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Structure-Based Drug Design of PPAR gamma Agonists for Anti-cancer Activity

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ABSTRACT

PPAR γ (Peroxisome proliferator Activated Receptors gamma) has physiological response in body upon its activation through different agonists which can be fruitful for management of cancer. Using Molecular docking we designed suitable agonists which could be the possible pharmacophores for PPAR γ activation. Some ester derivatives of rhodanine are designed using Structural Activity Relationship Studies by V Life MDS Software package version 4.2 and synthesized by *Schotten Bauman's Esterification* reaction. In current work we synthesized five derivatives and are characterized by spectroscopic techniques that validate the correct synthesis of the compounds. Further evluated their effect on MCF7 cell lines for Anticancer Potential

KEY WORDS: PPAR γ, Molecular Docking, Rhodanine, Schotten Bauman's esterification, pharmacophore

INTRODUCTION

PPAR-gamma receptor is a nuclear receptor that is expressed in a wide variety of cancer cells. In cancer cells, PPAR-gamma stimulation by its agonists can reduce cell growth and can induce the apoptosis by

- Targeting Cyclin-dependent Kinase (CDK) inhibitors such as p18, p21 and p27
- Thiazolidindiones and rhodanine derivatives activate PPAR gamma receptors and cause apoptosis by decreasing anti-apoptotic proteins such as bcl-2/ bcl-x and survivin while increasing the levels of the proapoptotic proteins, p53, Bcl-2-associated death promoter (BAD) protein and phosphatase and tensin homologs
- PPAR gamma ligands frequently downregulate the expression of proangiogenic factors VEGF, IL-8, Ang-1, and Cox-2, thus suspending tumor angiogenesis.
- phosphorylation by glitazones after binding with PPAR gamma causes inhibition of genetic mutation which leads to cancer.

In current research work, rhodanine derivatives are designed using SAR and molecular docking as possible agents for cancer treatment[1,2,3]. Because we compared the binding of rosiglitazone with PPAR gamma receptor and recorded its dock score then by considering the structural requirements we designed the rhodanine derivatives for proper binding to PPAR gamma. the designed derivatives showing dock scores more than or comparable to rosiglitazone were selected for synthesis.

Structural Activity Relationship of Thiazolidinediones

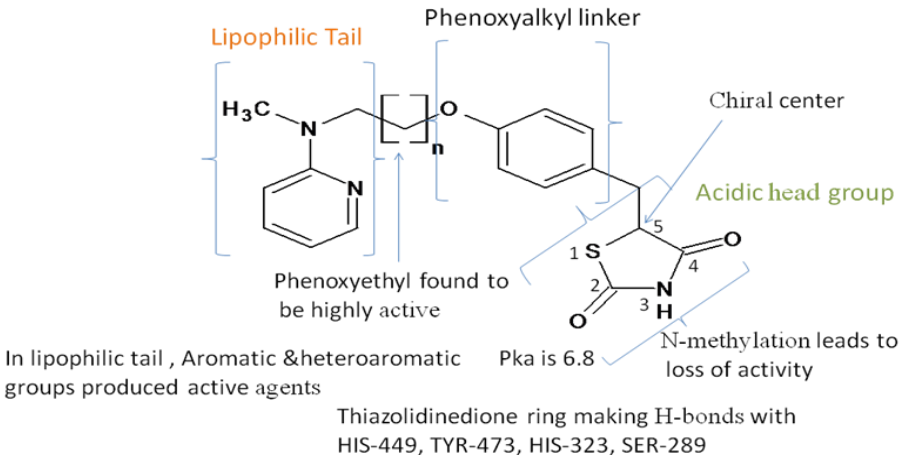


Fig. 1: Structure showing SAR of TZDs

1. Thiazolidinedione derivatives having an acidic head group connected to lipophilic tail by a phenoxy alkyl linker [5].
2. pKa of TZD IS 6.8 partially ionized at body pH [5].
3. Removal of acidic function by N-methylation leads to loss of activity.
4. Substituted carboxylic acids are often highly potent may not selective PPARγ agonist [5].
5. Though the majority of TZD containing glitazones possess a methylene group bridged to the TZD and phenyl group, it is revealed that compounds with its bioisostere rhodanine ring has too be made known to have activity to considerable levels [6].

