Design, synthesis and biological evaluation of novel thiadiazole derivatives as antihyperlipidemic agents

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Abstract
Thiadiazole derivatives are reported to have various biological activities including antihyperlipidemia. In the present study a series of N-phenyl-1, 3, 4-thiadiazol-2-amine derivatives were designed using various softwares like Molinspiration, Schrodinger etc. The derivatives with optimum properties were synthesized and characterized using FT-IR, ¹H NMR and MASS spectroscopy. In vivo screening for antihyperlipidemic activity in triton X-100 induced models showed moderate activity in comparison with standard.

Keywords: Thiadiazole, Antihyperlipidemia, Triton X-100, Atorvastatin.

Introduction
Hyperlipidemia or hyperlipoproteinemina is the presence of raised or abnormal levels of lipids and/or lipoproteins in the blood i.e., an increase (hyper) in the lipids (liji), which are a group of fatty substances in the blood (demia). Lipids (fatty molecules) are transported in a protein capsule, and the density of the lipids and type of protein determines the fate of the particle and its influence on metabolism. Cholesterol and the triglycerides are the two lipids in the blood. Elevation of one or both of these lipids is seen in hyperlipidemia. Hypercholesterolemia or hyperlipidemia is one of the greatest risk factors contributing to prevalence and severity of cardiovascular diseases. Serum cholesterol levels above 240 mg/dL and triglyceride levels above 150 mg/dL are associated with atherosclerosis. Atherosclerosis is the condition in which the lipid deposits on the lining of the blood vessels, eventually producing degenerative changes and obstruction of blood flow. Atherosclerosis is considered to be a major contributor in the development of heart disease.¹,²

CYP51A1: CYP51A1 is also referred to as lanosterol-14a-demethylase. This P450 enzyme is the only one of the eight enzymes that is involved in de novo cholesterol biosynthesis. Lanosterol-14a-demethylase is the cytochrome P450 monoxygenase, which oxidatively removes the 14α-methyl groups of lanosterol, resulting in the generation of at least two oxysterols that, in mammalian tissues, are efficiently converted into cholesterol as well as more polar sterols and steryl esters.³

Thiadiazole is a five-membered heterocyclic compound that contains two nitrogen atoms and one sulphur atom and shows various types of biological activities. The ending -azole designates a five-membered ring system with two or more heteroatoms, one of which is nitrogen. Recently a number of derivatives containing thiadiazoles which contain the nitrogen in different position say, 1,3,4-thiadazole, 1,2,3-thiadazole, 1,2,4-thiadazole, & 1,2,5-thiadiazole are reported with various pharmacological activities.⁴

Thiadiazole moieties reported to show major biological activities such as antibacterial, antitubercular, anti-inflammatory, anti-oxidant, anticancer, antifungal, antidepressant, antihyperlipidemia, antihypertensive etc.⁵ It was planned to design some novel thiadiazole derivatives by incorporating different substitutions to 1, 3, 4-thiadiazole (Fig.1) moiety to explore the possibilities of some altered biological actions. The main objective of the present study is to design and synthesize newer derivatives of thiadiazole and to perform the docking studies with molecular targets/receptors for antihyperlipidemic activity and to screen the compounds for antihyperlipidemic activity.

Fig. 1: Thiadiazole

Materials and Methods
The chemicals and reagents used in this study such as ethyl chloroacetate, anhydrous potassium carbonate, hydrazine hydrate, ammonia solution, carbon disulphide, sulphuric acid, tritonX100 and sulphuric acid were purchased from Sigma-Aldrich and were of analytical grade.

The melting points of the synthesized compounds were determined by Thiele melting point apparatus (open capillary tube method) and all the compounds...
gave sharp melting points and were uncorrected. Purity of the compounds was ascertained by single spot thin layer chromatography using silica gel G as stationary phase and appropriate mixtures of toluene and ethyl acetate as mobile phase. The spots resolved were visualized using iodine chamber. The 3-D structure of the protein was obtained from PDB using their specific PDB ID (3LD6). The X-ray crystal structure of lanosterol-14α-demethylase has been determined to a resolution of a 2.15Å.10

**Scheme for Synthesis:**

**Step 1: SYNTHESIS OF CARBOXYLIC ACID HYDRAZIDES (Ah)** (Fig.2)

**Procedure**

**Synthesis of ethyl aryl amino acetate:** Ethyl chloroacetate (0.012 mol) was refluxed with substituted aniline (0.01 mol) on water bath in dry acetone (25 ml) and anhydrous potassium carbonate (0.01 mol) for 6 h. The solvent was evaporated and the reaction mixture was poured into crushed ice to get the respective ethyl aryl amino acetate. The solid thus separated was filtered, dried and recrystallized from petroleum ether.

