IN SILICO, SYNTHESIS AND MAO-A INHIBITORY ACTIVITY OF KETONIC MANNICH BASES OF ISATIN

A Dissertation submitted to
THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY
CHENNAI- 600 032

In partial fulfillment of the requirements for the award of the Degree of
MASTER OF PHARMACY
IN
BRANCH – II - PHARMACEUTICAL CHEMISTRY

Submitted by
KATHIRAVAN .S
REGISTRATION No. 261815102

Under the guidance of
Mr. M. FRANCIS SALESHIER, M.Pharm.,
Department of Pharmaceutical Chemistry

COLLEGE OF PHARMACY
SRI RAMAKRISHNA INSTITUTE OF PARAMEDICAL SCIENCES
COIMBATORE – 641044

MARCH 2020

Certificate

This is to certify that the dissertation entitled **"*IN SILICO*, SYNTHESIS AND MAO-A INHIBITORY ACTIVITY OF KETONIC MANNICH BASE OF ISATIN "**was carried out by **S.KATHIRAVAN(reg.no:261815102)** in the Department of Pharmaceutical Chemistry, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore which is affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, under my direct supervision and guidance to my fullest satisfaction**.**

Prof. M. FRANCIS SALESHIAR, M. Pharm.,
Head of the Department,
Department of Pharmaceutical Chemistry,
College of Pharmacy, SRIPMS,
Coimbatore- 641 044.

Place: Coimbatore

Date:

Certificate

This is to certify that the pharmacological screening carried out and the dissertation entitled **"*IN SILICO*, SYNTHESIS AND MAO-A INHIBITORY ACTIVITY OF KETONIC MANNICH BASE OF ISATIN"** was carried out by **S.KATHIRAVAN (reg.no:261815102)** in the Department of Pharmaceutical Chemistry, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore being submitted to the Tamil Nadu Dr. M.G. R. Medical University, Chennai under my direct supervision and to my fullest satisfaction..

Dr.K. Asok Kumar, M. Pharm., Ph. D.,
Professor&Head,
Department of Pharmacology,
College of Pharmacy, SRIPMS,
Coimbatore-44.

Place: Coimbatore
Date:

Certificate

This is to certify that the dissertation entitled **"*IN SILICO*, SYNTHESIS AND MAO-A INHIBITORY ACTIVITY OF KETONIC MANNICH BASE OF ISATIN"** was carried out by **S.KATHIRAVAN (reg.no:261815102)** in the Department of Pharmaceutical Chemistry, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore which is affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, under the guidance of **Prof. M. FRANCIS SALESHIAR, M.Pharm** Professor, Department of Pharmaceutical Chemistry, College of Pharmacy, SRIPMS, Coimbatore.

Dr. T.K. RAVI, M.Pharm., Ph.D.,FAGE.,
Principal,
Department of Pharmaceutical Analysis,
College of Pharmacy, SRIPMS,
Coimbatore- 641 044.

Place: Coimbatore
Date:

ACKNOWLEDGEMENT

With the blessing of omnipresent God, let me write that the source of honor for the completion of the work embodied in the present dissertation is due to numerous persons by whom I have been inspired, helped and supported during my work done for M.Pharm degree.

My dissertation would not have been possible without the grace of The Almighty ***GOD*** *who gave me strength and wisdom to complete this project. First and foremost, I want to pay all my homage and emotions to my beloved parents* ***Mr. Subramanian .A*** *and* ***Mrs. Ruckmani .S*** *without whose blessings this task would not have been accomplished. I bow my head with utter respect to them for their continuous source of inspiration, motivation and devotion to me.*

I extend my profound gratitude and respectful regards to our Managing Trustee, ***Thiru. R.Vijayakumhar, Managing Trustee, M/s. SNR Sons Charitable Trust, Coimbatore*** *for providing the adequate facilities in this institution to carry out this work.*

It is my pleasure to express my sedulous gratitude to our Principal ***Dr. T.K.Ravi, M.Pharm., Ph.D., FAGE.,*** *College of Pharmacy, SRIPMS, Coimbatore for giving us an opportunity to do this project work and for providing all necessary facilities for it.*

