Asian Journal of Pharmaceutical and Health Sciences
Journal Homepage : www.ajphs.com

Design, Synthesis and Pharmacological Evaluation of Isoxazole Analogues Derived From Natural Piperine

Mary Sheeja T.L*, Anny Mathew, Joyamma Varkey

Department of Pharmaceutical Chemistry, College of Pharmaceutical Sciences, Medicalcollege, Thiruvananthapuram.

ARTICLE HISTORY

Received: 18-Jun-2011

Accepted: 05-Aug-2011

Available online: 10-Feb-2011

Keywords:

Piperine, piperonal, Isoxazole, Elevated Plus Maze, Mirrored Chamber, Anxiolytics

***Corresponding author:**

E-mail: annaserahs@yahoo.com
Phone: +91-9446695428

ABSTRACT

Piperine has attracted considerable attention Worldwide owing to its outstanding biological properties. Synthesis of some Isoxazole analogs of piperine under conventional and microwave conditions in moderate to excellent yields is reported here. Conversion of piperine to piperonal by hydrolysis and oxidation. Condensation of piperonal with substituted Acetophenone gave chalcone derivatives. Finally cyclized with hydroxylamine hydrochloride to form isoxazole analogues of piperine. Anxiolytic activity of the synthesized compounds were studied in Elevated plus Maze and Mirrored chamber using *Albino Mice.*

INTRODUCTION

There are many structural features to piperine, an acetal, an aromatic ring, a *trans, trans* diene and an amide. The *trans, trans* diene is, synthetically, the most interesting feature of the molecule. A series of Isoxazole analogues of Natural piperine were synthesized by removing the basic piperidine moiety from the piperine nucleus. Piperine known to posses anti-epileptic[1], anti depressants[2], anxiolytic activities[3]. Isoxazole also posses anxiolytic, antidepressant activities. Thus we became interested in the synthesis of piperine analogues that contains Isoxazole moiety. Here we also take the advantage of microwave technique and evaluation of biological activities. Microwave assisted reaction have received great interest because of their simplicity in operation, enhanced reaction rates, product with high purity and better yield compared to those conducted by conventional heating.

MATERIALS AND METHODS

EXPERIMENTAL

Melting points of the compounds were found out in an open capillary tube method by electrically heated melting point apparatus were uncorrected. The synthesised compounds were purified by Recrystallisation and Thin Layer Chromatography. IR spectra of the compounds were recorded using KBr pellets in the range of 4000- 500 cm^{-1} on a Jasco FTIR model 6200 in the College of Pharmaceutical Sciences, Medical College, Thiruvananthapuram. The ^{1}H NMR and ^{13}C NMR spectra's of the compounds were recorded in $CDCl_3$. Chemical shifts were reported in parts per million downfield with reference to internal standard Tetra Methyl Silane (TMS)on Bruker Ultra Sheild DPX 400 in the Institute of intensive research in Basic Sciences, Mahatma Gandhi University, Kottayam. SMILES and C Log P values, Physico Chemical Properties, Analysis of Lipinski rule of five, Drug Likeness Analysis, prediction of activity spectra (PASS) of the Novel proposed analogues were carried out by using Chemsketch and molinspiration software. *Insilico* ADME properties were screened by using the application Qikprop in maestro molecular modelling software and Docking studies were carried out against different targets like Cycloxygenase and farnasyl transferase receptors using Schrodinger software in college of pharmaceutical sciences, Medical college, Thiruvananthapuram.

The reactions were carried out in catalyst synthetic microwave oven and conventional method. The physical and spectral data of the synthesized compounds were reported in the table No. 4,5and 6.