6. Compounds with TZD as acidic head group has good action as compared to its bioisoster rhodanine, indicates that acidic head should be more polar [7, 8.]
7. There is a chiral centre at the 5th position of thiazolidinedione ring, but this is not configurationally stable body pH. PPAR gamma activity remain only in S-enantiomer [5].
8. Compounds with double bond at 5th place of thiazolidinedione or rhodanine yields active compounds [6, 7, 8].
9. A phenoxyethyl group (n=2) as the central Phenoxyalkyl linker generally produece highly active compounds[5].
10. Chain length n=1 or introduction of the phenoxyethyl group into a heterocyclic ring also produces active compounds [5].
11. The two-carbon acyl linker in the form of amide (-CH_2CONH) is the common structural part in all the derivatives creates good activity activity [7, 8].
12. An oxygen atom connected to the aromatic ring (hydrophobic trunk) in the form of ether is essential for activity [8].
13. Incorporation of thioethyloxy linkage with two carbon spacer connecting to triazole derivative and oxadiazole derivative displayed good activities [9].
14. In the lipophilic tail, inclusion of different aromatic and heteroaromatic groups can give active agents.
15. Reducing cyclic ring from six membered benzene to furan five membered rings found to reduce the activity[5].
16. Increased hydrophobicity and orientation of the substituents of the pyridine moiety shows a more potent activity[5].

MATERIALS AND METHODS

Molecular docking aids drug/ligand research by identifying suitable sites, obtaining optimal images of ligand-receptor complexes, and calculating interactions between ligands of different energies to create better ligands or receptor/protein interacting proteins.[9]

- Thiazolidinedione nucleus is critical for anticancer activity[8]
- Oxygen at second position of thiazolidindione is replaced by isoelectric Sulphur atom. It has low electronegativity as compated to O and has ability to build hydrogen bonds, which could be attributed for the activity[5,8]
- The exocyclic double bond is important for activity as there is delocalization of elcetrones between the this double bond and carbonyl group of rhodanine ring[8,10]
- 5-benzylidene ring at 5th position of is important for anticancer activity probably because of delocalization of electrons[8,10]
- Benzyloxy group is present at second position of benzylidene ring i.e. an ether group in glitazone being replaced by ester group in current design as we focous on aromatic ester linkage here which will not be susceptible for hydrolysis within the body [8,10].
- After observing the dock score and poses it is clear that the aromatic group after ester linkage is contributing to the proper bonding with PPAR gamma by aromatic interactions.

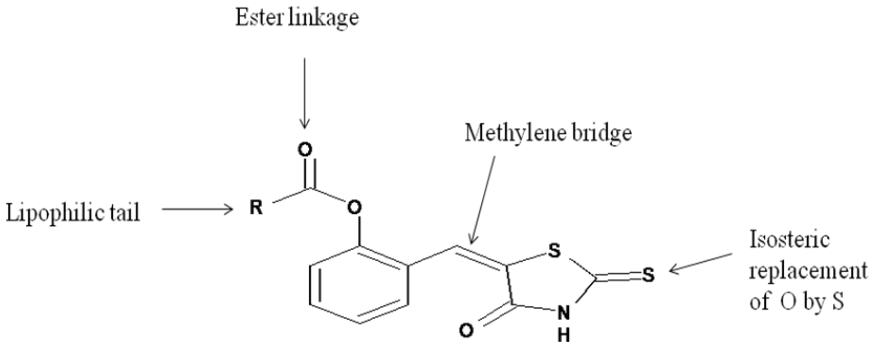


Fig. 2: Propsed structure of Rhodanine derivatives

Molecular Docking and software package

All drug design ie. virtual screening studies and structural studies were done using the Molecular Design Suite (VLife MDS software package, version 4.2; from VLife Sciences, Pune, India).

