5-Hydroxy Pyrazole Derivative as An Antihyperlipidemic Agent: Design, Synthesis and Evaluation with Npc1l1 Inhibitory Activity

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ABSTRACT: In order to investigate the SAR of 5-hydroxy pyrazole analogs for the cholesterol absorption inhibitors, electron- donating group were introduced to the C-(4) carbon chain of the 5-hydroxy pyrazole derivative. Nine new derivatives of the 5- hydroxy pyrazole for the cholesterol absorption inhibitor have been synthesized. All the synthesized new compounds were evaluated for the activity to inhibit cholesterol absorption in hamster and above all of them some showed comparable good effects in suppressing the level of total cholesterol in the blood.

KEYWORDS: Antihyperlipidemia, 5-hydroxy pyrazole, Docking, NPC1L1, Cholesterol absorption inhibitor.

1.0 INTRODUCTION

Cholesterol is the main constituent required for cell membrane permeability, penetration, and flexibility. It also performs as a source for the production of bile acids, Vit D, and steroid receptors in animals. Additionally, cholesterol acts as a vital key mediator. As a result, dysfunctional regulation of Cholesterol and its connected compounds have an impact on human health. A high blood cholesterol level in the blood is a key risk factor for atherosclerotic coronary heart disease, the biggest cause of mortality in developed nations(1, 2).

Hyperlipidemia means elevated levels of one or more plasma lipids that are a key warning sign of atherosclerosis and atherosclerosis-related disorders such as coronary heart disease (CHD), ischemic vascular disease, and peripheral arterial disease. These cardiovascular diseases (CVDs) are believed to be the root cause of more than 30% of all mortality worldwide(3, 4). Niacin (vitamin B3 or nicotinic acid) is one of the earliest antihyperlipidemic medicines (Fig. 1). It is reported to lower cholesterol and triglyceride levels while increasing high-density lipoprotein values (HDL) (5-7). HDL cholesterol > 60 mg/100 mL is known to be protective against coronary heart disease(5).

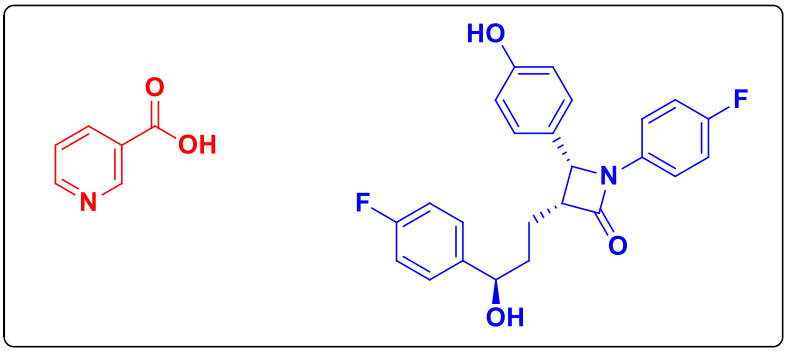


Fig.No.1 Structure of Nicotinic acid and Ezetimibe

Niemann-Pick C1-like 1 protein is one of the receptors that can be targeted to reduce plasma lipids (NPC1L1). The transmembrane protein NPC1L1 is present in enterocytes and

Hepatocyte (8-10). It carries sterol for gastrointestinal or hepatobiliary lipid metabolism or excretion. The inhibition of NPC1L1 by ezetimibe, a -lactam drug (fig. no.1) limits cholesterol

biosynthesis and hence limits diet-induced clinical symptoms, obesity, and lowers cholesterol levels(11).

The hypocholesterolemic medicine ezetimibe interferes with the interaction with NPC1L1 and flotillin, preventing the development of cholesterol-enriched microdomains (12).

In the development of compounds one famous strategy that is widely used in drug design and development is the hybridization of molecules. It works on the principle where two or more pharmacophoric moieties or two known nuclei are gathered together for the formation of a new molecular nucleus. It is prepared for improving affinity, intensity, efficacy, increasing target selectivity, reducing side effects (13, 14)

In the literature survey, we observed that the togetherness of nicotinic acid and ezetimibe analog in one moiety (fig.no. 2) replacing 2-azetidinone ring with pyrazoline ring and in the final structure formation forms 5-hydroxy pyrazole ring as a potential nucleus which offers a good synthesis and extra sites for possible interaction with NPC1L1 receptor. In the study, we observed that the addition of an electron-donating group in the para position of the carbon chain increases more affinity to bind molecules to the respective receptor site.

