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

Paul Jacob[1,*], Beena Thomas[2], Neethu Santhosh[3]

[1,2]Senior Lecturer, [3]Student, Dept. of Pharmaceutical Sciences, Mahatma Gandhi University RIMSR, Kottayam, Kerala, India

***Corresponding Author:**
Email: pauljacobdps@gmail.com

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, ^{1}HNMR 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 **hyperlipoproteinemia** is the presence of raised or abnormal levels of lipids and/or lipoproteins in the blood i.e, an increase (hyper) in the **lipids** (lipi), 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-14α-demethylase*. This P450 enzyme is the only one of the eight enzymes that is involved in *de novo* cholesterol biosynthesis. *Lanosterol-14α-demethylase* is the cytochrome P450 monooxygenase, 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-thiadaizole, 1,2,3-thiadiazole, 1,2,4- thiadiazole, & 1,2,5-thiadiazole are reported with various pharmacological activities.[4]

Thiadiazole moieties reported to show major biological activities such as antibacterial, anti-tubercular, anti-inflammatory, anti-oxidant, anticancer, antifungal, antidepressant, antihyperlipidemia, antihypertensive etc.[5-9] 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.

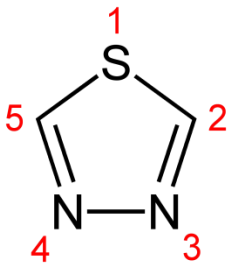


Fig. 1: Thiadiazole

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 2.15A^{0}.[10]

Scheme for Synthesis:

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

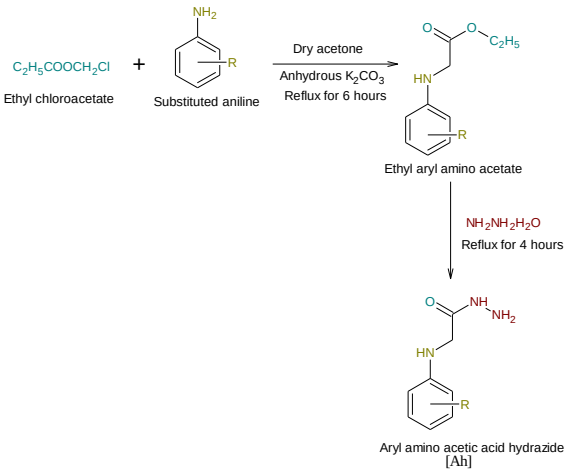


Fig. 2: Scheme for the synthesis of Ah

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)

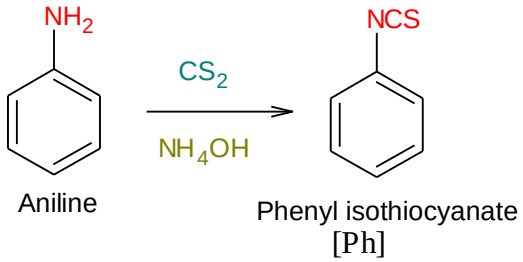


Fig. 3: Scheme for the synthesis of Ph

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 H_2SO_4 as long as any oil comes over. About 2–3ml of distillate was collected. The product was obtained from the water using a separating funnel.[12]

STEP 3: SYNTHESIS OF THIADIAZOLE DERIVATIVE (Td)[13] (Fig. 4)

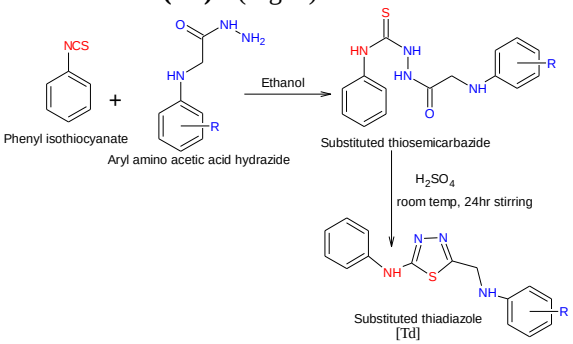


Fig. 4: Scheme for the synthesis of thiadiazoles

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 (Td_1, Td_2, Td_3 and Td_4) were synthesized using four different substituted anilines. (Table 1)

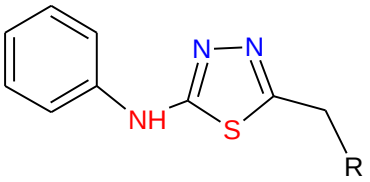
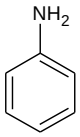
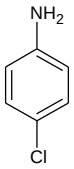
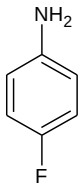
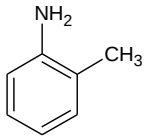


Table 1: V**arious anilines used in the preparation**

Compound	Td_1	Td_2	Td_3	Td_4
-R				

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 administerd as shown. (Table 2)[14-16]

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 Intra peritoneal injection at a dose of 100mg/kg body weight Serves as toxic control group	No drug was given
Group C	Single Intra peritoneal injection at a dose of 100mg/kg body weight	Standard drug Atorvastatin at effective dose 1.14mg/kg was given orally for 7 days.

