Research Article **ISSN: 2321-0923**

Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry

Journal home page: www.ajpamc.com

SYNTHESIS, DOCKING AND EVALUATION OF *IN-VITRO* HEPATOPROTECTIVE ACTIVITY OF TETRAHYDROQUINAZOLINE DERIVATIVES

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ABSTRACT

The heterocyclic amines are the carcinogenic chemicals. Quinazolines is stable in cold dilute acid and alkaline solutions, but it is destroyed when these solutions are boiled. O- Aminobenzaldehyde, ammonia and formic acid are formed when quinazoline is boiled with hydrochloric acid. Male Wistar Albino rats, weighed 200 ± 20 g were utilized for this study. They were housed in polypropylene cages under standard laboratory conditions (12-h light/ 12-h dark cycle, 21 ± 2 ºC, and relative humidity 55 %). The animals were given standard rodent pellets and tap water *ad libitum*. The resulting liver dysfunction as marked by the increase of SGPT, SGOT and ALP activities. $4a_1$ and $4a_4$ decreased the alcohol-induced increase of SGPT, SGOT and ALP activities dose dependently. However, 50 mg/kg bodyweight dose of $4a_4$ was found to be most effective. Molinspiration virtual screening toolkit miscreen one can easily develop a screening engine for arbitrary target, provided that several active ligands are known. With the trained model it is possible to screen large libraries of hundreds of thousands of molecules in less than hour, to identify molecules with highest chance to become active drugs or pesticides.

KEYWORDS

Molinspiration, Tetrahydroquinazoline, Recrystallized and Antimitotic.

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INTRODUCTION

Heterocyclic compound is an organic compound that contains a ring structure containing atom in addition to carbon, such as sulfur, oxygen or nitrogen as part of the ring. They may be either simple aromatic ring or non-aromatic rings[1,2]. Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence

docking plays an important role in the rational design of drugs[3]. Lipinski's Rule of Five is a rule of thumb to evaluate drug likeness, or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. The rule was formulated by Christopher A. Lipinski in 1997, based on the observation that most medication drugs are relatively small and lipophilic molecules[4]. The liver the largest solid organ in the body; and is also considered a gland because among its many functions, it makes and secretes bile2. The liver is located in the upper right portion of the abdomen protected by the rib cage. It has two main lobes that are made up of tiny lobules[5]. Each liver disease will have its own specific treatment regimen. For example, hepatitis A requires supportive care to maintain hydration while the body's immune system fights and resolves the infection[6-9].

MATERIALS AND METHODS

In the present study we have attempted the synthesis of tetrahydroquinazoline derivatives using ethanol, para amino phenol and aldehyde.

Synthesis of Compounds 1-4

Step – 1

A mixture of aldehydes (0.2 mol) and urea (0.4 mol) in absolute ethanol (100 ml) was heated under reflux for 4 hrs in such a manner that moisture air did not pass into the reaction mixture. Ethanol was removed by distillation and the residual solid was washed with water. The crude product was dried in and recrystallized using dilute methanol[10].

Step - 2

A mixture of 4-hydroxy-3-methoxyphenyl-bis-ureas (0.2mol) and p-amino phenol (0.2mol) were dissolved in glacial acetic acid (50 ml) by stirring and heating slowly. The acidic solution was subsequently heated under reflux for 5 hrs. The hot solution was cooled at room temperature and poured into ice-cold water (250 ml) and stirring vigorously for half an hour. It was filtered off and washed with water (3 × 25ml). The crude product was dried in vacuo and recrystallized using diluted ethanol[11].

Step – 3

A mixture of bromo aniline and compound 2 in ethanol(30ml) containing 3-4 drops of glacial acetic acid was heated for half an hour and left overnight at room temperature. The solid product thus obtained was recrystallized with methanol.

Step – 4

A mixture of compounds 3 (0.01mol) was suspended in a minimum quantity (10ml) of dimethyl formamide (DMF). To this solution slight more than 0.01mol of formaldehyde and various secondary amines were added with vigorous stirring. The reaction mixtures were heated on water bath for 20 minutes and left overnight was shown in Figure No.1. The product thus obtained was re-crystallized from ethanol solvent [12].

POLO-LIKE KINASE 1 (PLK1)

Antimitotics form the basis of the therapy for patients with both solid tumors and hematological malignancies. However, current antimitotic drugs affect both dividing and non-dividing cells. One of the emerging next generation antimitotic targets is Polo-like kinase 1 (PLK1). Among the four members of PLK family, PLK1 is the best characterized and it is recognized to be a key component of the cell cycle control machinery with important roles in the mitotic entry, centrosome duplication, bipolar mitotic spindle formation, transition from metaphase to anaphase, cytokinesis and maintenance of genomic stability. PLK1 is often over-expressed in many different tumor types and over-expression often correlates with poor prognosis. As an antimitotic target, PLK1 is only expressed in dividing cells while it is not expressed in differentiated postmitotic cells like neurons, where instead expression of PLK2 and PLK3 was reported. This indicates a potentially better safety profile for a PLK1 specific inhibitor. Thus, PLK1 is thought to be a promising target for anti-cancer therapy and indeed some PLK1 inhibitors are currently under evaluation in clinical trials[13].