Structure Drawing

All the proposed Structures of lead molecules were drawn using the 2D structure draw function of Vlife 2D draw and transformed to 3D structures. All the designed ligands were reduced and optimized with the Merck Molecular Force Field (MMFF) method taking the root mean square gradient (RMSD) and the iteration limit to 10,000. Structures were created using Monte Carlo by applying MMFF force field process and least energy conformer was chosen for further study**.**

Protein preparation

The Protein Data Bank structure 5YCP (www.rcsb.org) was downloaded and energy minimization of the protein complex was done using software. All the attached water molecules, ligands and cofactors were detached (pre process) from the proteins which were taken in pdb format. Incomplete and missing residues were added in the protein. The complex obtained was minimized using Merck molecular force field. The minimization was completed after either completion of 5,000 steps or after the energy slope converged below 0.05 kcal/mol.

Ligand preparation

Structures of the designed molecules were drawn by inbuilt Vlife 2D draw provision of the software and the format taken in .mol and changed it into 3D structure and a geometry minimization of the ligands was done. Merck Molecular Force Fields (MMFF) using default settings that were used for the ligand minimization.

Docking method

Virtual screening was done on Vlife MDS version 4.2 on Dell computer, i3 processor with 7 XP Vista operating system. The GRIP-based docking was done using 'Y' shaped cavity of the PPAR gamma receptor 5YCP. The dock scores of the complex were calculated by PLP scoring function. The ligand with minimum docking scores were choosen for further study.

PPARγ is having ‘Y’ shaped cavity, rosiglitazone shows key hydrogen bonding with His 323, His 449, Tyr 473 and Ser 289. Conformations, different interactions of various ligands with amino acid residues present in to the active site (ligand binding domain) of PPARγ was studied,

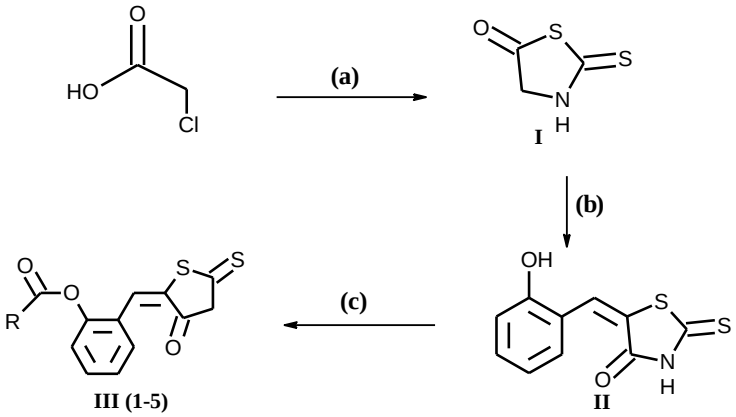
ligands which bind strongly (revealed from smaller dock score) with amino acid residues in to the binding domain of PPARγ where glitazones known to interact were identified, library of ligands was prepared by using different substituents using Craig plot, were docked in to the active site of protein PPARγ. Ligands which known to interact with same amino acid residues which are part of ligand binding domain to which standard glitazones interacts, have comparatively strong binding affinity for active site revealed from smaller dock score as well those which synthetically feasible were selected.

Rational for Schotten baumann esterification: Schotten Baumann reaction is between the phenol and acid chloride, here in this study the second step compound is **5-(2-hydroxybenzylidene)-2-thioxo-1,3-thiazolidin-4-one** having phenolic group which can be esterifed easily with different aromatic acid chlorides using pyridine as alkaline agent.

Benzoylation using 10% sodium hydroxide leads to hydrolyze the ester linkage itself hence we replaced sodium hydroxide by pyridine.

Criteria for synthesis of ester derivatives: Total 7 derivatives were designed based on their small dock scores signifies their proper binding to the PPAR gamma receptors and feasibility and availability of the chemicals.

SYNTHETIC SCHEME



(a) Sodium carbonate, ammonium dithiocarbamate, conc. Hydrochloric acid, heat [11].

(b) Salicylaldehyde, thiourea, 110 ^{0}C [12].

(c) Different benzoyl chlorides, reflux [13].