I would like to devote my sincere gratitude to my guide ***Mr.M.Francis Saleshier, M. Pharm., Professor & HOD, Department of Pharmaceutical Chemistry,*** *College of Pharmacy, SRIPMS, Coimbatore for his remarkable guidance and valuable suggestion during the tenure of my work. I wish to convey my deep sense of gratitude to her for all the guidance she has provided me over the time of my academic years. There is no doubt that without her effort that task would not be achieved. It is my great privilege to have such dedicated guide like her that provides dynamic encouragement to me.*

I would like to my sincere thanks to ***Dr. K. Asok Kumar, M. Pharm., Ph.D., Professor & Head of the Department, Department of Pharmacology,*** *College of Pharmacy, SRIPMS, Coimbatore for his guidance and valuable suggestion during my work.*

My solemn thanks to my dear teachers, ***Dr.Sonia George, M.Pharm., Ph.D., Mrs. K.Susila, M. Pharm.,*** *Department of chemistry for their timely help and guidance during the course of the work*

I express my gratitude to our esteemed and beloved stafss ***Dr.R.Vijayaraj M.Pharm., Ph.D, Mr.Sunnapu Prasad, M. Pharm.., Dr.K.P.Beena, M.Pharm., Ph.D.,*** *Department of chemistry for their valuable suggestions and encouragement .*

It is my privilege to express my sincere thanks to Dr. ***M. Gandhimathi, M.Pharm., PhD., PGDMM, Assistant Professor, Department of Pharmaceutical Analysis*** *for providing me all the facilities to carry out the spectral and in vitro studies.*

My special thanks to Dr. ***R. Venkatasamy M.Sc., Ph.D., Senior Lab Technician*** *Department of Pharmacognosy,* ***Mrs. R. Beula Hepsibah*** *Lab assistant Department of chemistry for providing me all the facilities to carry out studies.*

Words are not enough To my friends and Batch mates ***Gokul, Rajasekaran, Athish****,* ***Sai Krishna priya, Anvidha, Ansu, Ajin , Wassem ,*** *for their love, kind support and cooperation.*

To my lovable juniors ***Priyadharshini , Sathish kumar , Anbu Dinesh, Sujtiha, Kavitha*** *,* ***Sathya Pooja , Thaiyalnayagi*** *for their love,kind support and cooperation.*

I place my heartful love to ***my baby Aara,my brother Manoj , Aisuu*** *and my well wishers for* ***Anitha, Dhanya, Bharathi****,* ***Raksana*** *their valuable support.*

My special thanks to the office staff of our college ***Mrs. R.Vathsala, Mrs.Nirmala and Mrs. Rajeswari*** *for all the help and support given by them to me.*

S.Kathiravan

CONTENTS

SL.NO.	CONTENTS	PAGE NO.
1.	INTRODUCTION	1
2.	REVIEW OF LITERATURE	12
3.	PURPOSE AND PLAN OF WORK	17
4.	MATERIALS	20
5.	EXPERIMENTAL SECTION	22
6.	RESULTS & DISCUSSION	40
7.	SUMMARY & CONCLUSION	66
8.		
	REFERENCES	

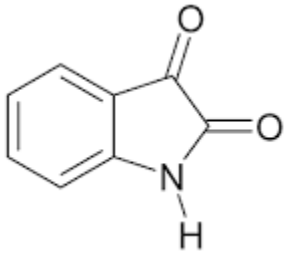
INTRODUCTION

Drug design

The drug discovery process involves the identification of the lead structure followed by the synthesis of its analogs, their screening to get candidate molecules for drug development against a target disease. Despite advances in understanding of biological systems, drug discovery is still a long process with low rate of new therapeutic discovery. It has been estimated that out of 10000 compounds synthesized in the laboratory only one achieves clinical use as a drug. The whole process takes around 10-15 years and costs nearly ~$1.2 billion today. This is due to the high quality and safety standards that are to be met before getting the FDA approval for marketing. In view of the failures in the late phases of drug development and the expensive novel technologies involved in the drug's effects on the molecular level and to find new targets, there is a continuous need of newer approaches for careful designing of the compounds and for predicting their biological activity to prioritize their synthesis. Since the activity of a molecule is the result of multitude of factors such as bioavailability, selectivity, toxicity and metabolism, the rational drug design has been a dream of medicinal chemists since a long time and for the last two decades the goal of medicinal chemistry has been dramatically focused on pinpointing the molecule for synthesis.(Leonardo G.Ferreira *et al*.,2015)