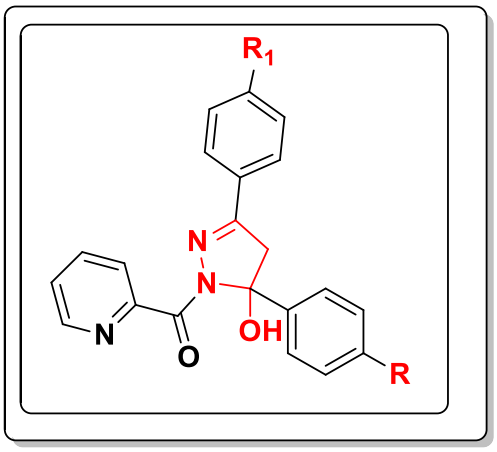
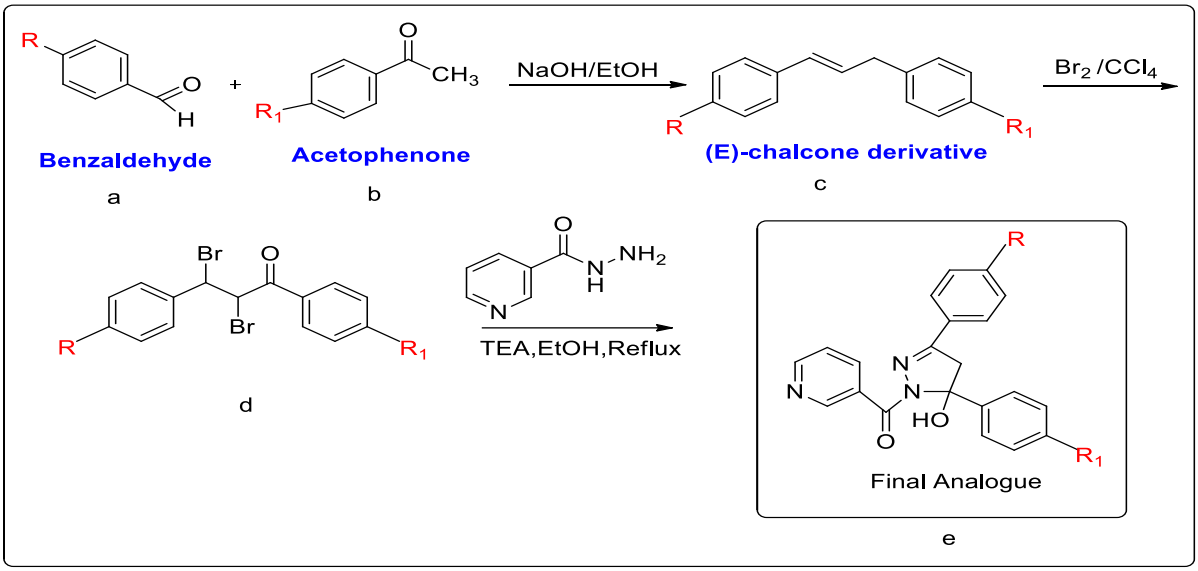