Hepato Protective activity procedure for synthesized tetrahydroquinazoline derivatives

Animals

Male Wistar Albino rats, weighed 200 ± 20 g were utilized for this study. They were housed in polypropylene cages under standard laboratory conditions (12-h light/ 12-h dark cycle, 21 ± 2 ºC, and relative humidity 55 %). The animals were given standard rodent pellets and tap water *ad libitum*. The rats were acclimatized to laboratory condition for 7 days before commencement of experiment. The hepatoprotective study was carried out by the Chromosoft Infotech, Chennai as per the guidelines set by Organization for Economic Co-operation and Development (OECD) received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA NO. 8926/242/PAHR). On eighth day, the rats were sacrificed by overdose of anaesthesis, liver was separated and used for estimation of biochemical parameters.

Experimental procedure

In vitro study was done using liver slice culture system following the protocol developed by Wormser and Ben Zakine (1990), with slight modification. Rat liver was perfused heavily by using Modified Hank's Medium to remove all the blood clots. The liver was then cut into small square slices, weighing 5–6 mg, and was cultured in 24 well plates (20–22 slices weighing about 100–120mg/well) using MEM 199 medium supplemented with 0.1-g/l penicillin, 0.07-g/l streptomycin and 0.2% BSA. Liver slices were incubated in this condition for 4 h at 37 ◦C in 95%O2/5% CO_2. The medium was changed at 2 h, this time gap was given to recover the shock from surgery. The control slices were kept in culture medium only, ethanol (50 mg/ml) or ethanol plus $4a_1$ and $4a_4$ (2.5, 5.0 and 10.0 mg/ml) or ethanol plus Silymarin (1.0, 2.0 and 3.0 mg/ml) were added to the incubation medium where mentioned. On termination of incubation at

4 h, the medium was collected and subjected to the determination of SGOT and SGPT for the assessment of liver damage. The liver slices from different incubations were separately collected, washed repeatedly and then homogenized in ice-cold phosphate (50mM, pH 7.4) buffer; the homogenate for each incubation was subjected to the estimation of lipid peroxidation [14]. Then further study like Serum Glutamate oxaloacetate transaminase, Serum glutamate - pyruvate transaminase (SGPT), Estimation of TBARS, Estimation of protein carbonyl content, Catalase, Measurement of PNP–UGT activity was done with its standard procedure [14,15].

RESULT AND DISCUSSION

The general synthesized compound 4 used for synthesis of various tetrahydroquinazoline derivatives was shown in Figure No.2, further docking studies was done with different substrates in R and R^1 positions was shown in Table No.1. To evaluate druglikeness better, the rules have spawned many extensions, for example one from a 1999 paper by Ghose et al,

- Partition coefficient log *P* in -0.4 to +5.6 range
- Molar refractivity from 40 to 130
- Molecular weight from 160 to 480
- Number of atoms from 20 to 70

At Molinspiration we believe that the strategy which leads to success is not a universal drug-likeness score, but focus on particular drug classes and development of specific activity score for each of these classes. The method we implemented uses sophisticated Bayesian statistics to compare structures of representative ligands active on the particular target with structures of inactive molecules and to identify substructure features typical for active molecules. With the Molinspiration virtual screening toolkit miscreen one can easily develop a screening engine for arbitrary target, provided that several active ligands (in the extreme case only single ligand) are known. With the trained model it is possible to screen large libraries of hundreds of thousands of molecules in less than hour, to identify molecules with highest chance to become active drugs or pesticides. Expert system for calculation of druglikeness score towards GPCR ligands, ion channel modulators, kinase

inhibitors, nuclear receptor ligands, protease inhibitors and other enzyme targets based on Molinspiration technology may be tested on-line was show in Table No.2 and 3 and spectral datas was shown in Table No.4 to 9

***In vitro* Hepatoprotective Study**

The resulting liver dysfunction as marked by the increase of SGPT, SGOT and ALP activities. $4a_1$ and $4a_4$ decreased the alcohol-induced increase of SGPT, SGOT and ALP activities dose dependently. However, 50 mg/kg bodyweight dose of $4a_4$ was found to be most effective. This dose was therefore selected for next experiment on time-dependent effect of alcohol. Treatment of alcohol for different time periods (0-35 days) showed a linear increase in the activities of the liver marker enzymes, SGPT, SGOT and ALP till 12 days and at 15th day there was no significant additional effect over day 15 was show in Table No.10-14 and Figure No 3-7. $4a_1$ significantly decreased alcohol-induced increase of enzyme activities, suggesting its hepatoprotective effect. UDP-glucuronosyl transferase (UGT) activity is known to prevent the generation of cellular oxidative stress by glucuronidation and subsequent exclusion of harmful metabolites. UGT therefore plays an important role this prompted us to investigate UGT in some detail in the liver. Since 50 mg/kg body weight dose of $4a_1$ was found to be most effective in protecting liver damage, this dose was selected for UGT-related studies. Alcohol treatment affected microsomal UGT activity that was evident from the reduction of PNP-glucuronidation and $4a_1$ prevented the deterioration of glucuronidation activity effectively. It could be seen from that alcohol treatment caused a significant decrease ($p < 0.001$) in UGT gene expression while co-treatment with $4a_1$ protected the down regulation of this gene expression.