Chemistry of Isatin

Isatin is a naturally occurring heterocylic compound. The structure of isatin comprises of 8 carbons atoms,indole ring and two oxygen in its ring system. Naturally it is found in plants of isatis genus and the species *Melochiatomentosaaubl, couroupitaguianensis and boronellakoniambonesis*. It is potential chemical scaffold which undergoes chemical transformation to give various products which makes it a synthetically important compound. The structure of isatinissimple hencesynthesizing it in laboratory is feasible.(Rajasree Gpai *et al*.,(2016).



Target Enzyme

The drug design involves selection of target disease as an essential step to find a potent lead to inhibit the enzymes responsible in their mechanism of action. The monoamine oxidase (MAO)inhibitors were first developed as antidepressants long back. The exact mechanism of action of the enzyme and its inhibitors have been studied vastly, and many early theories about their functions have been proved wrong. The crystal structures of the two MAO isoenzymes have only recently been elucidated, necessitating a radical revision of some long-held ideas about how the enzyme interacts with substrates and inhibitors. Some inhibitors of the enzyme have been shown to have potential uses in the treatment of several neurodegenerative conditions including Parkinson's and Alzheimer's disease. The reaction catalysed by MAO produces hydrogen peroxide, a source of hydroxyl radicals, and MAO inhibitors might, therefore, be useful in managing the outcome of stroke and other tissue damage associated with oxidative stress. The activity of the enzyme is essential for normal brain development, and there are indications that the genetically determined level of activity of this enzyme influences aspects of personality and addictive behaviour. Here, we consider these phenomena in terms of our current understanding of the functions of MAO and its inhibitors.

Mitochondrial MAO catalyses the oxidative deamination of a range of monoamines, including 5-hydroxytryptamine (5-HT, or serotonin), histamine and the catecholamines dopamine, noradrenaline and adrenaline. The reaction

produces hydrogen peroxide, the corresponding aldehyde, and either ammonia (in the case of primary amines) or a substituted amine (from secondary amines). For example, methylamine, rather than ammonia, is produced from adrenaline. Although MAO was suggested to be a metallo-enzyme, containing either iron or copper ions essential for activity, it is now known that this is not the case. Two isoenzymes of MAO (MAOA and MAOB) are present in most mammalian tissues. These were originally distinguished by their sensitivities to the acetylenic inhibitors clorgyline and *l*-deprenyl (Selegiline), and by their substrate specificities. Typically, MAOA is inhibited by low concentrations of clorgyline and catalyses the oxidation of 5-HT.

MAO in peripheral tissues, such as the intestine, liver, lungs and placenta, seems to protect the body by oxidizing amines from the blood or by preventing their entry into the circulation. MAO-B in the micro vessels of the blood–brain barrier presumably has a similar protective function, acting as a metabolic barrier. It has been suggested that in the PNS and CNS intra neuronal MAOA and MAOB protect neurons from exogenous amines, terminate the actions of amine neurotransmitters, and regulate the contents of intracellular amine stores. However, studies on the localization of MAO-A and MAO-B in the brain necessitated a re-evaluation of earlier ideas that in nerve terminals MAO is dominant in neurotransmitter metabolism, whereas in glia it is only involved in scavenging after excessive release. The low level of MAO-A in serotonergic neurons suggests that glial cells might have an important role indegrading the neurotransmitter 5-HT.