The reaction was carried using sodium hydroxide but it was found that it hydrolyses the ester linkage of target compounds. Then process was corrected by using pyridine in place of sodium hydroxide.

PROTOCOL FOR *IN-VITRO* SCREENING OF THE SYNTHESIZED LIGANDS

Materials[16]

1. Cell line: MCF7 cell (Human breast Carcinoma Cell line) procured from **National Centre for Cell Sciences** (NCCS), Pune.

2. Culture media: MEM medium with Antibiotics and 10% FBS (Himedia)

3. 96 well Tissue culture plate

4. Neubauer's chamber

5. MTT Reagent (Himedia)

6. Phosphate buffer saline of pH 7.4

7. Acidic isopropanol

8. ELISA reader (Erba-Lisascan)

Procedure

Day 1

1. 1×105 cells/ml were added in 96 well tissue culture plate (cell count was taken on Neubauer's chamber).

2. The plate was then incubated in a CO_2 incubator (5% CO_2 at 37^0C) for 24 hrs.

Day 2

1. After 24 hrs incubation, the plate was observed.

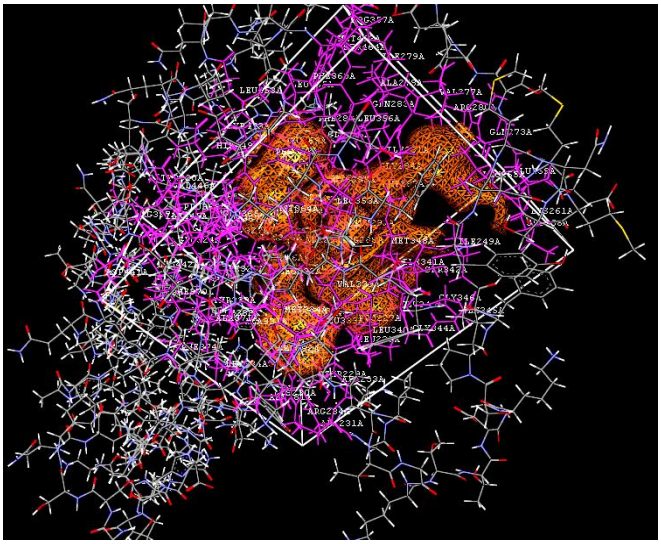
2. The concentrations were decided by considering the molecular weights of the repsctive samlples and dissolved in DMSO in micromole range.

3. The media was replaced with the media containing different dilutions (1 µM - 200µM/well) of test samples in 100 µl were added in duplicates.

RESULTS AND DISCUSSION

A library of ligands was docked by using Molecular Design Suit (*V.4.3*) in to the 3D 'Y' shaped cavity of PPARγ receptor (5YCP). Class of drug, glitazones which activates PPARγ pathway was initially analyzed by molecular docking analysis in order to validate pharmacophoric points of the protein under investigation. The protein-ligand complex was constructed based on the X-ray crystallized structure of receptor. The designed compounds built using Vlife2Ddraw converted into 3D structure and energy minimized by using Merck Molecular Force Field (MMFF). Conformers were generated by using Monte Carlo conformational search ring flip method[9].

GRIP method employed in software fruitfully to dock the designed agonists into 'Y shape' cavity of the PPAR gamma 5YCP receptor and to were well correlated with the obtained binding score to anticancer activities of compounds. In this correlative virtual screening experiments of designed compounds with known Rosiglitazone (as a standard) with dock score calculated -44.07. Obtained results were screened in terms of docking score in to the Y shape cavity of 5YCP. The software provides access of the batch docking of the optimized molecules with the simulated receptor. All ligands are selectively docked against Y shaped cavity of 5YCP. Each molecules takes a time for the completion of docking. Molecules which show minimum dock score shows more attraction for PPAR gamma receptor and Dock score shown in Table 1[9]