Table No.1: Characterisation Data of Synthesised Compounds $4a_1$- $4a_{12}$

S. No	Compound code	Molecular formula	Molecular weight gm	Melting point (°C)	Rf value	Percentage yield (%)	λ max	Elemental analysis				
								C	N	O	H	Br
1	$4a_1$	$C_{26}H_{27}BrN_4O_3$	523.42	272	0.5	69	245	59.66	10.70	9.17	5.20	15.27
2	$4a_2$	$C_{24}H_{25}BrN_4O_2$	481.38	270	0.4	68	245	59.88	11.64	6.65	5.23	16.60
3	$4a_3$	$C_{27}H_{30}BrN_5O_2$	536.46	200	0.5	75	240	60.45	13.05	5.96	5.64	14.89
4	$4a_4$	$C_{33}H_{27}BrN_4O_2$	591.50	270	0.5	68	244	67.01	9.47	5.41	4.60	13.51
5	$4a_5$	$C_{26}H_{27}BrN_4O_4$	539.42	272	0.3	69	245	57.89	10.39	11.86	5.05	14.81

6	$4a_6$	$C_{34}H_{29}BrN_4O_2$	605.52	280	0.4	71	245	67.44	9.25	5.28	4.83	13.20
7	$4a_7$	$C_{35}H_{32}BrN_5O$	618.57	282	0.4	73	245	67.96	11.32	2.59	5.21	12.92
8	$4a_8$	$C_{25}H_{28}BrN_5O$	494.43	260	0.5	68	248	60.73	14.16	3.24	5.71	16.16
9	$4a_9$	$C_{24}H_{25}BrN_4O_3$	497.38	285	0.4	70	245	57.95	11.26	9.65	5.07	16.06
10	$4a_{10}$	$C_{34}H_{29}BrN_4O_3$	621.52	240	0.4	72	240	65.70	9.01	7.72	4.70	12.86
11	$4a_{11}$	$C_{25}H_{25}BrN_4O_3$	509.39	250	0.3	74	240	58.95	11.00	9.42	4.95	15.69
12	$4a_{12}$	$C_{25}H_{23}BrN_4O_2$	467.36	283	0.4	69	240	59.11	11.99	6.85	4.96	17.10

Table No.2: Application of lipinski rule of 5 to our ligand test set

S.No	Ligand	Molecular weight	Number of Hydrogen bond acceptors	Number of Hydrogen bond donors	Logp (oct/wat)	Lipinski Violations
1	$4a_1$	523.431	7	2	5.024	2
2	$4a_2$	481.394	6	2	5.178	1
3	$4a_3$	536.474	7	2	5.069	2
4	$4a_4$	591.509	6	3	8.036	2
5	$4a_5$	539.43	8	3	4.309	1
6	$4a_6$	618.579	6	2	8.481	2
7	$4a_7$	605.536	6	2	8.453	2
8	$4a_8$	494.437	6	2	5.223	1
9	$4a_9$	497.393	7	3	4.46	0
10	$4a_{10}$	546.798	0	0	9.497	2
11	$4a_{11}$	509.404	7	3	4.488	1
12	$4a_{12}$	446.367	6	3	4.642	0

Table No.3: Molinspiration bio activity score of $4a_1$ – $4a_{12}$

S.No	Ligand	GPCR ligand	Ion channel modulator	Kinase Inhibitor	Nuclear Receptor ligand	Protease inhibitor	Enzyme inhibitor
1	$4a_1$	-0.11	-0.17	-0.35	-0.51	-0.34	-0.16
2	$4a_2$	-0.10	-0.10	-0.36	-0.47	-0.36	-0.13
3	$4a_3$	-0.08	-0.14	-0.30	-0.46	-0.31	-0.13
4	$4a_4$	-0.03	-0.22	-0.25	-0.32	-0.22	-0.11
5	$4a_5$	-0.10	-0.17	-0.32	-0.52	-0.36	-0.15
6	$4a_6$	-0.07	-0.39	-0.33	-0.40	-0.22	-0.20
7	$4a_7$	-0.07	-0.33	-0.32	-0.38	-0.24	-0.18
8	$4a_8$	-0.07	-0.07	-0.32	-0.42	-0.33	-0.10
9	$4a_9$	-0.09	-0.10	-0.33	-0.49	-0.38	-0.12
10	$4a_{10}$	-0.07	-0.44	-0.39	-0.11	-0.10	-0.23
11	$4a_{11}$	-0.08	-0.12	-0.34	-0.51	-0.31	-0.13
12	$4a_{12}$	-0.07	-0.05	-0.34	-0.46	-0.33	-0.10

Table No.4: IR Spectral Data of Compound $4a_1$

S.NO	FREQUENCY	MODE OF VIBRATION
1	1448	m CH_3 bending
2	1405	m O-H in plane bending and C-O Stretching
3	3215	m N-H Stretching
4	1300-1253	s Asymmetric C-O-C stretching
5	1339-1314	m C-N stretching
6	589-514	C-Br stretching

7	1680	C=N stretching
8	1589	C=C stretching
9	976	s C-H out of plane bending
10	3070	C-H stretching