Fig.No.2 Structure of Target derivative

2.0 RESULT AND DISCUSSION

2.1 General chemistry

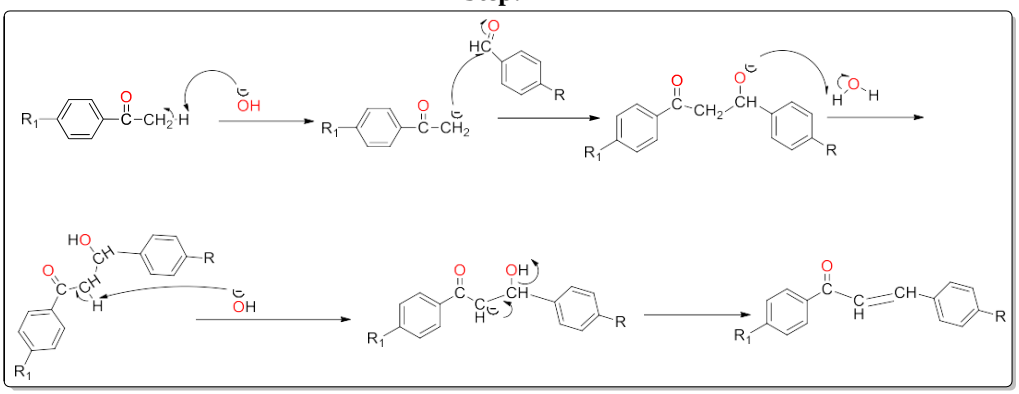
The procedure of the entire synthesis is summarized in Scheme 1. The Claisen-Schmidt condensation process between benzaldehyde derivatives and acetophenone derivatives in the presence of NaOH yielded the chalcone derivatives, which were recognized by their stated melting point and thin layer chromatograph(15). Then with the chalcone intermediate, formation of chalcone dibromide derivatives were formed(16). Cyclization of intermediate compound with nicotinic acid hydrazide in the presence of base tri ethyl amine with refluxing ethanol for 20-48 hours(17) to obtained the desired 5-hydroxy pyrazole derivatives.



Scheme 1:- Scheme of designed compound

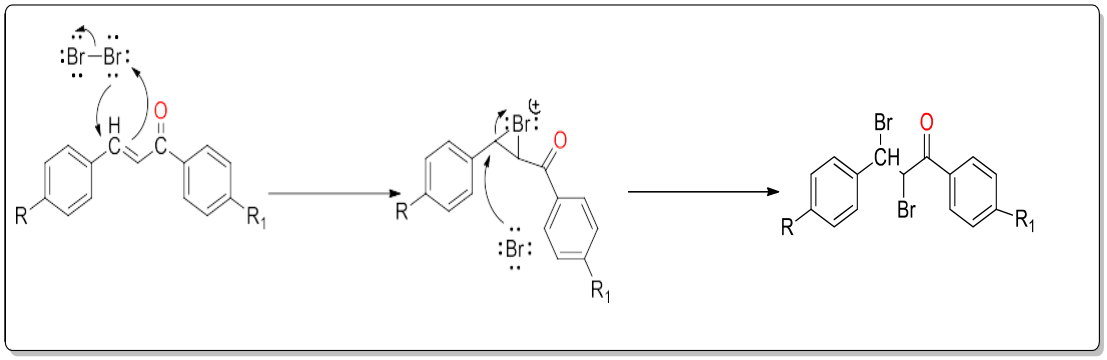
MECHANISM

Step: - 1



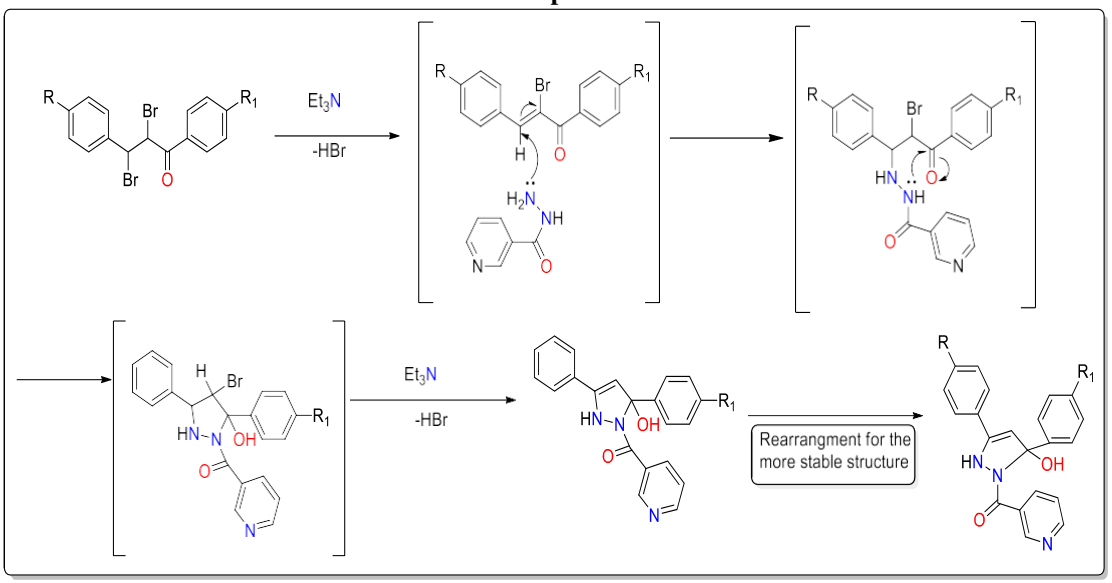
Substituted acetophenones have α hydrogen and thus they can be deprotonated to give a nucleophilic enolate anion. The nucleophilic enolate anion attacks the electron- deficient carbon of the substituted benzaldehyde to produce alkoxide β-hydroxy ketone, which undergoes base-catalyzed dehydration to yield substituted chalcone.