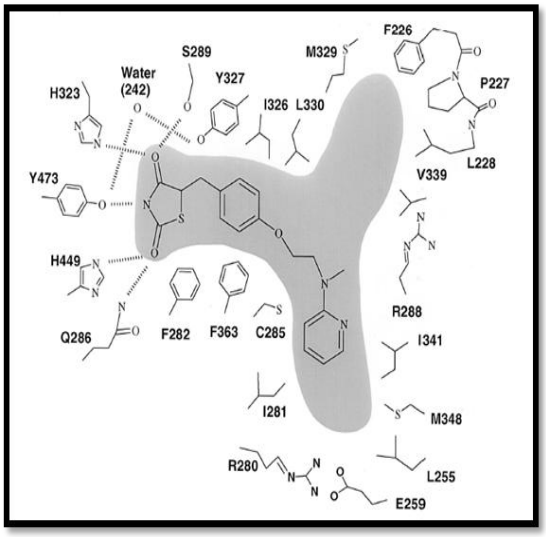


Fig. 3 (a) Rosiglitazone docked in Y shape cavity of PPAR (b) Y shaped cavity as per literature

Table. 1: Standard rosiglitazone and Newly designed Molecules with all characterization

No.	Compounds	Dock Score KCal/mole	Molecular wt. calculated	Spectral Characterisation
	Rosiglitazone	**-44.072427**	NA	NA
No.		Dock Score KCal/mole		
1	Compound 1	-40.791003	279.33476	**Yield** = 56% **m. p.** = 182-184 ºC $\mathbf{R_f}$ = 0.543 **IR:** 3344 (-NH *str*.), 3050 (C-H *str*.), 2845 (C-H *str*.), 1726 (C=O *str*.), 1685 (C=O), 1600 cm^{-1} (C=C *str*.), 1175 cm^{-1} (C=S *str*.), 1014 (C-O *str*.) **^{1}H NMR:** 13.6 (s, 1H), 7.50 (s, 1H), 7-8 (m, 4H), 2.3 (s, 2H, -CH_3) **Mass:** 279 (molecular ion peak $M^{\cdot +}$), 236 (base peak)
2	Compound 2	-52.518931	341.40414	**Yield** = 60 % **m. p.** = 242- 244 ºC $\mathbf{R_f}$ = 0.476 **IR:** 3203 (*str*.), 3050 (-C-H *str*.), 1724 (C=O *str*.), 1600 (C=C *str*.), 1200 (C-O *str*.), 1061 cm^{-1} (-C=S *str*.) **^{1}H NMR:** 13.7 (s, 1H), 7.58 (s, 1H), 7-8 (m, 9H) **Mass:** 341 (molecular ion peak $M^{\cdot +}$), 341.2 (base peak)
3	Compound 3	-40.021477	355.43072	**Yield** = 58 % $\mathbf{R_f}$ = 0.636 (c) **m. p.** = 234- 236 ºC **IR:** 3100 (-C-H *str*.), 2844 (-C-H *str*.), 1732 (-C=O *str*.), 1600 cm^{-1} (-C=C *str*.), 1105 cm^{-1} (-C-O *str*.)

				^{1}H NMR: 13.6 (s, 1H), 7-8 (m, 8H), 7.5 (s, 1H,), 2.51 (s, 3H) **Mass:** 355 (molecular ion peak $M^{\cdot +}$), 354.2 (base peak) **Element Analysis: (% cal.) found:** C (60.83) 59.36, H (3.69) 3.50, N (3.94) 4.41, S (18.04) 17.79
5	Compound 4	-48.459820	386.4017	**Yield** = 51 % **m. p.** = 192-194 ºC **R_f** = 0.285 **IR:** 3573 (-NH *str*.), 3040 (C-H *str*.), 1683 (C=O *str*.), 1601(C=C *str*.), 1540 (-NO_2 *str*.), 1455 (-NO_2 *str*.), 1107 (C=S *str*.) **^{1}H NMR:** 13.7 (s, 1H), 7-8 (m, 8H), 6.9 (s, 1H) **Mass:** 385 (molecular ion peak $M^{\cdot +}$), 236.0 (base peak) **Element Analysis: (% cal.) found:** C (52.84) 49.12, H (2.61) 2.68, N (7.25) 6.75, S (16.60) 17.8

^{1}HNMR and mass spectroscopic techniques were used to validate the synthesised ligands and results obtained are as follows

Compound 1:
Mass spectroscopy: Theoretical molecular wight is 279.33 and the molecular ion peak in mass spectrum obtained at m/z ratio at 279 which confirmes the proper synthesis of compound 1[14,15].