Table No.5: IR Spectral Data of Compound $4a_2$

S.NO	FREQUENCY	MODE OF VIBRATION
1	1448	m CH3 bending
2	1405	m (O-H in plane bending and C-O Stretching
3	3215	m (N-H Stretching)
4	1122	S (Asymmetric C-O-C stretching)
5	1314	m (C-N stretching)
6	589-514	C-Br stretching
7	1680	C=N stretching
8	1589	C=C stretching
9	820 - 803	s C-H out of plane bending
10	3070	C-H stretching

Table No.6: IR Spectral Data of Compound $4a_3$

S.NO	FREQUENCY	MODE OF VIBRATION
1	2810	m CH3 bending
2	1410 - 1400	m (O-H in plane bending and C-O Stretching
3	3430	m - w(N-H Stretching)
4	1120	s (Asymmetric C-O-C stretching)

5	1314	m (C-N stretching)
6	593-509	C-Br stretching
7	1659	C=N stretching
8	1599	C=C stretching
9	834 - 815	s C-H out of plane bending
10	1227 - 1003	w C-H in plane bending

Table No.7: IR Spectral Data of Compound $4a_4$

S.NO	FREQUENCY	MODE OF VIBRATION
1	2810	m CH3 bending
2	1416	m (O-H in plane bending and C-O Stretching
3	3392	m - w(N-H Stretching)
4	1120	s (Asymmetric C-O-C stretching)
5	1314	m (C-N stretching)
6	589 - 502	C-Br stretching
7	1659	C=N stretching
8	1594	C=C stretching
9	816	s C-H out of plane bending
10	1224 - 1005	w C-H in plane bending

Table No.8: Mass Spectral Data of Compound $4a_1$ and $4a_4$

S.No	Compound Code	Mass fragmentation pattern
1	$4a_1$	523.13 (28.5%), 524.12 (97.12%), 522.13 (100%)
2	$4a_4$	593.13 (36.2%), 592.13 (97.8%), 590.13 (100%)

Table No.9: Spectral Data of Synthesized Compounds

COMPOUND	1H NMR ($CDCL_3$-d, δppm)
$4a_1$	2(d, 1H, -NH_2), 4.62 (S, 4H, -CH_2)7.55-7.92 (M, 4H, Ar-CH), 7.2 (S, 1H, -NH).
$4a_2$	2.35(S, 3H, -CH_3), 4.62 (S, 2H,-CH_2), 7.0(S, 1H, -NH), 7.3-7.98(M, 9H, Ar-H).
$4a_3$	4.62(S, 2H, CH_2), 5(S, 1H, -OH), 6.8-7.98(M, 8H, Ar-H), 8.0(S, 1H, -NH), 8.1(S, 1H, CH).
$4a_4$	4.82(S, 2H, CH_2), 6.8-8.1 (M, 8H, Ar-H), 8.7(S, 1H, -NH), 8.2(S, 1H, CH), 2.35(S, 6H, CH_3).

Table No.10: Effect of dosage on ALP activity

S.No	Drug	Optical Density
1	Control	18 ± 0.9
2	Ethanol	59 ± 6.3
3	Ethanol+$4a_4$ -2.5	42 ± 7.3
4	Ethanol+$4a_4$-5.0	27 ± 1.9
5	Ethanol+$4a_4$-10.0	29 ± 1.7

Table No.11: Effect of dosage on SGOT activity

S.No	Drug	Optical Density
1	Control	45 ± 4.2
2	Ethanol	135 ± 10.5
3	Ethanol+4a_4-2.5	127 ± 11.83
4	Ethanol+4a_4-5.0	65 ± 5.2
5	Ethanol+4a_4-10.0	68 ± 4.8

Table No.12: Effect of dosage on SGPT activity

S.No	Drug	Optical Density
1	Control	22 ± 2.0
2	Ethanol	196 ± 15.3
3	Ethanol+4a_4-2.5	136 ± 9.54
4	Ethanol+4a_4-5.0	72 ± 4.6
5	Ethanol+4a_4-10.0	81 ± 8.7