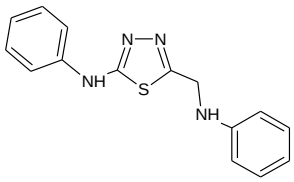
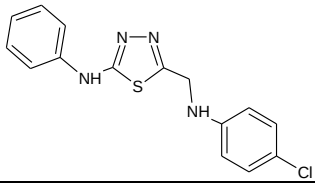
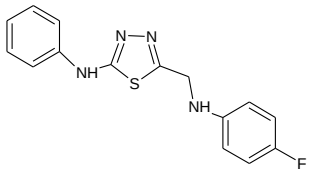
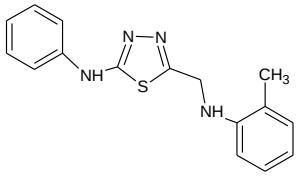
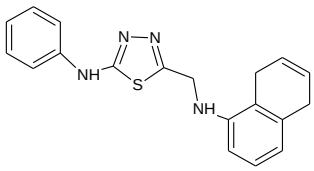
Group D	Single Intra peritoneal injection at a dose of 100mg/kg body weight	Animals received thiadiazole derivative 100mg/kg/p.o. in 1% sodium CMC for a period of 7 days.

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).

Table 3: Docking scores of designed compounds

Compound	Structure	Glide score
Td_1		-9.45308
Td_2		-8.72432
Td_3		-8.13892
Td_4		-7.52065
Td_5		-6.81097

The docked image and ligand interaction diagram of the best derivative Td_1 with receptor ***lanosterol-14α-demethylase*** **(PDB ID 3LD6)** is shown (Fig.5).

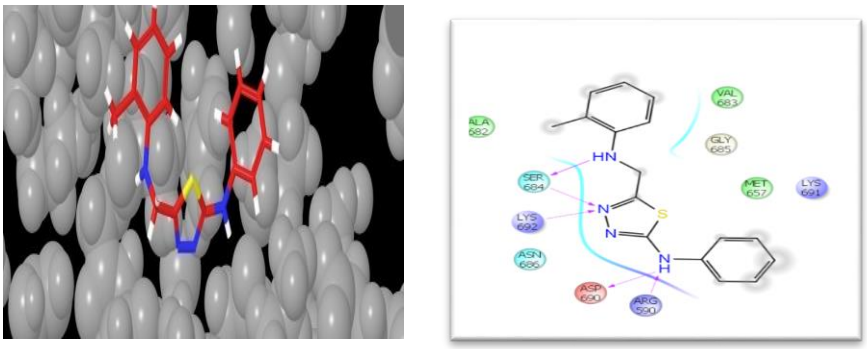


Fig. 5: Docked image and ligand interaction diagram of Td_1 with *lanosterol 14α-demethylase*

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
Td_1	3	100	1564.924	6	0.213
Td_2	3	100	1737.744	5	0.295
Td_3	3	100	2004.048	5	0.177
Td_4	3	100	1857.843	7	0.312
Td_5	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 (Td_1): **Molecular Weight**-282**, TLC System**: Toluene: Ethyl acetate (2:3)**,** Percentage yield-70%**,** Melting Point-312 ˚C,**IR** : C=S group at 648.08cm^{-1} ,C=S group at 694.34cm^{-1} ,C-H Aromatic group at 763 cm^{-1}, C-C (b) group at 817.82cm^{-1} , C-C (b) group at 1072 cm^{-1},C-H (b) group at 1180cm^{-1},C-N (b) group at 1311cm^{-1},C=C ring stretching at 1458.18cm^{-1}, N-H (b) group at 1597cm^{-1}, C=N (s) group at 1689 cm^{-1}, N-H (s) group at 3502.73cm^{-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).