^{1}HNMR: ^{1}H NMR spectrum of compound exhibits singlet at 13.6 ppm indicates ring –NH, singlet at 7.56 ppm indicate one benzylidene proton, shows multiplet in the range of 7-8 ppm, equivalent to 4 aromatic protons, singlet at 2.3 ppm must be equivalent to 3 methyl protons but actual spectrum shows 2 protons instead of 3 protons. Peak extra at 2.5 ppm could be due to presence of impurities[14,15].

Compound 2:
Mass Spectroscopy: Theoretical molecular wight is 341.40 and the molecular ion peak in mass spectrum obtained at m/z ratio at 341 which confirms the proper synthesis of compound 2[14,15].

^{1}HNMR: It shows singlet at 13.7 ppm corresponds to ring –NH, singlet at 7.5l ppm correspond to benzylidic proton, multiplet at 7-8 ppm range corresponds to aromatic protons. Peak at 2.5 ppm is due to *DMSO* and at 3.5 ppm is due to moisture[14,15].

Compound 3:
Mass spectroscopy: Theoretical molecular wight is 355.43 and the molecular ion peak in mass spectrum obtained at m/z ratio at 355 which confirms the proper synthesis of compound 3[14,15].

^{1}HNMR: It 13.6 (s, 1H) singlet indicates –NH in 2-thioxo-1,3-thiazolidin-4-one ring, 7-8 (m, 8H) multiplet is equivalent to 8 aromatic protons, 7.5 (s, 1H,) singlet indicates benzylidene proton, 2.51 ppm (s, 3H) singlet equivalent to 3 protons of methyl group at para position[14,15].
Compound 4:
Mass Spectrum: The mass spectrum of compound III 4 shows molecular ion peak at m/z 385 which confirm molecular weight of compound, base peak at m/z 374.2, fragment1 at m/z 234.2, fragment 2 at m/z 218.1 and fragment 3 at m/z 175[14,15].

^{1}HNMR: 13.7 ppm (s, 1H) ring –NH proton, 7- 8.3 ppm (m, 7H) eight aromatic protons, but it shows one aromatic proton less than the actual, 7.51 ppm (s, 1H) one benzyidene proton, 7.41 ppm (s, 1H) one -OH proton[14,15].
There are some extra peaks in both mass spectrums and NMR spectrums which indicate that compound require more purification via recrytallisation.

In-vitro MTT Cytotoxicity Assay

Table 2: Summarised IC 50 value and % inhibition of MCF7 Cell lines

Conc. (µm)	Comp. 1	Comp. 2	Comp. 3	Comp. 4Co	STD
1	34.0988	19.7789	15.9040	27.5001	53.2452
10	40.5493	29.3322	19.5196	37.3896	68.2318
50	46.4300	48.6723	28.7367	48.1167	86.7229
100	52.0115	42.3044	31.1528	48.2820	92.7631
200	52.1796	79.8536	65.6847	67.9868	98.7464
IC50	**133.7100**	**94.7500**	**147.4600**	**95.7200**	**78.9700**

CONCLUSION

- All the selected compounds show proper binding with the similar residues on PPAR gamma recptors and having best fit with the target.
- As these bind properly with the pharmaophoric points hence they can be possible leads for PPAR γ agonism
- After spectral characterization it is evident that synthesized molecules are showing maximum correlation with the mass spectrometry as the data practically obtained is pretty close to the theoretically calculated molecular weights of the compounds.
- In future we will screen the synthesized compounds for cytotoxicity assay to see wheather they are suitable for cancer therapy or not.

- As shown in Table 2 the designed Compounds show cell inhibition particularly Compound 2 and Compound 4 are showing IC50 value less than 100 µM concentration which indicate that these two compounds could find their way as Anti cancer Molecules.

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