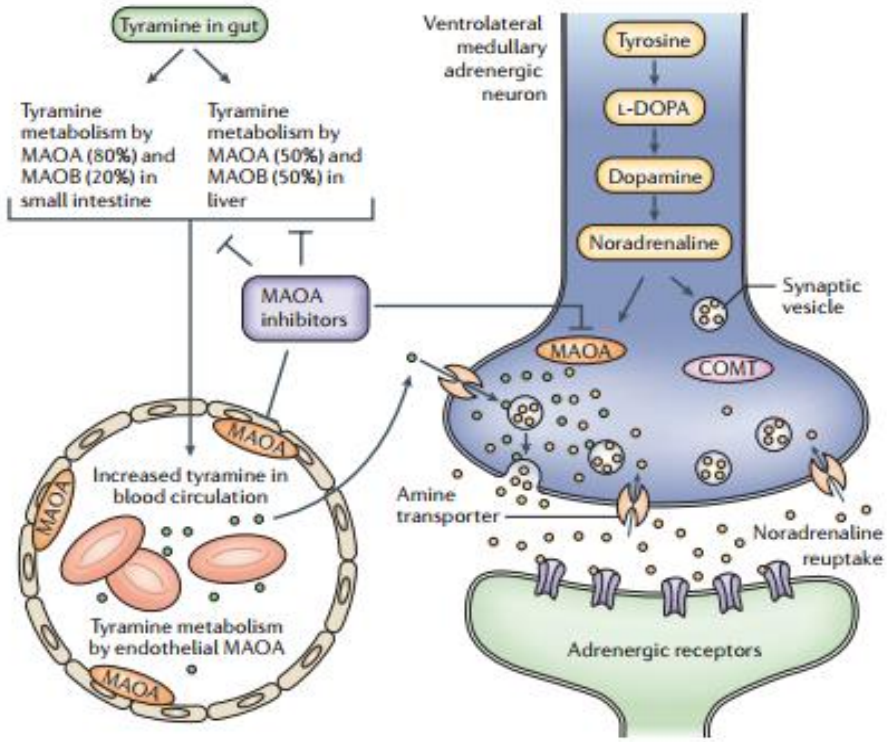


Fig 1:Mechanism of MAO-A

Hence in the present study MAO-A enzyme was selected as a target and the crystal structure of it was obtained from the RCSB protein data bank for further studies. The potential lead moiety fit to inhibit the MAO-A enzyme was identified using the computational tools which could be a effective drug candidate for depression. (Moussa B. H. Youdim,2006,P-295)

Drug Discovery and Development Process

The important steps involved in the process of drug discovery are:-

Lead identification:

A critical factor is the search for lead structures. Leads can be obtained from natural products, structure directed molecular design, modification of natural products, biochemical understanding of the disease process and broad screening of synthetic compounds etc.

Lead optimization:

It involves the identification of lead molecule through the synthesis, and testing of derivatives of leads to develop structure activity relationships (SARs), calculation of physico-chemical properties and using them for lead refinement from techniques like quantitative structure activity relationships (QSARs). Modern techniques such as combinatorial chemistry coupled with high throughput screening (HTS) provide us an enormous number of new chemical entities (NCEs) but these techniques have not been proved cost effective due to high costs of reagents in addition to the involvement of costly modern equipments, hence there has been a strong demand for the computer assisted techniques which are fast, reliable and cheap to rationalize these early steps in drug development.

Pre-clinical lead development:

Drug formulation experiments, in-vivo studies in animals, animal safety studies, drug metabolism studies and large scale synthesis come under the umbrella of pre-clinical lead development.

Clinical lead development:

It involves the small scale safety and dose ranging test in healthy human volunteers (phase I), short term toxicity and followed by development of clinical study protocols employing clinical investigations on patients (phase II) and comparative double blind studies on patients' studies (phase III).

The process of finding a new drug against a chosen target for a particular disease often involves random screening usually by high-throughput screening (HTS), wherein large libraries of chemicals are tested for their ability to modify the target. For example, if the target is a novel GPCR, compounds will be screened for their ability to inhibit or stimulate that receptor. Novel pharmacophores can emerge very rapidly from these exercises.