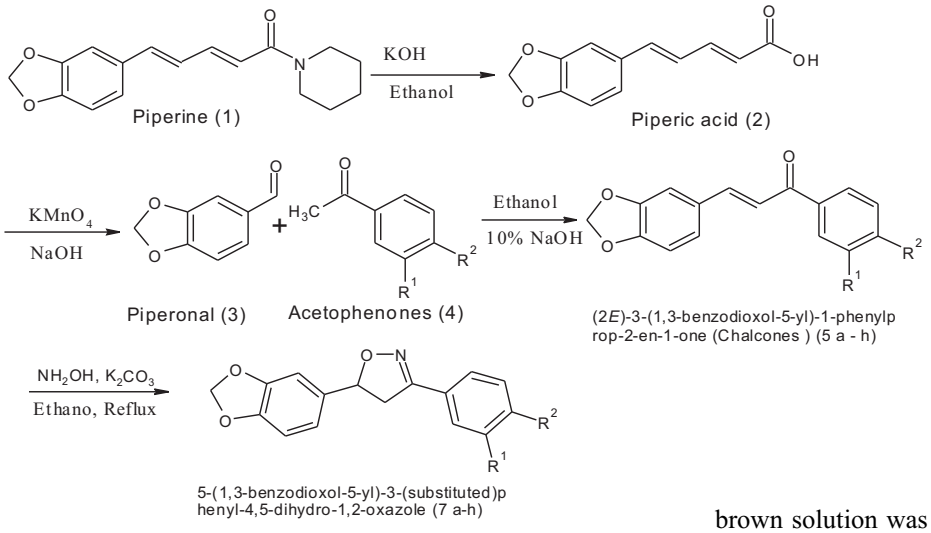
Synthesis of piperic acid (2) from piperine (1) [4,5,6]

Piperine 1 (11.4 g, 0.04 mol) was refluxed with methanolic KOH (20%, 500 ml) for 24 hrs. After completion of hydrolysis, methanol was distilled under reduced pressure. The resulting reaction mixture was suspended in hot water and acidified with HCl to pH<1. Yellow precipitate obtained was collected by filtration, washed with cold water and recrystallized from methanol to yield crystals of piperic acid in 59% yield, mp 215-217^{0}c.

Synthesis of piperonal (3) from piperic acid [7,8]

Piperic Acid 2.18g (.01 Mol) was suspended in 150ml boiling H_20, containing 4.2g (.05 Mol) Sodium Bicarbonate. To this hot solution, 3.16g (.02 Mol) $KMnO_4$ in 75ml warm H_20 added with a dropper over about 40 min, with constant stirring. Added 25ml of Isopropyl alcohol. (To kill any remaining oxidizer) The warm

SCHEME



Comp	R1	R2
a	H	H
b	H	O-CH_3
c	H	NH_2
d	NH_2	H
e	OCH_3	H
f	OCH_3	OCH_3
g	NO_2	H
h	H	NO_2

Table No.1: Characterization data of synthesized derivatives

Compound Code	Molecular formula	Molecular Weight	m.p (^{0}C)	Rf value
7a	$C_{16}H_{13}NO_3$	267.27932	178-180	0.68
7b	$C_{17}H_{15}NO_4$	297.3053	186-189	0.78
7c	$C_{16}H_{14}N_2O_3$	282.29396	182-185	0.46
7d	$C_{16}H_{14}N_2O_3$	282.29396	179-182	0.58
7e	$C_{17}H_{15}NO_4$	297.3053	186-289	0.56
7f	$C_{18}H_{17}NO_5$	327.33128	172-275	0.72
7g	$C_{16}H_{12}N_2O_5$	312.27688	187-280	0.61
7h	$C_{16}H_{12}N_2O_5$	312.27688	189-282	0.61

Table No.2: Comparison of Different Synthetic Methods.

Compounds	Conventional		Microwave	
	Time (hr)	Yield %	Time (min)	Yield %
7a	8	68	3	92
7b	8	66	3	91
7c	8	69	3	94
7d	8	65	3	92
7e	8	70	3	92
7f	8	77	3	90
7g	8	66	3	92
7h	8	68	3	89

Table No.3: Glide score of synthesized compounds

Targets	PDB ID	Compounds	G-Score
Monoamino Oxidase	3P07	7b	-8.05
		7d	-7.25
		7c	-6.74

brown solution was filtered to leave a slightly yellow solution. This was chilled overnight and recrystallized.