Step: - 2



As the π electron of the alkene approach a molecule of Br_2.Bromine accepts those electrons and releases the shared electrons to the other bromine, which leaves as a bromide ion. Because bromine's electron cloud is close enough to other sp^2 carbon to form a cyclic brominium ion intermediate is formed rather than a carbon cationintermediate. The cyclic bromonium ion intermediate is unstable because of the strain in the three-membered ring and the positively charged bromine, which withdrawelectrons strongly from the ring carbons. Therefore, the cyclic bromonium ion reacts with a nucleophile (br^-).

Step:-3



Triethyl amine facilitates the dehydro-halogenation of the dibromo-chalcone derivative,which leads to the formation of the transitional state 1. The lone pair electron presents on the electron

ring of the nicotinic acid hydrazide attacks the β carbon of the transition state 1 to form transition state 2. Transition state 2 undergoes 1, 4 addition with nicotinic acid hydrazide to form pyrazole derivative (transitional state 3). Transition state 3 undergoes dehydro-halogenation in the presence of triethyl amine to yield 5-hydroxy pyrazole derivatives, which undergo rearrangement to yield the final compound (18).

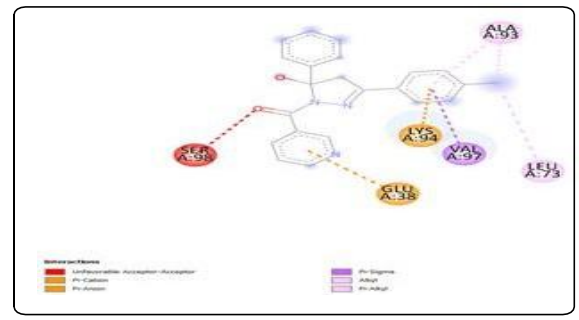
2.2 MOLECULAR DOCKING STUDY

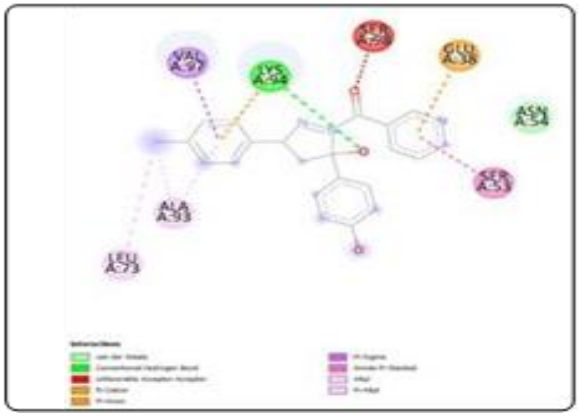
AutoDock Vina Software was used to dock compounds RA 01- 09 and the reference compound ezetimibe into the active site of the Niemann-Pick C1-like 1 protein (NPC1L1, PDB code: 3QNT)(19). Docking scores of compounds with electron donating group substitutions were better than those having electron withdrawing group substitution. In the docking study, somecompounds show good scores against the reference once. RA-01, RA-03, and RA-05 show a good enough docking score

S. No.	Compound Code	Docking score
1.	RA-01	-6.8
2.	RA-02	-6.7
3.	RA-03	-6.9
4.	RA-04	-6.9
5.	RA-05	-6.7
6.	RA-06	-6.8
7.	RA-07	-6.9
8.	RA-08	-6.8
9.	RA-09	-6.8
10.	Reference	-4.9

RA 03

RA 05





3.0 EXPERIMENTAL

3.1 General Procedure

Chemicals and the solvents used were purchased from Avra Synthesis Pvt Ltd, Chempura Pvt Ltd..Thin Layer Chromatography (TLC) (Merck 60F254 silica 90gel plates supported on aluminum) were used to monitor the progress of the reaction.The TLC plates were visualized under UV-light/Kmno4 and Iodine Staining reagent.