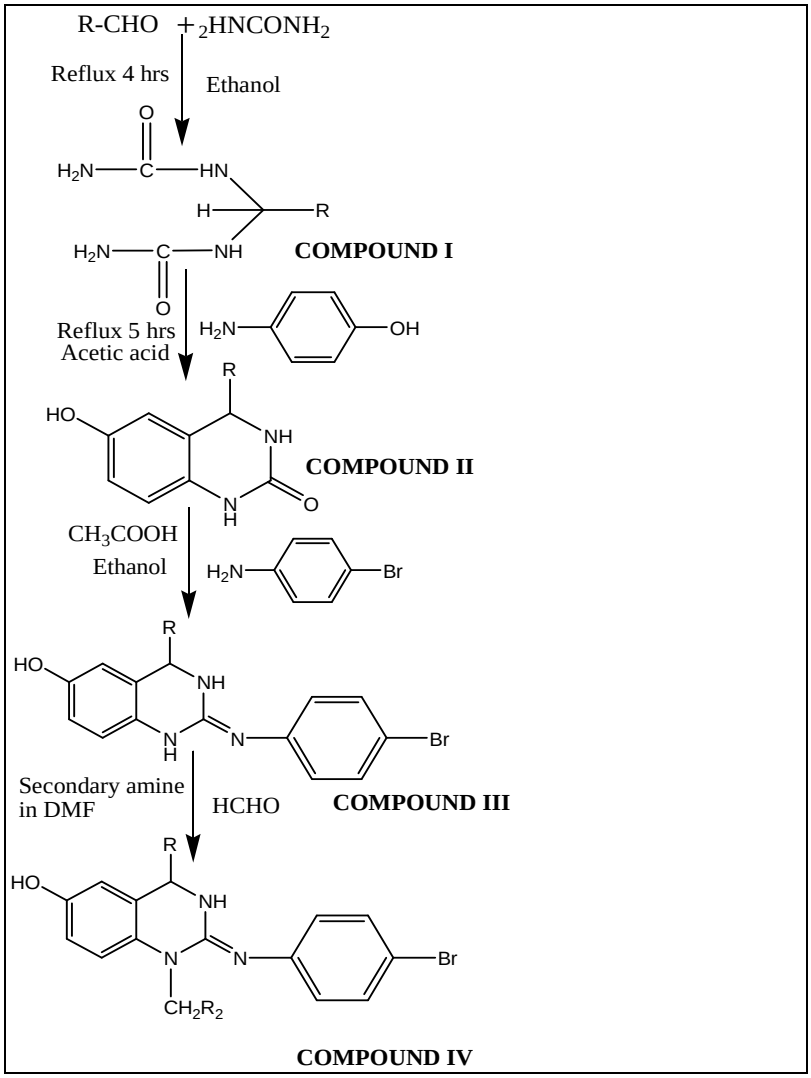
Table 13: Measurement of PNP–UGT activity against synthesized compound 4a_1

S.No	Drug	PNP-UGT activity of non activated microsomes	PNP-UGT activity of activated microsomes
1	Control	61 ± 5.2	23 ± 1.1
2	Ethanol	40 ± 3.5	17 ± 0.9
3	Eth+4a_1	67 ± 4.7	29 ± 1.2

Table 14: Treatment of alcohol extract for different time periods (0–35 days)

S.No	Days	0	7	14	21	28	35
1	Control	51	48	43	45	51	48
2	Ethanol	51	47	58	76	68	69
3	Ethanol+ $4a_1$	51	66	71	101	143	141