Procedure for the Synthesis of (2*E*)-3-(1, 3-benzodioxol-5-yl)-1-phenylprop-2-en-1-one (chalcone)[9]

Into a 10 ml Erlenmeyer flask placed 0.24 g (2.0 mmoles) of acetophenone, 0.30 g (2.0 mmoles) of piperonal, 1 ml of 95% ethanol, and 1 ml of 10% sodium hydroxide solution. The mixture is stirred for 30 minutes during which time a solid forms. The reaction mixture is cooled and suction filtered using a Hirsch funnel, and recrystallized from a small amount (< 1mL) of 95% boiling hot ethanol and then allowed to slowly cool to room temperature.

Procedure for the Synthesis of 5-(1, 3-benzodioxol-5-yl)-3-[(substituted) phenyl]-1-2-oxazole (7a-h).

Conventional Method[10]

A mixture of 2.77 g of chalcone 5(a-h) (10 mmoles), 0.7 g of hydroxylamine hydrochloride (10 mmoles) and 1.4 g of anhydrous potassium carbonate (10 mmoles) in (50 ml) of ethanol was refluxed for 8 hr., then left to cool. The reaction mixture was poured into cold water and the solid product was filtered off, washed with water, dried and finally crystallized from ethanol to afford isoxazole derivative 7 (a-h).

Microwave method[11]

Chalcone 5 (a-h) (0.01mol), hydroxylamine hydrochloride (0.01 mol) and NaOH, as a catalyst, in methanol (30 ml) were taken. The reaction mixture was taken in RBF placed in a microwave oven and irradiated for 3.0 min at 400 watt and then cooled and acidified with glacial acetic acid. The solvent was removed and residue was recrystallized from absolute alcohol to get compound 7 (a-h), as a yellow brown powder.

ANIMAL STUDIES

Experimental animals

Clearance from the Institutional Animal Ethics Committee (IAEC No.04/02/2011/MCT, dt. 03/02/2011) was obtained for carrying out the animal experiments. Swiss albino mice (20-25 g) maintained at a room temperature of 25±5˚C with relative humidity of (60±5%) was used. The animals were housed in polypropylene cages with access to standard rodent pellet diet and water *ad libitum*. A twelve hour, light/dark cycle was maintained for experiments.

Table No.4: Characteristic IR peaks of the Synthesized Compounds

Compound	IR (KBr v cm^{-1})
Piperonal (3)	2985(Ar-CH), 1601.59(Ar C=C), 1037.52(sym C-O-C), 1261.22(asym C-O-C),1686.44(C=O), 2820,2720(CH)
5a	2956(Ar-CH) 1659.45(C=O), 1590.32(Ar-C=C), 1018.23(Sym C-O-C Str), 1253.5, 1105.98(asym C-O-C).
5b	2956(Ar-CH) 1652.7(C=O), 1583.27(Ar-C=C), 1018.23 (SymC-O-CStr), 1253.5, 1105.98(asym C-O-C),1098.26(Ar-OCH)
5c	1642(C=O), 1597.73(C=C), 3457.74, 3346.85 (ArNH), 1034.02 (sym C-O-C), 1243(asy m C-O-C).
5d	3424.96(Ar-CH), 1655.59(C=O), 1580.38 (C=C), 1036.55 (sym C-O-C), 1267.97 (asym C-O-C)
5f	2925.1 (Ar-CH) 1652.7 (C=O), 1583.27 (Ar-C=C), 1018.23 (Sym C-O-C Str), 1253.5, 1105.98 (asym C-O-C), 1495.38 (Ar-OCH)
5g	2956(Ar-CH)1659.45(C=O),1590.32(Ar-C=C), 1018.23(Sym C-O-C Str), 1253.5,1105.98(asym C-O-C).1521.41(Ar-NO_2)
7a	1256.4(asym C-O-C str),1030(sym C-O-C str), 816(N-O), 1590(C=N), 2900 (Ar str).
7b	1042 (aryl-O-CH), 2922.59, 1607.38(C=N) 1249.65(asym CH C-O-Cstr) 828.277 (N-O), 936.27(C-O)
7c	3400 (m w Ar NH_2)2919.7, 1595.81(C=N) 832.133(N-O), 1254.47 (asym C-O-C), 1038.48 (sym C-O-C),
7d	3342.03(Ar-NH_2)1574.59(C=N).
7f	1475.14 (aryl-O-CH), 2922.59, 1650.12 (C=N) 1249.65 (asym CH C-O-Cstr) 828.277 (N-O), 936.27(C-O)
7g	1595.81 (m w Ar NO_2)2954.47, 1595.81 (C=N) 832.133(N-O),1254.47(asym C-O-C), 1038.48 (sym C-O-C),