Synthetic procedure of Intermediate I:

In a flat bottom flask, containing (0.01mol) of benzaldehyde derivative and (0.01mol) of acetophenone derivative, followed by the addition of 10ml ethanol in it. Then the addition of 10ml NaOH solution dropwise to the same reaction mixture on continuous stirring for 30min when the solution became turbid. Then the whole reaction mixture temperature was maintained at 20-25° C while using a cold water bath on the magnetic stirrer. Mixture with vigorous stirring for 5-6 hours, then the reaction was neutralized by 0.1-0.2N HCl meanwhile the ppt was obtained. The progress of the reaction was monitored using TLC (Hexane: Ethyl Acetate – 9.5:0.5).

Synthetic procedure of Intermediate II:

In a 500ml flask, added intermediate I and react with the specific amount of acetic acid. Then the addition of 20% bromine solution. To the flask, a stir bar was addedand the flask was placed on a stirring plate. After adding the bromine solution the flask was slowly filled with distilled water to precipitate out the product. The mixture was filtered out to separate the product. Then the product was washed with a copious amount of water to remove any excess acetic acid. Then the product was allowed toair dry.

Synthetic procedure of Intermediate III:

Nicotinic acid hydrazide (0.01mol) was added in intermediate II (0.01mol) with the appropriate quantity of ethanol. Then triethylamine was added. The prepared mixture was heated on reflux for 24-48 hours. The progress of the reaction was monitored using TLC (Hexane: Ethyl Acetate – 9.5:0.5).

Compound	R	R_1
RA-01	4-methyl benzaldehyde	Acetophenone
RA-02	4-methyl benzaldehyde	4-methyl acetophenone
RA-03	4-methyl benzaldehyde	4- hydroxy Acetophenone
RA-04	4-methyl benzaldehyde	4- amino Acetophenone
RA-05	benzaldehyde	4-methyl Acetophenone
RA-06	benzaldehyde	4- hydroxy Acetophenone
RA-07	4- chloro benzaldehyde	4- amino Acetophenone
RA-08	4- methoxy benzaldehyde	Acetophenone
RA-09	4- methoxy benzaldehyde	4-methyl acetophenone

- **RA-01 ((E)-1-phenyl3 (p-tolyl) prop-2-en-1-one)** yield 55.648% ,IR 1343.48 (C-N), 1589.41 (C=C), 1674 (C-C), 2278 (C=N), 1701 (C=O), ^{1}H NMR (500 MHz, DMSO) δ 5.65 (s, 1H), -0.01 (s, 2H), -0.25 (qd, *J* = 7.3, 4.8Hz, 4H), -2.17 (td, *J* = 7.3, 1.6 Hz, 7H).
- **RA-02 ((E)-1, 3-di-p-tolylprop-2-en-1-one)** yield 50.378%, IR 1289(C-N), 1601 (C=C), 1529 (C-C), 2015 (C=N), 1651 (C=O), 3605 (-OH), ^{1}H NMR (500 MHz, DMSO) δ -0.25 (s, 3H), -2.13 – -2.20 (m, 4H).
- **RA-03 ((E)-1-(4-hydroxyphenyl)-3-(p-tolyl) prop-2-en-1-one)** yield 54.201%, IR 1339 (C-N), 1546 (C=C), 1505 (C-C), 1674 (C=N), 1707 (C=O), 3643 (-OH), ^{1}H NMR (500 MHz, DMSO) δ 5.56 (s, 0H), 0.01 (s, 11H), -0.23 (qd, *J* = 7.3, 4.8Hz, 2H), -0.30 (s, 1H), -2.16 (t, *J* = 7.3 Hz, 3H).
- **RA-04 ((E)-1-(4-aminophenyl)-3-(p-tolyl) prop-2-en-1-one)** yield IR 49.284%, IR 1126 (C- N), 1674 (C=C), 1661(C-C), 1655(C=N), 1752(C=O), 3565 (-OH), ^{1}H NMR (500 MHz, DMSO) δ 10.83 (s, 3H), 9.09 (d, *J* = 2.2 Hz, 2H), 8.80 (dd, *J*= 4.8, 1.6 Hz, 2H), 8.28 (d, *J* = 8.2 Hz, 3H), 7.60 (dd, *J* = 7.8, 4.8 Hz, 2H), 7.51(s, 2H), 7.25 (s, 2H), 7.04 (s, 1H), 3.54 (s, 7H), 2.15 (s, 1H), 1.26 (t, *J* = 7.3 Hz,3H), 1.16 (t, *J* = 7.2 Hz, 1H).
- **RA-05 ((E)-3-phenyl-1-(p-tolyl) prop-2-en-1-one)** yield 59.234%, IR 1341 (C-N), 1577(C=C), 1673(C-C), 1694(C=N), 1722(C=O), 3701(-OH), ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.03 (s, 1H), 3.15 (q, *J* = 7.3 Hz, 6H), 1.44 (tt, *J* =8.2, 4.1 Hz, 9H).