Figure No.1: SCHEME – I



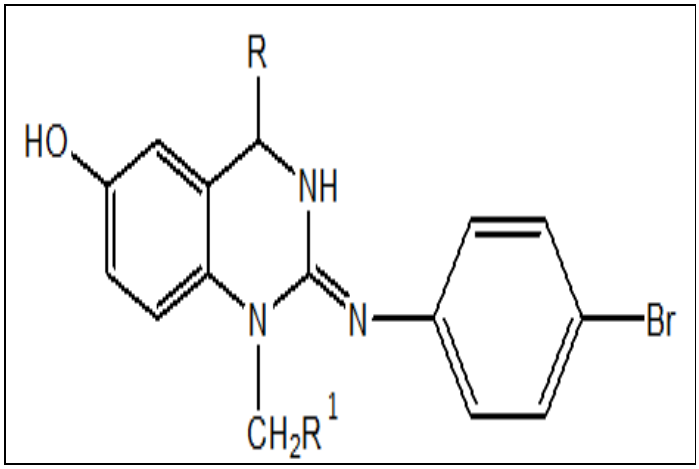


Figure No.2: Synthesized compound 4

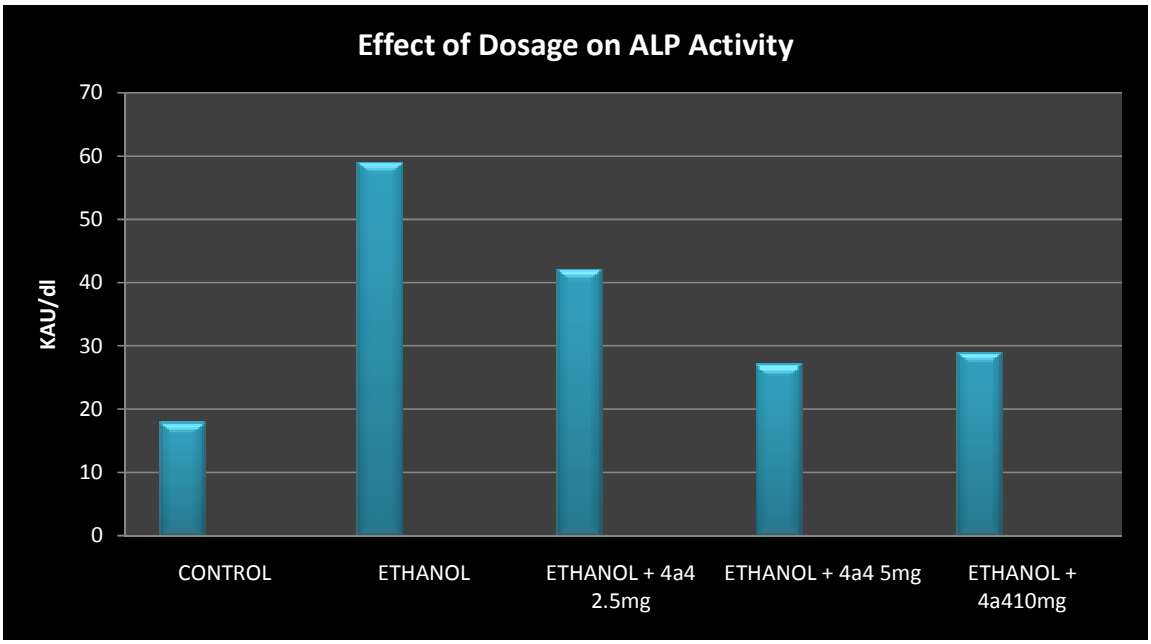


Figure No.3: Effect of Dosage on ALP activity

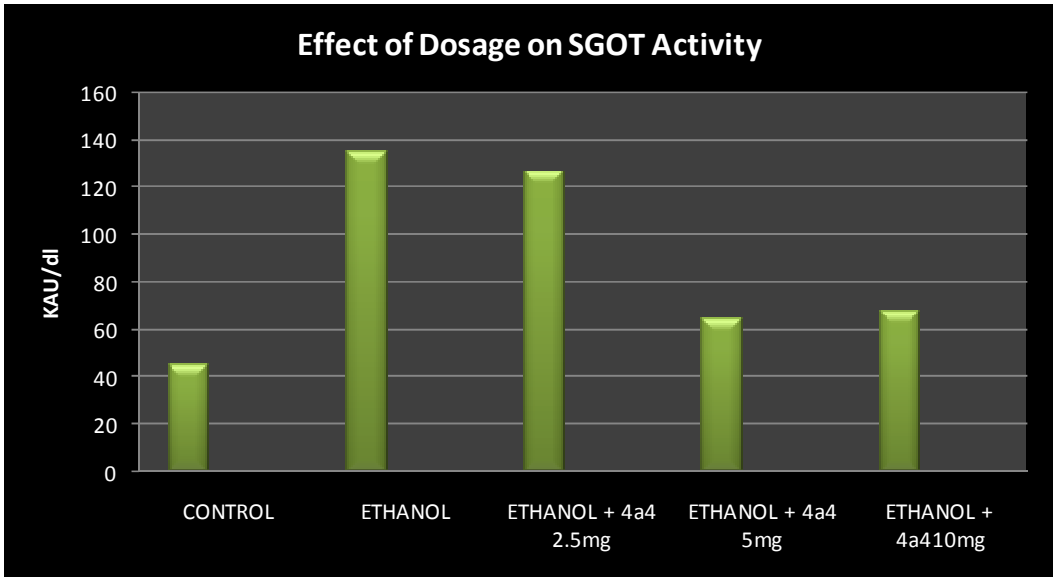


Figure No.4: Effect of Dosage on SGOT activity

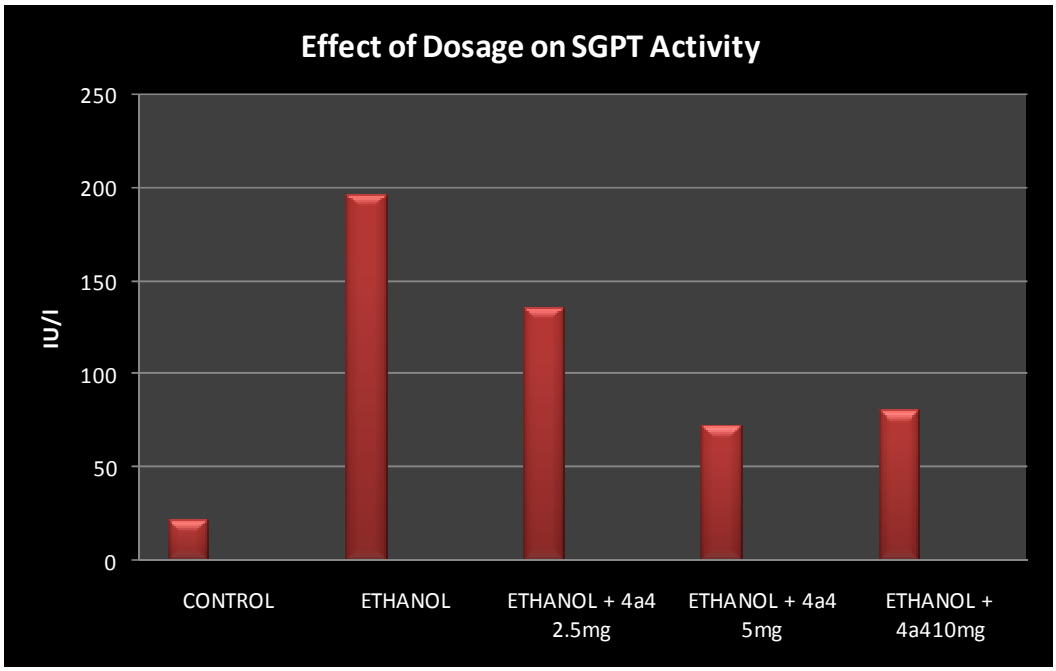