Acute Toxicity Study[12]

Albino mice of both sex, weighing between 20 and 25 gm were starved overnight. They were divided in to 6 groups of 5 mice each. The first group served as the control and was given 0.5% CMC orally at a dose of 10ml/kg body weight. The derivatives 5-(1, 3-benzodioxol-5-yl)-3-(4-methoxyphenyl)-1, 2-oxazole (7b) and 3-[5-(1, 3-benzodioxol-5-yl)-4, 5-dihydro-1, 2-oxazol-3-yl] aniline (7d) were selected for the toxicity studies. The dose for the five study groups were selected after an initial pilot acute toxicity tests using five groups of two animals each. (7b) and (7d) in CMC (0.5%) at a dose of 250mg/kg, 500mg/kg, 1000mg/kg, 1500mg/kg and 2000mg/kg were given orally as a suspension (0.2ml) to the 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} group respectively. All the animals were observed continuously for 2 hours, then intermittently for another 4 hours and at the end of 24 hours. The number of animals which died at the end of 24 hour was noted. LD50 of these derivatives were determined graphically by Miller and Tainter (Karber's) method.

Antianxiety study

Anxiolytic activities of the proposed analogues 5-(1, 3-benzodioxol-5-yl)-3-(4-methoxyphenyl)-1,2-oxazole (7b) and 3-[5-(1,3-benzodioxol-5-yl)-4,5-dihydro-1,2-oxazol-3-yl]aniline (7d) were carried out by using mirrored chamber[3,8] and elevated plus maze[13,14] apparatus. Two doses were selected according to the acute toxicity study such as 231.1 mg/kg, 316.98mg/kg and 200mg/kg, 300mg/kg for 7b and 7d respectively. *Diazepam (2mg/kg)* was used as the standard drug. Control group was given 0.5% CMC Sodium (10ml/kg). Statistical analysis was carried out by one way ANOVA followed by Dunnett's't' test. A value of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Insilico molecular analysis of different Isoxazole analogues of piperine were done, all these compounds obeyed "Lipinski rule of five". These analogues were taken for computing molecular descriptors, and then for synthesis. The designed analogues were synthesized by conventional and microwave procedures. Microwave procedure was simple efficient and showed better yield. The purity of the synthesized molecules was ascertained

