (micro- and macro-vascular) leads to blindness, kidney failure, heart disease, stroke and amputations. Diabetes mellitus is one of the most common endocrine disorders affecting almost 6% of the world's population. The number of diabetic patients will reach 300 million in 2025. More than 97% of these patients will have type II diabetes. "Glitazones," bind to ppar-γ, a type of nuclear regulatory proteins involved in transcription of genes regulating glucose and fat metabolism. These PPAR-γ acts on Peroxisome Proliferator Responsive Elements (PPRE). In the present article, Rhodanine analogs were screened for their anti diabetic activity. Rosiglitazone is used as a reference standard. The compounds R2, R5 and R7 shows better activity than the reference compound and was found to be effective as compared to the other synthesized compounds. The determined fasting blood glucose levels were monitored and the decrease in the blood glucose level was calculated. Some of the synthesized Rhodanine derivatives have shown considerable improved efficacy and improved biological response which is enough reason to believe that PPARγ is a promising target for the management of type II diabetes and that Rhodanine analogs have it in them to illicit a biological response out of the receptor.

Key-Words: Diabetes, Synthesis, PPARγ

Introduction

Diabetes mellitus (DM) is a progressive disease characterized by hyperglycemia due to insulin deficiency and insulin resistance or both. The insulin-insensitive form of diabetes, type 2 diabetes mellitus (T2DM), characterized by hyperglycemia (elevated blood glucose concentrations), most frequently arises as a consequence of obesity, represents approximately 95% of the overall incidence of diabetes. Additionally, diabetes-related complications exert a heavy toll on patients with poor metabolic control[13, 14, 16] . The regulation of blood glucose homoeostasis is complex. T2D is characterized by elevated blood glucose levels or hyperglycemia, and results from failure of pancreatic β-cell to secrete sufficient insulin to overcome insulin resistance (mainly in liver, adipose and skeletal muscle). PPAR γ has been known to be associated with the control of Diabetes and has been used as a target for the management of the disease.

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In the presence of a ligand, PPARs heterodimerize with RXR, a retinoid receptor, and modulate transcription of target genes by binding to PPAR response elements in the promoter region of target genes. PPAR α influences glucose homeostasis. Under fasting conditions, PPAR α null mice suffer from hypoglycaemia and hyperinsulinaemia. Mostly Thiazolidinediones and their analouges have been shown to activate this enzyme with various degrees of efficacy and specificity. But often fail to proceed any further either because of side effects such as weight gain, edema etc. or as a result of poor efficacy. Therefore it is worthwhile to develop new PPAR γ activators. Thus for this purpose identification of compounds is essential that are aimed at resolving this issue. The objective is to synthesize the compounds which are efficacious than those present in the market and possess minimum side effects.

Material and Methods

The interaction study of Rhodanine analogs and PPAR γ was performed through docking using *MOLEGRO*

VIRTUAL DOCKER 2010.4.2. Docking was performed through following step:

Ligand Preparation

The initial structure of molecules was build through ChemDraw and subsequently energy was minimized using MM2 and MOPAC. Minimized structured are saved as "mol" file format for further study

Protein Preparation

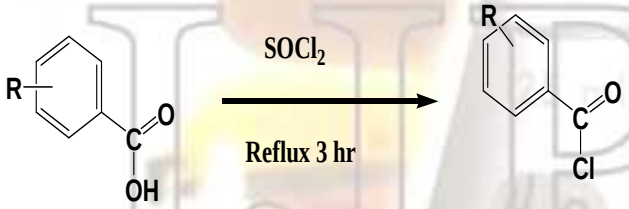
The X-ray crystal structure of the PPAR γ enzyme (PDB ID: 4EMA) was retrieved from protein data bank. The enzyme consisted of two chins and the chain with more number of amino acids was used for docking of the ligand. The surface of the protein was created and the cavities were detected. The largest cavity in terms of volume was chosen for docking.

Docking of Design Compounds

The minimized structures of ligands and prepared structure of protein were subsequently used in docking simulation. The default parameters were considered for docking. The MolDock score and ReRank score of the compounds and ligand-macromolecular interactions were considered for the selection of compounds.