Figure No.5: Effect of Dosage on SGPT activity

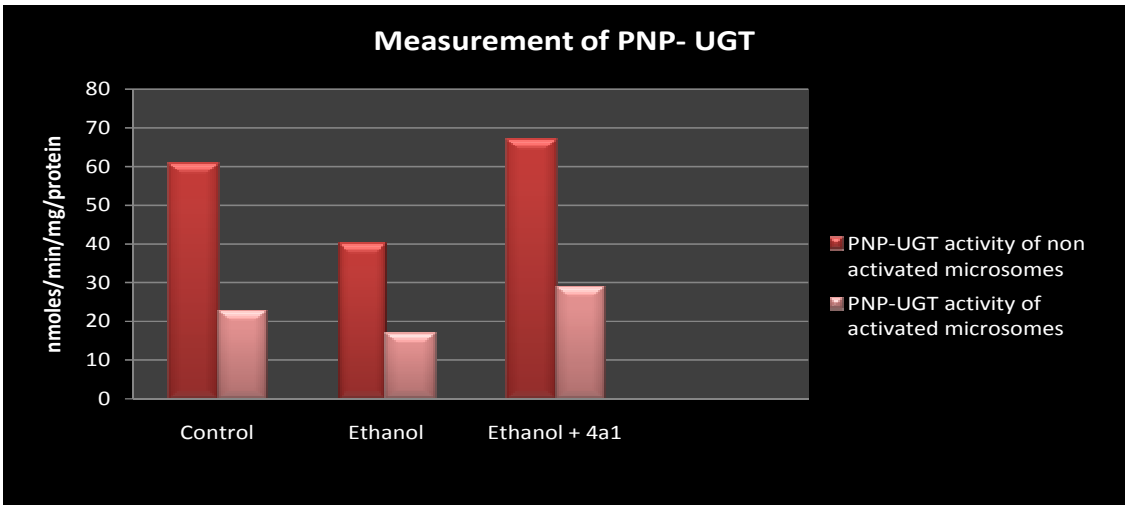


Figure No.6: Measurement of PNP–UGT activity against synthesized compound $4a_1$

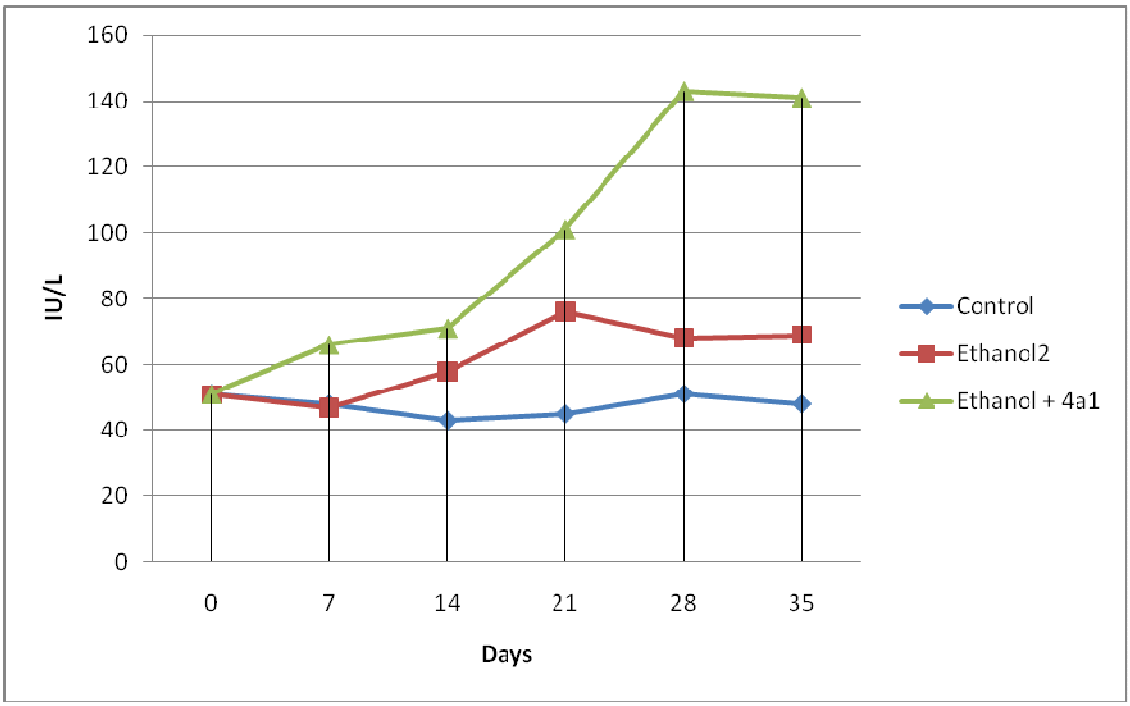


Figure No.7: Treatment of alcohol extract for different time periods (0–35 days)