**Synthesis of aryl amino acetic acid hydrazide (Ah):** Ethyl aryl amino acetate (0.01mol) was refluxed on water bath with excess of hydrazine hydrate (0.02mol) in alcohol (25 ml) for 4 h. The solvent was evaporated; the product thus obtained was washed with cold water, dried and purified by recrystallization with suitable solvent. (11)

**Step 2: SYNTHESIS OF PHENYL ISOTHIOCYANATE (Ph) (Fig. 3)**

**Procedure:** Synthesis of phenyl isothiocyanate (Ph): In a 500ml round-bottomed flask, fitted with a mechanical stirrer and surrounded by an ice-salt cooling bath, placed 0.71 mole of carbon disulfide and 1.3 moles of conc. aqueous ammonia. While stirring, 0.6 mole of aniline was added into the mixture from a separating funnel at such a rate that the addition was completed in about twenty minutes. The stirring was continued for thirty minutes after all the aniline has been added, and then the reaction mixture was allowed to stand for another thirty minutes. During this time a heavy precipitate of ammonium phenyl dithiocarbamate separated. The salt was dissolved in 800ml of water and transferred to a 5.0L round-bottomed flask. To the solution with constant stirring a solution of 0.6 mole of lead nitrate in 400ml of water was added. Lead sulphide was separated as a heavy brown precipitate which soon turned black. The mixture was then distilled with steam into a receiver containing 5–10ml of 1N H2SO4 as long as any oil came over. About 2–3ml of distillate was collected. The product was obtained from the water using a separating funnel. (12)

**Step 3: SYNTHESIS OF THIADAIZOLE DERIVATIVE (Td)** (Fig. 4)

**Synthesis of substituted thiosemicarbazide:** A mixture of the carboxylic acid hydrazide (Ah) (0.01mol) and phenyl isothiocyanate (Ph) (0.01mol) in ethanol (10 ml) was heated under reflux for 4 h. On cooling, the precipitated crystalline solid was filtered,
washed with cold ethanol, dried and crystallized from ethanol to yield compounds in 75-85% yields.

**Synthesis of thiadiazole derivatives (Td):** Sulphuric acid (98%) (6 ml) was added drop wise to the appropriate substituted thiosemicarbazides (0.01mol) and the mixture was stirred at room temperature for 24 h. The resulted homogenous mixture was poured into crushed ice (100 g), neutralized with ammonium hydroxide solution. The separated precipitate was filtered, washed with water, dried and crystallized to yield the expected thiadiazoles (Td).

Four different thiadiazole derivatives (Td1, Td2, Td3 and Td4) were synthesized using four different substituted anilines. (Table 1)

![Fig. 1: Structure of Td1](image)

**Table 1: Various anilines used in the preparation**

| Compound | Td1 | Td2 | Td3 | Td4 |
|----------|-----|-----|-----|-----|
| -R | NH2 | NH2 | NH2 | NH2 |

**Biological Screening**

This study was conducted in the department of Pharmacology, DPS, Puthuppally, Kerala, India. The study was conducted in accordance with CPCSEA guidelines and the study was approved by the Institutional Animal Ethical Committee (Regd. No. 1702/PO/C/CPCSEA).

**Acute Toxicity Study:** The acute toxicity study was carried out as per the OECD 423 guidelines. The animals were selected randomly and grouped, three animals per group. They were kept fasting four hours prior to the treatment and the test substance was administered in a single dose by the oral route. Subsequently, the dose was gradually increased with each step, starting from 5 mg / kg then to 50, 300, 2000 mg/kg. After the substance had been administered, the food could be withheld for a further one-to-two hours, in mice.

**In Vivo Antihyperlipidemic Study:** Healthy albino rats of male sex weighing between 200-250 gm were collected from Animal house, Department of Pharmacology, RIMSR, Kottayam. The animals were housed in a group of 6 animals and were allowed to acclimatize to the environmental for 7 days and supplied with standard pellet diet & water ad libitum.