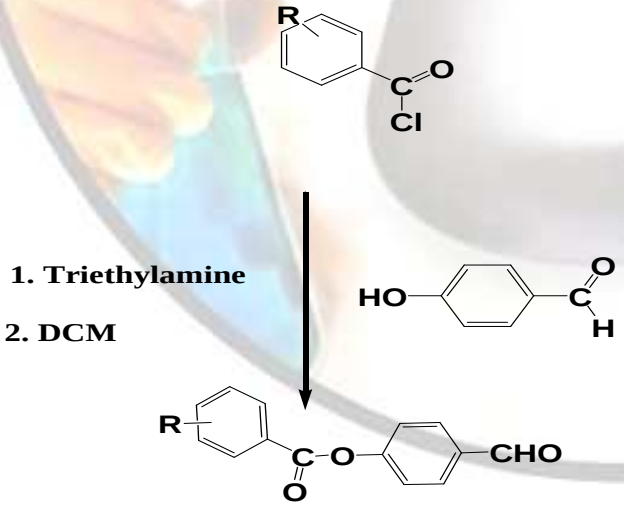
Synthesis

Step I – Synthesis of acid chloride



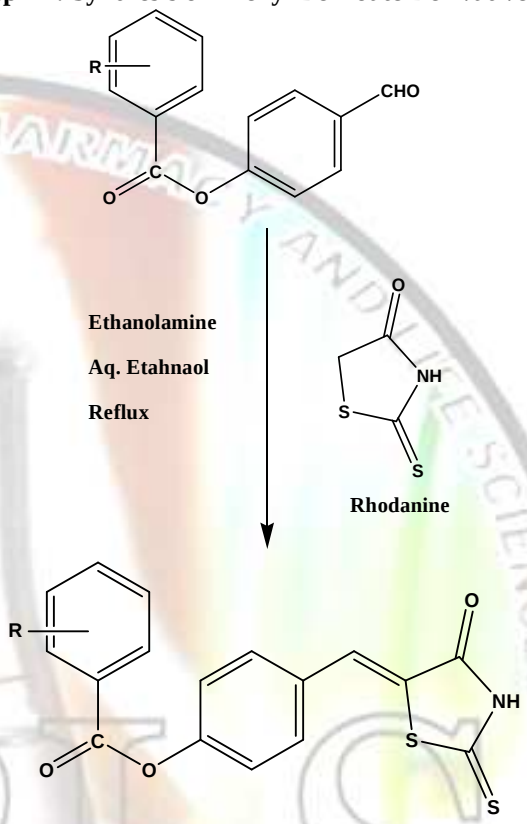
Substituted Benzoic Acids **Acid Chloride**

Step-II: Synthesis of aldehyde ester



Aldehyde Ester

Step III: Synthesis of Phenyl Benzoate Derivatives



(Z)-4-((4-oxo-2-thioxothiazolidin-5-ylidene) methyl) phenyl substituted benzoate

General synthetic procedure

General Synthesis of Comp. (Step I)

Benzoic acid derivatives (0.01 mole) were refluxed with thionyl chloride (10 ml.) for 3- 4 hrs and progress of the reaction was monitored through TLC. On completion of the reaction, excess of thionyl chloride was removed under vacuum. The residue was used as such in next step.

General Synthesis of Comp. (Step II)

Benzoyl chloride analogs were taken into 20 mL of dichloromethane in RBF and cooled to 0°C. To this reaction mixture triethylamine (0.03 mole) was added slowly with constant stirring. Followed by p-hydroxy benzaldehyde (0.01 mole), was added with stirring. The reaction mixture was stirred at 0°C for another 2 hrs. and stirring continued at RT for overnight. Progress of the reaction mixture was checked through TLC. Then reaction mixture washed with saturated solution of sodium bicarbonate, brine solution and water. Organic phase was separated and pass through anhydrous Na_2SO_4. Solvent was removed under vacuum and recrystallized by ethanol.

General Synthesis of Comp. (Step III)

Rhodanine (7.4 mM) was dissolved in 10 mL water at 70°C on oil bath with continuous stirring. After complete dissolution the pH was adjusted to 7.0 using saturated sodium bicarbonate solution. The temperature was then raised to 90°C after the addition of 0.9 mL ethanolamine. Equimolar quantity of the 4-chlorobenzaldehyde solution in 5 mL ethyl alcohol was then added drop wise with continuous stirring. The reaction was kept under reflux for approximately 8h. The reaction was monitored by TLC. After complete depletion of the starting aldehyde, the mixture was cooled and the precipitate was filtered and washed with ethanol/water (1:5).

Results and Discussion

On the basis of literature study Rhodanine analogs were designed. The The basis of selection of compounds was their docking score; compounds (Der01-Der07) were synthesized by Knoevengal condensation reaction. The progress of the reaction was monitored through thin layer chromatography. The synthesized compounds were characterized by TLC, melting point, IR, NMR & Mass spectroscopy. Structure of synthesized compounds was elucidated by ^{1}H NMR, Mass spectral data and IR findings. All spectral data were in accordance with assumed structures.