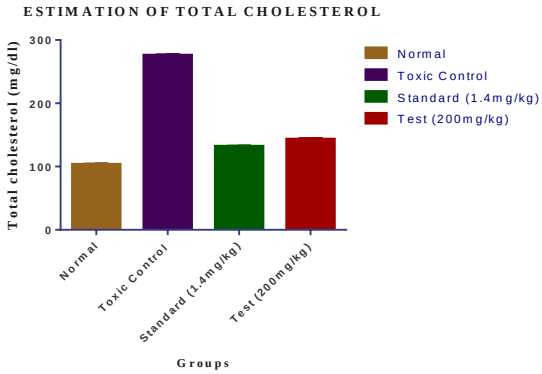
Table 5: Effect of prepared derivatives on lipid profile

Group	Total Cholesterol	HDL	TG	LDL	VLDL
Normal	103.5± 0.727	37.666± 0.2108	77.166± 0.8724	35.666± 0.2108	15.433± 0.8724
Triton	276.00± 0.7303	21.00± 0.3651	262.66± 0.085	150.66± 0.4216	52.532± 0.085
Atorvastatin	132.00± 0.4472 **	35.166± 0.3073 **	155.166± 0.579 **	47.666± 0.2108 **	31.0322± 0.579 **
Thiadiazole derivative	143.50± 0.8851 **	32.00± 0.2582 **	163.666± 0.085 **	56.833± 0.4014 **	32.733± 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.

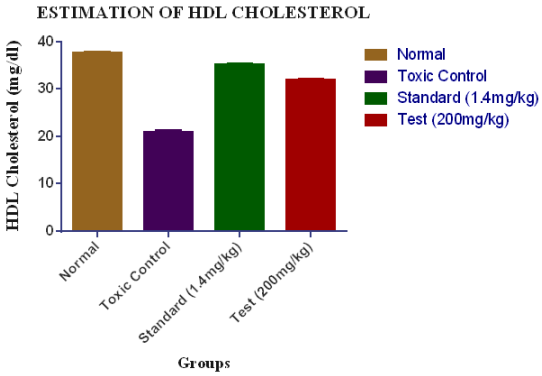
The bar graph showing the effect on total cholesterol in each group administered with various agents is shown below (Graph1).

Graph 1: Effect of derivative Td_1 on Total Cholesterol in hyperlipidemic rats



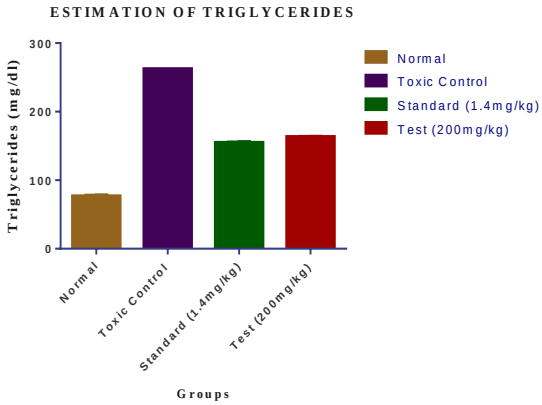
The bar graph showing the effect on HDL cholesterol in each group administered with various agents is shown below (Graph 2).

Graph 2: Effect of derivative Td_1 on HDL Cholesterol in hyperlipidemic rats



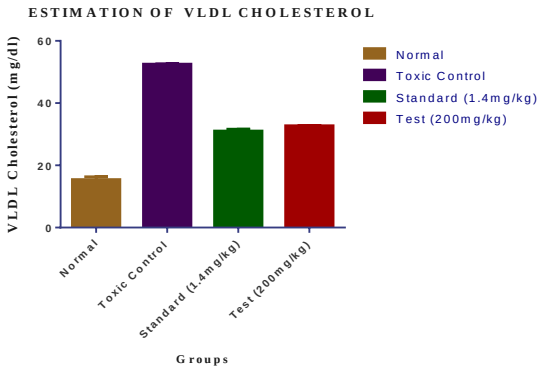
The bar graph showing the effect on triglycerides in each group administered with various agents is shown below (Graph3).

Graph 3: Effect of derivative Td_1 on Triglycerides in hyperlipidemic rats



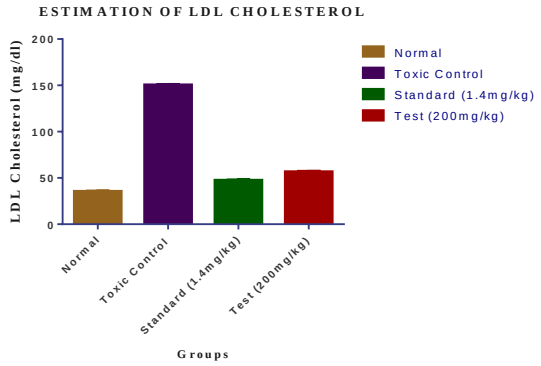
The bar graph showing the effect on VLDL cholesterol in each group administered with various agents is shown below (Graph 4).

Graph 4: Effect of derivative Td_1 on VLDL Cholesterol in hyperlipidemic rats



The bar graph showing the effect on LDL cholesterol in each group administered with various agents is shown below (Graph5).