The rats were fasted over night and divided into 4 groups each containing 6 animals viz., normal control group, toxic group treated with triton, standard group (Atorvastatin1.14mg/kg) and test drug group (thiadiazole derivatives 100mg/kg). The treated groups both test group and positive control group were given daily prophylactic dose of the test drug and standard drug atorvastatin respectively for 7 days. Hyperlipidemia was induced in rats by single I.P injection of freshly prepared solution of Triton X -100 in physiological saline after overnight fasting for 18hrs. Throughout the experiment the experimental rats were processed in accordance with the instructions given by our institutional animal ethical committee, CPCSEA.

Thirty albino rats of male sex weighing about 200 – 250 gm were selected in the present study. The animals were kept under observation for one week and grouped into four in such a way that each group consisted of six animals and various drugs are administered as shown. (Table 2)

![Fig. 2: Experimental Design](image)

**Table 2: Experimental design**

| Groups | Triton X-100 | Drug dose & duration |
|--------|-------------|---------------------|
| Group A | No administration of triton Serves as normal control group | No drug was given |
| Group B | Single Intraperitoneal injection at a dose of 100mg/kg body weight Serves as toxic control group | No drug was given |
| Group C | Single Intraperitoneal injection at a dose of 100mg/kg body weight | Standard drug Atorvastatin at effective dose 1.14mg/kg was given orally for 7 days. |
Group D  | Single Intra peritoneal injection at a dose of 100mg/kg body weight | Animals received thiadiazole derivative 100mg/kg/p.o. in 1% sodium CMC for a period of 7 days.

**Statistical Analysis:** The collected data on different parameters from each subject were expressed as the mean ± SEM. One-way ANOVA followed by post-hoc analysis with Dunnett’s test was performed to test the significance of differences between means using standard statistical technique Graph pad version, 6.

**Results and Discussion**

**Molecular docking:** The derivatives are docked with receptor *lanosterol-14α-demethylase* (PDB ID: 3LD6) and the docking scores obtained are as follows (Table 3).

| Compound | Structure | Glide score |
|----------|-----------|-------------|
| Td₁      | ![Structure](image1.png) | -9.45308    |
| Td₂      | ![Structure](image2.png) | -8.72432    |
| Td₃      | ![Structure](image3.png) | -8.13892    |
| Td₄      | ![Structure](image4.png) | -7.52065    |
| Td₅      | ![Structure](image5.png) | -6.81097    |

The docked image and ligand interaction diagram of the best derivative Td₁ with receptor *lanosterol-14α-demethylase* (PDB ID 3LD6) is shown (Fig.5).
ADME Profile of the Analogues by QikProp: The ADME profile of the derivatives is shown below (Table 4).

Table 4: ADME profile of the synthesized compounds

| Compound | Human Oral Absorption | Percent Human Oral Absorption | QPPCaco       | #metab | QPlogKhsa |
|----------|------------------------|------------------------------|---------------|--------|-----------|
| Td1      | 3                      | 100                          | 1564.924      | 6      | 0.213     |
| Td2      | 3                      | 100                          | 1737.744      | 5      | 0.295     |
| Td3      | 3                      | 100                          | 2004.048      | 5      | 0.177     |
| Td4      | 3                      | 100                          | 1857.843      | 7      | 0.312     |
| Td5      | 3                      | 100                          | 2015.624      | 6      | 0.506     |

**QPPCaco** - Caco-2 cell permeability, **#metab** - number of likely metabolic reactions, **QPlogKhsa** - Prediction of binding to human serum albumin.

Characterization

N-phenyl-5-[(phenylamino) methyl]-1,3,4-thiadiazol-2-amine (Td1): Molecular Weight-282, TLC System: Toluene: Ethyl acetate (2:3), Percentage yield-70%, Melting Point-312 °C, IR: C=S group at 648.08 cm\(^{-1}\), C=C (b) group at 1458.18 cm\(^{-1}\), N=H (s) group at 3502.73 cm\(^{-1}\). NMR: δ 7.261-7.602 (m, 5H phenyl attached to thiadiazole nucleus), 4.276-4.398 δ (t, 1H, NH), 2.989-2.999 δ (d, 2H, CH2). MASS: Molecular ion peak- 282.3451, Base peak- 235.7753.

In Vivo Antihyperlipidemic Activity: The effects seen in each group administered with various agents on total cholesterol, HDL, triglycerides, LDL and VLDL are shown below (Table 5).