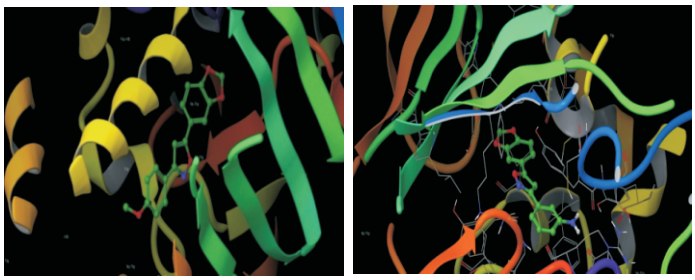


Fig.1: Compounds 7B and 7D in 3P07 showing stronger binding with the receptors

Table No.5: Characteristic 1H NMR peaks of the synthesized analogues

Compound	^{1}H NMR($CDCl_3$) δ ppm
7a	6.5-7 (m 8H, Ar-H), 5.45(2H,CH_2), 4.5 (1H,CH) 1.9,1.7(2H CH_2)
7b	6.5-7(m7HAr-H)5.45(2H, CH_2), 4.5 (1H,CH), 3.73(3H,Ar-OCH_3), 1.9, 1.7(2H, CH_2)
7c	6.5-7 (7H,Ar-H),5.45(s 2H,CH_2),4- 4.5(m 3H,CH ,Ar-Nh_2)1.9,1.7(2H, CH_2).
7f	6.5-7(6H,Ar-H),5.9(2H,CH_2)3.9-4(7H,CH, Ar-OCH_3), 1.8-2(2H(CH_2).
7g	6.5-7(7H, Ar-H), 5.9(2H,CH_2), 3.9(1H,CH), 1.5-2.2(2H,CH_2).

Table No.7: Acute Toxicity study of 5-(1, 3-benzodioxol-5-yl)-3-(4-methoxyphenyl)-1, 2-oxazole (7b)

Group	Dose mg/kg	Log dose	Dead/total	Dead %	Corrected %	Probit
1	250	2.398	0/5	0%	5	3.36
2	500	2.699	0/5	0%	5	3.36
3	1000	3.000	1/5	20%	20	4.16
4	1500	3.176	2/5	40%	40	4.75
5	2000	3.30	3/5	60%	60	5.25

Animal: *Albino mice.* Route of administration: Oral No. of animals in a group: 5

Corrected Formula: For the 0% dead =100(0.25/n)
Where n =5 (no. of animals in the group)

Table No.8: Acute Toxicity study of 3-[5-(1, 3-benzodioxol-5-yl)-4, 5-dihydro-1,2-oxazol-3-yl]aniline (7d)

Group	Dose mg/kg	Log dose	Dead/total	Dead %	Corrected %	Probit
1	250	2.398	0/5	0%	5	3.36
2	500	2.699	0/5	0%	5	3.36
3	1000	3.000	0/5	0%	5	3.36
4	1500	3.176	2/5	40%	40	4.75
5	2000	3.30	4/5	80%	80	5.84

Animal: *Albino mice.* Route of administration: Oral No. of animals in a group: 5

Corrected Formula: For the 0% dead =100(0.25/n)
Where n =5 (no. of animals in the group)

routinely by TLC, and melting point determinations were noted with an open capillary tube method and are uncorrected.

Docking studies were carried out against targets Monoamino Oxidase receptors. Majority of the synthesized chemical compounds showed good fit with the active site of targets. Compounds 7b, 7d, which showed a maximum G-score were taken out for wet laboratory validations for their anxiolytic activity. Purity of the compound was done routinely by TLC and melting points. Acetone: Chloroform (3: 1) system was found to be ideal system for the development of compounds in TLC. The characterizations of the derivatives were carried out by various spectroscopic methods such as FTIR and NMR spectroscopy.

Acute Toxicity Study

The derivatives 5-(1,3-benzodioxol-5-yl)-3-(4-methoxyphenyl)-1,2-oxazole (7b) and 3-[5-(1,3-benzodioxol-5-yl)-4,5-dihydro-1,2-oxazol-3-yl]aniline (7d) were selected according to the glide score for the study of safety dose range. The doses taken were 250, 500, 1000, 1500 and 2000mg/ kg bodyweight of the animal. The Table No: 7 and 8 gives the result of Acute toxicity study of the derivatives (7b) and (7d) in mice using Miller and Tainter (Karber's method). A correction factor was applied to 0 % mortality group. The percent mortality values are converted to probit values by reading the corresponding probit units from the probit table. The probit values were plotted against log doses and read LD_{50} value as the dose that corresponds to probit 5.The LD_{50} value of the (7b) was found to be 1584.89 mg/kg and (7d)1995.26 mg/kg of body weight.