Another important function of these screens is to show the selectivity of the compounds for the chosen target by simultaneously screening the hits against other targets (cross-screening). Crossscreening is important as the more unrelated targets a compound hits, the more likely it is that, it may show toxicity once it reaches the clinic. However, it is very unlikely that a perfect drug candidate will emerge from these early screening runs. Apart from high throughput screening other methods such as virtual high throughput screening (vHTS), where screening is done using computer-generated models and attempts to "dock" virtual libraries to a target, are also very important as the virtual high throughput screening offers very fast, reliable and cost effective solution for large drug discovery projects. 12 recent Once a lead molecule series has been established with sufficient target potency, selectivity and favourable drug-like properties, one or two compounds may then be proposed for drug development. The best of these is generally called the "lead" compound. The lead compound is further optimized by random trials or more judiciously by rational drug design methods. The leads are examined for whether these compounds share some common features, chemists/modelers then use structure-activity relationships (SARs) to improve certain structural features of the lead molecules in order to (i) increase activity against the chosen target (ii) reduce activity against unrelated targets (iii) improve the "drug-like" or ADME properties of the molecule. A major target in the drug discovery process is to develop a high degree of “rationality.” This would represent an intellectually rigorous approach incorporating computer-assisted molecular design (CAMD), limited but highly focused chemical synthetic effort, and sophisticated biological assays. Thus, the drug design process may become significantly less risky and more resource efficient. .(Leonardo G.Ferreira *et al*.,2015)

Properties calculated using Molinspiration

In Molinspiration, we worked with lipophilicity, expressed as clogP, PSA index, nRotb and HBA/HBD counts. However, this website offers tools to calculate other properties, such as volume and total number of atoms in the

molecule. Analyzing the different approaches, the practical activities on the computer were directed to the evaluation of chemical and physicochemical data that influence the pharmacokinetic properties of a drug using computational techniques. Specific objectives were to allow the students to know these tools, to learn how to access them, to search for the structure of drugs and to analyze results. The advantage of Molinspiration is to provide more data (polar surface area, hydrogen bond donor and hydrogen bond acceptor) than Osiris. The advantage of Osiris is to provide values for drug-likeness and drug scores. Both are free software easily accessed by any student. (Monique Araújo de Brito,2011,P-788)

PreADMET

Over the last 30 years methods of computer aided drug design / discovery played a pivotal role in the development of therapeutic drugs. The potential of any compound used in therapeutics depends not only on the physical and chemical properties but also on Pharmaco dynamics [PD] and pharmaco kinetics [PK] aspects of the compounds. Pharmacodynamics correlates health effect of drugs on an individual patient while pharmacokinetics records the course of Absorption, Distribution, Metabolism and Excretion of a given drug, both are interrelated. Over the past 5 decades ADME played a major role in drug design process. ADME means absorption, distribution, metabolism and excretion which explain about the pharmacokinetics aspects of a drug molecule. There are several incidents reporting the attrition of drug discovery projects just because of the poor ADME profiles Therefore prior to synthesis and invivo studies, ADME profiling found to be more effective. Determination of ADME properties of compounds involves lot of experimental procedures to be followed which is time consuming and expensive. Therefore Insilco ADME models have been developed. (Ravi Kumar K. et al.,2018).

Molecular Docking

Molecular docking is one of the most frequently used methods in SBDD because of its ability topredict, with a substantial degree of accuracy, the conformation of small-molecule ligands within theappropriate target binding site. Following the development of the first algorithms in the1980s, molecular docking became an essential tool in drug discovery. For example, investigations involving crucial molecular events, including ligand binding modes and the corresponding intermolecularinteractions that stabilize the ligand-receptor complex, can be conveniently performed. Furthermore,molecular docking algorithms execute quantitative predictions of binding energetics, providing rankingsof docked compounds based on the binding affinity of ligand-receptor complexes.