Table 5: Effect of prepared derivatives on lipid profile

| Group                  | Total Cholesterol | HDL   | TG       | LDL      | VLDL     |
|------------------------|-------------------|-------|----------|----------|----------|
| Normal                 | 103.5±0.727       | 37.66±0.2108 | 77.16±0.8724 | 35.66±0.2108 | 15.43±0.8724 |
| Triton                 | 276.00±0.7303     | 21.00±0.3651 | 262.66±0.085 | 150.66±0.4216 | 52.53±0.085 |
| Atorvastatin derivate  | 143.50±0.8851 **  | 32.00±0.2582 | 163.66±0.085 | 56.83±0.4014 | 32.73±0.085 |

Values are presented as mean ± SEM by one-way Analysis of Variance (ANOVA) followed by Dunnett’s multiple comparisons, n=6, **P<0.01, when groups compared with control.

The bar graph showing the effect on total cholesterol in each group administered with various agents is shown below (Graph1).
Graph 1: Effect of derivative Td₁ on Total Cholesterol in hyperlipidemic rats

The bar graph showing the effect on HDL cholesterol in each group administered with various agents is shown below (Graph 2).

Graph 2: Effect of derivative Td₁ on HDL Cholesterol in hyperlipidemic rats

The bar graph showing the effect on triglycerides in each group administered with various agents is shown below (Graph 3).

Graph 3: Effect of derivative Td₁ on Triglycerides in hyperlipidemic rats

The bar graph showing the effect on VLDL cholesterol in each group administered with various agents is shown below (Graph 4).
Discussion

From the docking studies of various thiadiazole derivatives performed with lanosterol-14α-demethylase (PDB ID: 3LD6), it is seen that the derivative Td₁ containing unsubstituted aniline has maximum binding affinity than the derivatives with substituted anilines. This is indicated by the glide score which is lowest for Td₁ (-9.45308).

The interactions in the ligand interaction diagram is mainly stabilized by the hydrogen bonding interaction of the –NH group with the protein back bone of Ser684. Moreover four hydrogen bond interactions with side chain are seen. The nitrogen atom of the thiadiazole ring with Ser 684 and with Lys 692 and –NH of the aniline molecule with Asp 690 and Arg590.

The compounds which obeyed Lipinski’s rule of five and showed good ADME profile were taken for wet lab synthesis. Physicochemical parameters of the synthesized compounds like melting point were determined. The purity was also confirmed by single spot TLC. The synthesized compounds were characterized by various spectroscopic techniques IR, 1HNMR, 13CNMR and MASS. The corresponding values confirm the formation of the expected derivative.

The analogue Td₁ (100mg/kg), which showed the best glide score was screened for in vivo antihyperlipidemic activity. From this study it can be concluded that the antihyperlipidemic activity of Td₁ is significant. The results were expressed as mean ± standard error of mean. Statistical analysis was done using one-way ANOVA followed by Dunnet’s multiple comparison test. It is significant at p<0.01.

Conclusion

The present investigation was aimed for the design of novel 1, 3, 4-thiadiazole containing compounds in search of potential drugs. The objective of the present work was to perform the in silico screening, synthesis, characterization and biological activity studies of newly synthesized N-phenyl-1, 3, 4-thiadiazol-2-amine derivatives. Molecular docking experiments were carried out to identify potential drug candidates among the thiadiazole derivatives. The compounds which obeyed Lipinski’s rule of five and showed good ADME profile were taken for wet lab synthesis. Four different thiadiazole analogues were synthesized by conventional method. The yield of the newly synthesized compounds was found to be in the range of 60-75%. Synthesized compounds were purified by recrystallization. Purity of the compounds was confirmed by single spot TLC and
by melting point determination. The structure of the newly synthesized compounds was confirmed by spectral data viz. IR, \(^1\)HNMR and MASS. Preliminary pharmacological screening of the synthesized analogues was performed. The analogue which showed promising glide score was screened for \textit{in vivo} antihyperlipidemic activity. From this study it can be concluded that the thiadiazole derivative prepared from aniline, Td, showed good antihyperlipidemic activity. The above results establish the fact that thiadiazole can be a rich source for exploitation. Therefore in search of new generation of active compounds, it may be worthwhile to explore the possibility in this area by fusing and substituting different moieties to increase the potency. All the synthesized compounds can be further explored for structural modifications to improve their activity so that they can be converted to prospective drugs.

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