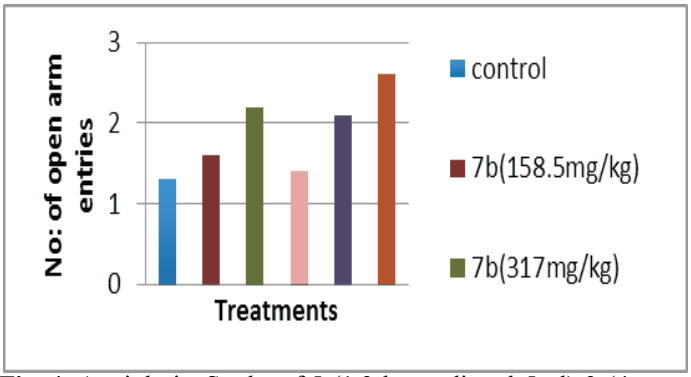


Fig. 4: Anxiolytic Study of 5-(1,3-benzodioxol-5-yl)-3-(4-methoxyphenyl)-1,2-oxazole (7b) and 3-[5-(1, 3-benzodioxol-5-yl)-4, 5-dihydro-1,2-oxazol-3-yl]aniline (7d)

Animal – *albino mice* No. of animals in a group: 6
Route of administration: Oral

Anxiolytic Study of 5-(1,3-benzodioxol-5-yl)-3-(4-methoxyphenyl)-1,2-oxazole (7b) and 3-[5-(1,3-benzodioxol-5-yl)-4,5-dihydro-1,2-oxazol-3-yl]aniline (7d)

The LD_{50} value of the derivative (7b) was found to be 1584.89 mg/kg of body weight and derivative (7d) showed an LD_{50} of 1995.26mg/kg. The $1/5^{th}$ and $1/10^{th}$ of the LD_{50} of compound 7b and 7d were chosen for the study. Diazepam was used as standard. Control group was given 1% CMC. The analysis of the result showed that the compound (7b) at doses of 316.98mg/kg, and (7d) at a dose of 399.052 mg/kg showed significant anxiolytic activity. Study of the biological activity shows that the title compound is having significant antianxiety effect similar to that of Standard drug Diazepam. The results obtained were shown in Fig 4,5 and 6.

Animal – *albino mice* No. of animals in a group: 6 Route of administration: Oral

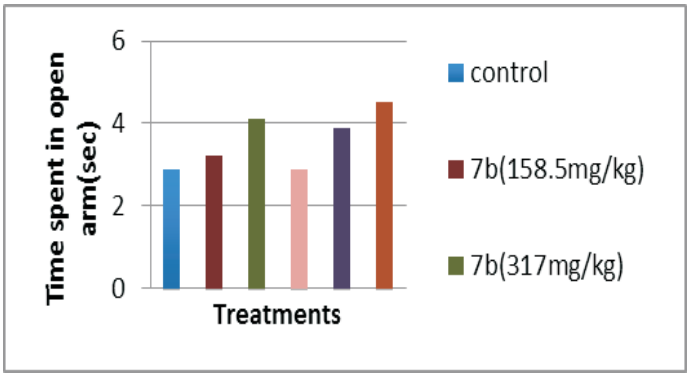


Fig. 5: Anxiolytic Study of 5-(1,3-benzodioxol-5-yl)-3-(4-methoxyphenyl)-1,2-oxazole (7b) and 3-[5-(1, 3-benzodioxol-5-yl)-4, 5-dihydro-1,2-oxazol-3-yl]aniline (7d)

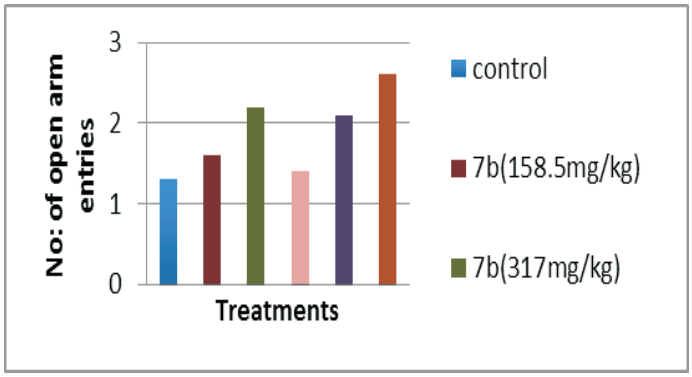


Fig. 6: Anxiolytic Study of 5-(1, 3-benzodioxol-5-yl)-3-(4-methoxyphenyl)-1, 2-oxazole (7b) and 3-[5-(1, 3-benzodioxol-5-yl)-4, 5-dihydro-1, 2-oxazol-3-yl] aniline (7d).