Graph 5: Effect of derivative Td_1 on LDL Cholesterol in hyperlipidemic rats



Discussion

From the docking studies of various thiadiazole derivatives performed with *lanosterol-14α-demethylase* (PDB ID: 3LD6), it is seen that the derivative Td_1 containing unsubstituted aniline has maximum binding affinity than the derivatives with substituted anilines. This is indicated by the glide score which is lowest for Td_1 (-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, ^{1}HNMR,^{13} CNMR and MASS. The corresponding values confirm the formation of the expected derivative.

The analogue Td_1 (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 Td_1 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 *in vivo* antihyperlipidemic activity. From this study it can be concluded that the thiadiazole derivative prepared from aniline, Td_1 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.

Acknowledgements

We express our sincere thanks to the Principal, Department of Pharmaceutical Sciences, Mahatma Gandhi University Regional Institute of Medical Science and Research, Puthuppally Campus for providing the facility for the successful completion of this work.

References

1. What is Hyperlipidemia? Available from http://www.news-medical.net/health/What-is-Hyperlipidemia.aspx downloaded on 2013 December 8.
2. Antihyperlipidemic Drugs. Chapter 43. Available from: http://mlearning. zju.edu.cn/G2S/eWebEditor/uploadfile/20120529102107199.
3. The medical biochemistry, 1996–2013. Available from: http://themedicalbiochemistrypage.org/cholesterol.php
4. Kempegowda, Kumar SGP, Prakash D, Mani TT. Thiadiazoles: Progress Report on Biological Activities. Der Pharma Chemica. 2011;3(2):330-41.
5. Mathew V, Giles D, Keshavayya J, Vaidya V P. Studies on Synthesis and Pharmacological Activities of 1,2,4-Triazolo[3,4-*b*]1,3,4-thiadiazoles and their Dihydro Analogues. Arch Pharm.2009;342:210–22.
6. Farghaly TA, Abdallah MA, Aziz MR. Synthesis and antimicrobial activity of some new 1, 3, 4-thiadiazole derivatives. Molecules. 2012;17(12):14625-36.
7. Syed MA, Ramappa AK, Alegaon S. Synthesis And Evaluation Of Antitubercular And Anti Fungal Activity Of Some Novel 6-(4-Substituted Aryl)-2-(3,5-Dimethyl-1h-Pyrazol-1-Yl) Imidazo[2,1-B] [1,3,4] Thiadiazole Derivatives. Asian J Pharm Clin Res. 2013;6(3):47-51.
8. Luo Z, Chen B, He S, Shi Y, Liu Y, Li C. Synthesis and antitumor-evaluation of 1,3,4-thiadiazole-containing benzisoselenazolone derivatives. Bioorganic & Medicinal Chemistry Letters.2012;22(9):3191–3.
9. Soni BK , SinghT , Bhalgat CM, KamleshB , Kumar SM , Pavani M. In-vitro antioxidant studies of some 1,3,4-thiadiazole derivatives. International Journal of Research in Pharmaceutical and Biomedical Sciences.2011; 2(4):1590-2.
10. Schrodinger suite user manual.Maestrov9.3. 2012.
11. Sammaiah G, Sarangapani M. Synthesis and Biological Activity of Phenyl Amino Acetic Acid (2-Oxo-1, 2-dihydroindol-3-ylidene) hydrazides .Asian Journal of Chemistry. 2008;20(1):75-80.
12. Dains FB, Brewster RQ, Olander CP. Phenyl Isothiocyanate .Org Synth. 1926;6:72.
13. Kadi AA, El-Brollosy NR, Al-Deeb OA, Habib EE, Ibrahim TM, El-Emam AA. Synthesis, antimicrobial, and anti-inflammatory activities of novel 2-(1-adamantyl)-5-substituted-1,3,4- oxadiazoles and 2-(1-adamantylamino)-5-substituted-1,3,4-thiadiazoles European Journal of Medicinal Chemistry. 2007;47:235-42.
14. Sudha SS, Karthic R, Naveen, Rengaramanujam J. Antihyperlipidemic activity of *Spirulina platensis* in Triton x-100 induced hyperlipidemic rats. Hygeia J D Med.2011;3(2):32-7.
15. Ara J, Sulthana V, Qasim R, Ahmed VU. Hypolipidemic activity of seaweed from Karachi coast. phytother Res. 2002;16(5):479-83.
16. Keshetty V, Pabba S, Gudipati R, Kandukuri JM, Allenki V. Antihyperlipidemic activity of methanolic extract of garlic (*Allium sativum L.*) in Triton X-100 induced hyperlipidemic rats. Journal of Pharmacy Research. 2009;2(5):777-80.