Table 1: Docking Score of Synthesized Compound

S/No.	Compound	Moldock Score	ReRank Score
1	R_1	-126.549	-98.199
2	R_2	-138.839	-99.085
3	R_3	-129.537	-99.122
4	R_4	-121.558	-95.640
5	R_5	-132.876	-104.614
6	R_6	-120.341	-98.846
7	R_7	-130.358	-98.685
8	Standard	-119.917	-99.194

Table 2: Characterization data of newly synthesized Derivatives

S/No	Mol. Weight	Melting Point (^{0}C)	% yield
R_1	375.85	172-176	60.2
R_2	386.4	184-188	70.3
R_3	359.39	192-196	65.5
R_4	386.	180-184	72.5
R_5	409.4	186-192	60.2
R_6	341.4	162-166	65.1
R_7	359.39	182-186	75

R_1 compound shows characteristic C=O aromatic acid stretching strong peak at 3456 cm^{-1}, C=C alkene stretching peak at 1617 cm^{-1}, C=C benzene stretching peak at 1589 cm^{-1}, C=S thiocarbonyl stretching peak at 1082 and 1238 cm^{-1} & C-O ester stretching peak at 1097cm^{-1} an and C-X halide stretching peak at around 800 cm^{-1}. R_2 compound shows characteristic C=O aromatic acid stretching strong peak at 3552 cm^{-1}, C=C alkene stretching peak around 1650 cm^{-1}, C=C benzene stretching peak at 1594 cm^{-1}, C=S thiocarbonyl stretching peak at 1074 and 1251 cm^{-1} & C-O ester stretching peak at 1112cm^{-1} and NO_2 stretching peak at 1340 cm^{-1}.,δ- 6.8-8.3 ppm, m/z 385. R_3 the compound shows characteristic C=O aromatic acid stretching strong peak at 3534 cm^{-1}, C=C alkene stretching peak at 1668 cm^{-1}, C=C benzene stretching peak at 1521 cm^{-1}, C=S thiocarbonyl stretching peak at 1069 and 1263 cm^{-1} & C-O ester stretching peak at 1068cm^{-1} and C-X halide stretching peak at around 800 cm^{-1}.δ-6.8-8.2 ppm, m/z 358. R_4 compound shows characteristic C=O aromatic acid stretching strong peak at 3512 cm^{-1}, C=C alkene stretching peak around 1653 cm^{-1}, C=C benzene stretching peak at 1590 cm^{-1}, C=S thiocarbonyl stretching peak at 1071 and 1240 cm^{-1} & C-O ester stretching peak at 1000cm^{-1} and NO_2 stretching peak at 1356 cm^{-1}.δ-7.0-8.4 ppm, m/z 385. R_5 compound shows characteristic C=O aromatic acid stretching strong peak at 3486 cm^{-1}, C=C alkene stretching peak at 1647 cm^{-1}, C=C benzene stretching peak at 1558 cm^{-1}, C=S thiocarbonyl stretching peak at 1076 and 1244 cm^{-1} & C-O ester stretching peak at 1031cm^{-1} and C-X halide stretching peak at around 800 cm^{-1}.δ-7.0-8.4, m/z 408.R_6 compound shows characteristic C=O aromatic acid stretching strong peak at 3523 cm^{-1}, C=C alkene stretching peak at 1617 cm^{-1}, C=C benzene stretching peak at 1583 cm^{-1}, C=S thiocarbonyl stretching peak at 1074 and 1257 cm^{-1} & C-O ester stretching peak at 989cm^{-1}.δ-7.4-8.2 ppm , m/z 340.

R_7 compound shows characteristic C=O aromatic acid stretching strong peak at 3487 cm^{-1}, C=C alkene stretching peak at 1624 cm^{-1}, C=C benzene stretching peak at 1589 cm^{-1}, C=S thiocarbonyl stretching peak at 1078 and 1246 cm^{-1} & C-O ester stretching peak at 1172cm^{-1} and C-X halide stretching peak at around 800 cm^{-1}.δ-6.8-7.8 ppm, m/z 358.

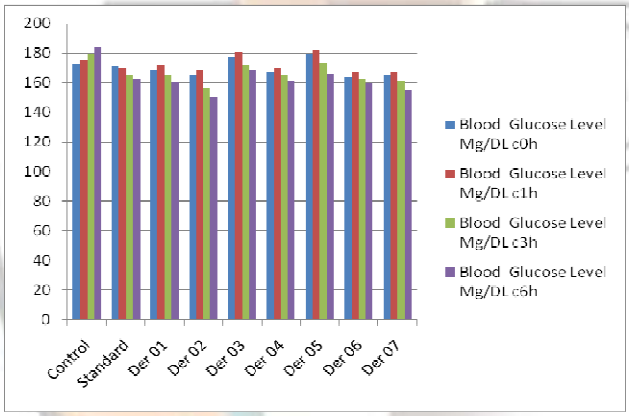
***In-vivo* Biological Activity**

Rhodanine analogs were screened for their antidiabetic activity by alloxan induced diabetic model and blood glucose level was measured by taking out blood from the retro orbital plexus and blood glucose was measured using a glucometer. There were ten groups each consisting of six rats. Male rats were used only.