CONCLUSION

This research work was focused on design and development of Isoxazole analogues of piperine as novel anxiolytic drugs. The present research work involved the preliminary insilico screening of various synthesized analogues for quantifying their drug likeness using molinspiration software. Eight analogues were synthesized in the wet lab by conventional and microwave procedures and comparative study for yield and reaction time was also carried out. Purity of the compounds were ascertained by consistency in melting point and Rf value and characterized by IR and ^{1}HNMR and ^{13}CNMR spectral studies.

The analogue also showed good binding affinity with mono amino oxidase receptors, which was proved from the docking studies. The present study also highlights the importance of the structural features and C log P responsible for the activities.

Acute toxicity studies showed that the analogue 7d and 7b were safe up to 1995 mg and 1584.89 mg respectively.

The compound having best Glide score -8.05, indicating good binding affinity with monoamino oxidase target, showed good activity in anxiolytic study

Hence we consider these derivatives as future leads for anti anxiety drug discovery. Further detailed studies may prove beneficial as useful drug candidate.

REFERENCES

1 Liu G. Q, S. Algeri, A. Ceci, Garattini S, Gobbi M, And Murai S, Stimulation Of Serotonin Synthesis In Rat Brain After Antiepilepsirine, An Antiepileptic Piperine Derivative. *Bmchemical Pharmoco/Ogy*. 1984:(33): No. 23.3883-3886.

2 Song Lia, Che Wangab, Wei Lic, Kazuo Koikec, Tamatsu Nikaidoc, Min -Wei Wangb, Antidepressant-like effects of piperine and its derivative, antiepilepsirine. *Journal of Asian Natural Products Research.* 2009. (July–August) No. 5. 421–430.

3 Song Li, Che Wang b, Minwei Wang c, Wei Li d, Kinzo Matsumoto e, Yiyuan Tang. Life Sciences . 2007: (80):1373–1381.

4 Amritpal Singh and Sanjiv Duggal. Piperine- Review of Advances in Pharmacology. *International Journal of Pharmaceutical Sciences and Nanotechnology*. 2009:(2):3.

5 Reilly KM, Mclaughlin AM. Piperine- Review. *American family physician.* 2007:(2):37.

6 Feychting M,Ahlbom A, Piperine. *Annual review of public health.2005:165.*

7 Available from: URL: http://www.scribd.com /doc/ 51167636/ anxiety.

8 Available from: URL:www.scfbio-iitd.res.in/tutorial/drug discovery.html

9 Available from: URL: http://en.wikipedia.org/wiki/Drug-development

10 Ekhlass Nassar, Synthesis, (in vitro) Antitumor and Antimicrobial Activity of some Pyrazoline, Pyridine, and Pyrimidine Derivatives Linked to Indole Moiety. *Journal of American Science* 2010:(6):8.

11 J.T. Desai, C.K. Desai and K.R. Desai.*J. Iran. Chem. Soc:* March 2008:(5): 67-73.

12 Mohammed Shaharyar, Abdullah M. Synthesis of a series of N 1- nicotinoyl- 3- (4-hydroxy-3-methyl phenyl)- 5-(substituted phenyl)-2- pyrazoline. *Europian Journal of Medicinal Chemistry.* 2010:(45):114-119.

13 Khalid Saifuddin, Kature Dattatraya, Suresh DKet al.Research *Journal of Pharmaceutical, Biological and Chemical Sciences.* 2010.(3):524.

14 Kulkarni S.K,Hand book of experimental pharmacology.3. Vallabh Prakashan:Delhi;1999. 135- 137.