SUMMARY AND CONCLUSION

1. The present study was carried out with the aim of synthesis screening of hepatoprotective activity of some novel 1, 2, 3, 4-tetrahydroquinazoline derivatives. Using the developed schemes various derivatives of 1, 2, 3, 4-tetrahydroquinazoline were prepared in good yield.
2. The test compounds $4a_2$, $4a_5$, $4a_8$, $4a_9$, $4a_{11}$ and $4a_{12}$ a better solubility, diffusion, Log P, molecular weight, etc., with no more than 1 violation making the ligands pharmacodynamically active and better oral absorptive series.
3. The spectral studies were performed for all the title compounds for their conformation. The synthesized compounds were further subjected to IR, Mass and ^{1}H NMR study for confirmation of functional group, molecular weight, number of protons present in the compounds.
4. The hepatoprotective screening of title compounds ($4a_1$ and $4a_4$) were evaluated by *in-vitro* method and the title compounds $4a_4$ were found to good hepatoprotective activity.
5. The binding mode of the title compounds with the Polo-like kinase inhibitor was clarified by flexible docking method and the title compounds $4a_1$, $4a_2$, $4a_4$, $4a_5$, $4a_7$, $4a_8$ and $4a_{12}$ were active against Polo-like kinase inhibitors. Binding energy values obtained were very high when compared to previous analysis.
6. It may conclude that further beneficial pharmacophore modifications in the design of novel tetrahydroquinazoline derivatives may be synthesized by designing novel ligands for therapeutic targets by substituting different functional group and also examine with the help of NMR and X-ray which provide three dimensional frame works which can analyze structure activity data, can guide the design and synthesis of future potential therapeutic towards other chronic disorder along with *in vivo* of different animal model and screening against different cancer lines and restore the existing different types cancerous disease.

ACKNOWLEDGEMENT

I am heart fully thanks to our J. K. K. Nattraja College of Pharmacy, Kumarapalayam, TN, India, to provide all the facilities and supports for doing this research work.

REFERENCE

1. Thevendren PS, Palnirajan VK, Chinasamy R P. Synthesis and anti microbial activity of novel thazolin quinazoline derivatives, *IJPS*, 2(2), 2010, 118-122.
2. Omar AL-deeb and Ahmed MA. Synthesis of some New 3H-quinazoline-4-one derivate. *Journal of World Applied Sciences*, 5(1), 2008, 94-99.
3. http://en.wikipedia.org/wiki/Druglikeliness.
4. http://en.wikipedia.org/wiki/Lipinski's_Rule_of _Five.
5. National Digestive Diseases Information Clearinghouse, National Institute of Diabetes and Digestive and Kidney Diseases. "Liver." http://digestive.niddk.nih.gov/ddiseases/topics/li
6. Su Hui Y, Daulat B K, Suk Hee C, Hye-Kyung J, Kwang Youl L, Ho Jae H , Kyung-Tae L, Won-Jea C. Virtual screening and synthesis of quinazolines as novel JAK2 inhibitors, *Bioorganic & Medicinal Chemistry*, 19, 2011, 968–977.
7. Vouy LT & Michelle M. Mild and efficient ligand-free copper-catalyzed condensation for the synthesis of quinazolines. *Tetrahedron Letters*, 51, 2010, 758–760.
8. Patil JP, Amrutkar SV, Ranawat MS. Synthesized micro wave assisted synthesis of quinazoline using different bases, *J. Pharma. Sci & Res*,1(3), 2009, 52-54.
9. Omar A and Ahmed MA. Synthesis of some new 3H-quinazolin-4-one derivatives as potential Antitubercular agents. *Journal of World Applied Sciences*, 5 (1), 2008, 94-99.
10. Siddappa K, Tukaram R, Mallikarjun M and Reddy V. Synthesis, Characterization and antimicrobial studies of 3-[(2-Hydroxy-quinolin-3-ylmethylene)-amino]-2-phenyl-3

and its Metal (II) Complexes. *E-Journal of Chemistry*, 5(1), 2008, 155-162.

11. Cedric L, Alexandra T, Valerie T, Olivier L, Melina B, Jean-Michel R, Laurent M, Thierry B. Synthesis, biological evaluation and molecular modeling studies of quinazoline derivatives, *European Journal of Medicinal Chemistry*, 43, 2008, 1469-1477.
12. Gloria DG, Rocío EF, Ana ME, Hernán PM, Carlos DPM and Roberto AE. Biological evaluation of novel 6-Arylbenzimidazo [1, 2-c]quinazoline derivatives as inhibitors of LPS-induced TNF-alpha secretion. *Biol Res*, 41, 2008, 43-50.
13. Piyush K, Keshav, Dhawan N, Satya V, Krishna, Bhargava P and Kesari K. Synthesis of 6-substituted-2-phenyl-3-(5substitutedmercapto-1,3,4-thiadiazol-2-yl)quinazoline-4-(3H)-ones as Antitubercular agents. *Archiv der Pharmazie*, 16(9), 1983, 759-763.
14. MutluGenckal H, Pazarli N & Irez G. Syntheses Structural Characterization, and Thermal Properties of New 1, 2, 3, 4-Tetrahydro quinazoline ligands and their Co(III) Complexes, *Journal of Science*, 2(5), 2014, 627-633.
15. Sedlack J and Lindsay RN. Estimation of total protein bound and non-protein sulphydryl groups in tissues with Ellman reagent, *Annal.Biochem*, 25, 1968, 192-205.