3.2 Molecular weight, Molecular formula, % yield

Compound Code	Molecular Weight	MolecularFormula	Yield ofCrude Product	Yield ofPure Product
RA-01	357.41	$C_{22}H_{19}N_3O_2$	67.345%	55.648%
RA-02	371.43	$C_{23}H_{21}N_3O_2$	70.652%	50.378%
RA-03	373.14	$C_{22}H_{19}N_3O_3$	71.365%	54.201%
RA-04	372.42	$C_{22}H_{20}N_4O_2$	67.210%	49.284%
RA-05	357.41	$C_{22}H_{19}N_3O_2$	77.252%	59.234%
RA-06	359.38	$C_{21}H_{17}N_3O_3$	68.402%	40.100%

RA-07	392.84	$C_{21}H_{17}ClN_4O_2$	54.933%	46.91%
RA-08	373.40	$C_{22}H_{19}N_3O_3$	17.650%	15.120%
RA-09	387.16	$C_{23}H_{21}N_3O_3$	33.611%	13.765%

3.3 ADMET Prediction:

ADME Results:

ADME prediction results of all synthesized compounds

Compound Code	Lipinski Rule	Log P[a]	Caco-2[b]	PPB[c]	BBB Penetration	Clearance[e]
RA-01	Accepted	2.707	-4.733	91.34%	0.9-1.0	3.170
RA-02	Accepted	3.195	-4.792	93.84%	0.9-1.0	2.8811
RA-03	Accepted	2.39	-4.757	91.09%	0.5-0.7	4.63
RA-04	Accepted	2.07	-4.730	83.66%	0.9-1.0	5.92
RA-05	Accepted	2.68	-4.731	91.34%	0.9-1.0	3.222
RA-06	Accepted	1.817	-4.741	88.76%	0.7-0.9	4.992
RA-07	Accepted	2.296	--4.680	87.32%	0.9-1.0	5.294
RA-08	Accepted	2.300	-4.675	91.22%	0.9-1.0	4.943
RA-09	Accepted	2.804	-4.733	92.96%	0.9-1.0	4.581
RA-10	Accepted	2.159	-4.674	88.64%	0.9-1.0	3.508

*a- Optimal: 0-3, b- Optimal higher than -5.15 log unit, c-Optimal-15ml/min/kg; moderate: 5-15 ml/min/kg; low:

As predicted by ADMET Lab 2.0, all the compounds follow Lipinski rule of five suggesting that they have drug likeliness. The HIA Value of all the compounds lies below the 0.3 suggesting that can be absorbed in the intestine very well. All the valuesof BBB lie beyond 0.7 suggesting that these compounds can poorly cross the blood brain barrier and compounds RA-01, RA-03 and RA-05 had a higher value of plasma protein binding suggesting that they may have poor distribution in the body.