The animals were kept fasting for 18 hrs before the blood withdrawal and glucose testing and the Fasting blood glucose level was monitored.

Table 3: Antidiabetic Activities of Synthesized Compounds

Comp	Blood Glucose Level Mg/DL			
	[c]0h	[c]1h	[c]3h	[c]6h
Control	172.83± 5.98	175.16±6. 67	179.50±6. 56	183.50± 5.08
[a] Standard	171.16± 5.77	169.50±5. 31	162.50±5. 35	157.83± 4.99
[b] Der 01	168.50± 5.95	171.66±6. 15	165.16±5. 84	160.16± 4.87
[b] Der 02	165.33± 4.13	168.50±3. 61	156.83±3. 18	149.83± 2.04
[b] Der 03	177.16± 4.53	180.50±3. 61	175±3.34	171.16± 3.31
[b] Der 04	167.33± 5.27	169.50±4. 88	165.16±4. 91	160.83± 5.23
[b] Der 05	178.83± 4.40	181.50±4. 76	173±4.93	166.16± 4.99
[b] Der 06	163.83± 4.16	166.66±3. 61	162.50±3. 20	159.66± 3.14
[b] Der 07	164.83± 5.91	167.5±4.8 4	161.16±5. 45	155.16± 5.49



Graph 1: Biological activity data chart of the compounds

Although all the compounds selected for synthesis exhibited better docking score than the reference compound i.e. Rosiglitazone, after the in vivo evaluation only the three derivatives were found to be lowering the blood glucose level to a greater extent than that of the reference compound and the onset of action of all the synthesized compounds were found to be slow than that of Rosiglitazone.

Der02, Der05and Der07 showed better efficacy than Rosiglitazone with Der02 exhibiting best results followed by Der05 and Der07 respectively. Der01 and Der03 showed activity profile similar to that of Rosiglitazone giving a nearby glucose lowering value where as Der04 and Der06 showed poor efficacy with Der06 being the poorest of the lot.

Thus, some of the synthesized Rhodanine derivatives have shown considerable improved efficacy and improved biological response which is enough reason to believe that PPAR γ is a promising target for the management of type II diabetes and that Rhodanine analogs have it in them to illicit a biological response out of the receptor

References

1. World Health Organization. *World Health Statistics 2011* - Table 6: Health workforce, infrastructure and essential medicines. Geneva, 2011.
2. US Federal Food, Drug, and Cosmetic Act, SEC. 210., (g)(1)(B).
3. Robert T. Morrison, Robert N. Boyd, and Robert K. Boyd, *Organic Chemistry*, 6th edition/
4. Madsen, Ulf; Krogsgaard-Larsen, Povl; Liljefors, Tommy (2002). *Textbook of Drug Design and Discovery*. Washington, DC: Taylor & Francis.
5. Vogel, A.I., Tatchell, A.R., Furnis, B.S., Hannaford, A.J. and P.W.G. Smith. *Vogel's Textbook of Practical Organic Chemistry, 5th Edition*. Prentice Hall, 1996.
6. Bastaki, S., *Int. J. Diabetes Metabolism*, 2005, 13, 111-134.
7. Ballard, A.M., *Clin. Diabetes*, 2000.
8. Gerald, I. S., J. *Clin. Invest*., 2000, 106, 171-176.
9. Modi, P., *Curr. Drug Discov. Tech*., 2007, 4, 39-47.
10. Clifford, J. B.; Caroline, D., Br. *J. Cardiol*., 2003, 10,128–136.
11. Sarabu, R.; Tilley, J., Annu. Rep. *Med. Chem*., 2005, 40, 167-178.
12. Khanfar, A. M.; Bilal, A. A.; Mudit, M.; Kaddoumi A.; Khalid A. E., *Bioorg. Med. Chem*., 2009, 17, 6032–6039.
13. Sivaprakasama, P.; Aihua, X.; Robert, J. D., *Bioorg. Med. Chem*., 2006, 14, 8210–8218.
14. Denise, M. F.; David, K., *Dev. Biol*., 2000, 225, 471–479.
15. Vats, R. K.; Kumar, V.; Kothari, A.; Mital, A.; Ramachandran, U., *Curr. Sc*., 2005, 88, 241-249.
16. Pattan, S. R.; Suresh, C.; Pujar, V. D.; Reddy, V. V. K.; Rasal, V. P.; Koti, B. C., *Indian J. Chem*., 2005, 44B, 2404-2408.