3.4 Toxicity Results:

Toxicity prediction results of all synthesized compounds

Compound Code	hERG Blockers[a]	H-HT(Human Hepatotoxicity)[b]	Carcinogenicity	Respiratory Toxicity[d]

RA-01	0-0.1	0.3-0.5	0.1-0.3	0.1-0.3
RA-02	0-0.1	0.3-0.5	0.1-0.3	0-0.1
RA-03	0-0.1	0.1-0.3	0.1-0.3	0.1-0.3
RA-04	0-0.1	0.5-0.7	0.1-0.3	0.3-0.5
RA-05	0-0.1	0.3-0.5	0.1-0.3	0.1-0.3
RA-06	0-0.1	0.1-0.3	0.1-0.3	0.1-0.3
RA-07	0-0.1	0.5-0.7	0.1-0.3	0.3-0.5
RA-08	0-0.1	0.5-0.7	0.1-0.3	0-0.1
RA-09	0-0.1	0.5-0.7	0.3-0.5	0-0.1
RA-10	0-0.1	0.3-0.5	0-0.1	0.1-0.3

*a-Category 1: active; Category 0: inactive, b-Category 1: H-HT Positive (+); Category 0; H-HT negative (-), c- Category1: carcinogens; Category 0: non- carcinogens, Category 1: respiratory toxicants, Category 0: respiratory nontoxicants.

As predicted by ADMET Lab 2.0, all the compounds belongd to the category of hERG blockers, category of carcinogenity and category of respiratory toxicity suggesting that all these compounds are non-carcinogenetic, respiratory nontoxicants and cannot block hERG significantly.

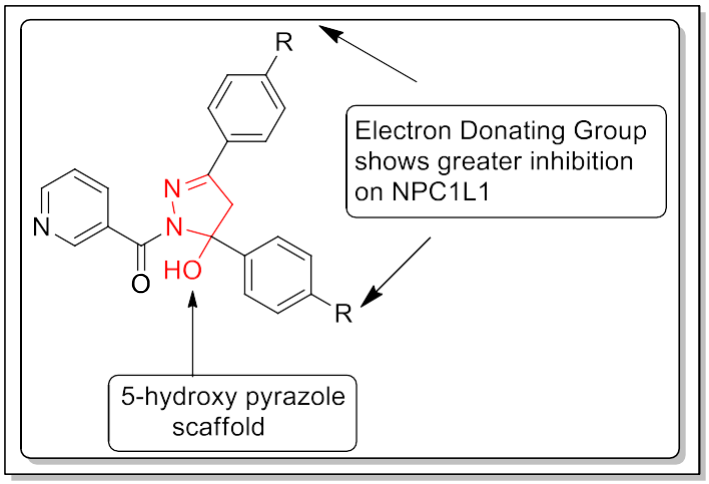
4.0 CONCLUSION

Hyperlipidemia is a chronic syndrome that is categorized by variation of lipid profiles (e.g. increased cholesterol level, elevated triglycerides, raised up flux of Free Fatty Acids, increment in the intensity of Low-Density Lipoprotein (i.e. bad cholesterol), decrease the level of High-Density lipoprotein (i.e. good cholesterol, familial combined hyperlipidemia) that may result in major health effects (e.g. arterial disorder, coronary heart disease, overweight etc.).Due to the several health issues it is important to discover newer, rapid action showing drug for future use.

In the entire research work, we mainly focused to target the Niemann-Pick C 1- Like 1 (NPC1L1) protein which is present on the membrane of the intestine this is a crucial protein for the absorption of dietary cholesterol absorption.

In the literature survey, we obtained many scaffolds which act to inhibit the activity of the protein. After a complete survey and through docking studies we conclude that the 5-hydroxy pyrazole scaffold had good activity to inhibit the absorption of protein.

So the main aim of the study was to build up 5-hydroxy pyrazole scaffolds which show an anti-hyperlipidemia activity. A literature survey suggested that substituting electron-donating groups on the C-4 position of substituted benzaldehyde and acetophenone had comparatively good action on the targeted protein. All 28 compounds are docked against the targeted protein.



Then synthesis was performed under the appropriate conditions and the entire reaction process was monitored by thin-layer chromatography. Characterization of the synthesized compounds was performed through Melting point, IR Spectroscopy, 1H NMR Spectroscopy, and Mass Spectroscopy. And after the above characterization compounds were subjected to the Oil Red O Staining method.

Out of ten synthesized compounds, two compounds show prominent antihyperlipidemic activity.RA-03 and RA-05 synthesized compounds, show goodenough activity.

So these derivatives RA-03 and RA-05 can be the best-synthesized compounds that could be useful for the progress and development of new anti-hyperlipidemia agents

This field is further open for research and the development of potent anti- hyperlipidemia drugs.

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