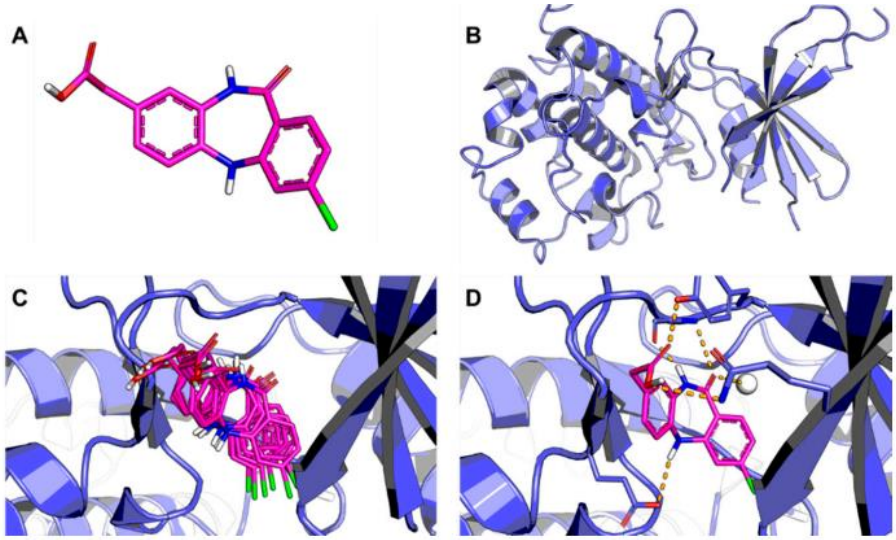
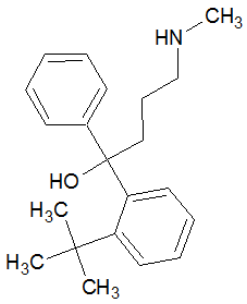


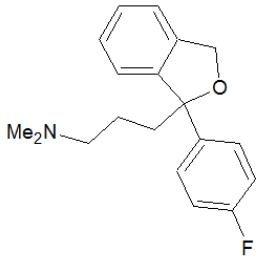
Fig 2: Molecular docking process. (**A**) Three-dimensional structure of the ligand; (**B**) Three-dimensional structure of the receptor; (**C**) The ligand is docked into thebinding cavity of the receptor and the putative conformations are explored; (**D**) The mostlikely binding conformation and the corresponding intermolecular interactions are identified.The protein backbone is represented as a cartoon. The ligand (carbon in magenta) and activesite residues (carbon in blue) are shown in stick representation. Water is shown as a whitesphere and hydrogen bonds are

indicatedasdashedlines. The identification of the most likely binding conformations requires two steps: (i) exploration of a large conformational space representing various potential binding modes; (ii) accurate prediction of theinteraction energy associated with each of the predicted binding conformations. Molecular dockingprograms perform these tasks through a cyclical process, in which the ligand conformation is evaluated by specific scoring functions. This process is carried out recursively until converging to a solution of minimum energy.

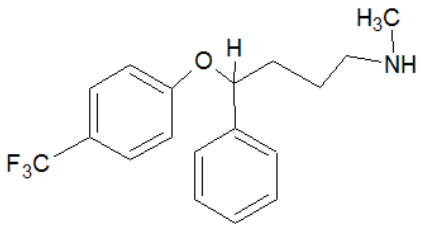
These approaches were based on an increased knowledge of the biochemistry involved in depression. This knowledge allowed the various teams to decide to develop drugs, now referred to as selective serotonin reuptake inhibitors (SSRIs) that selectively inhibited the amine pump that was responsible for the reuptake of serotonin in the presynaptic sites in the brain. This would increase the concentration of serotonin in contact with the postsynaptic receptor and, as a result, should reduce the patient's depression. In 1971 researchers at H. Lundbeck A/S selected talopram as the lead structure for a project to develop selective serotonin reuptake pump inhibitors. Talopram had been investigated by them as a potential antidepressant, however it had been found to be clinically unsatisfactory. A SAR investigation eventually revealed citalopram as the most clinically acceptable analogue. This was after less than 50 analogues of talopram had been synthesised. It was marketed by Lundbeck in 1989. Other companies soon followed suite and a number of SSRIs are now regularly used to treat mild and medium depression. Citalopram was first marketed as its racemate but it was found that the S-isomer was very much more active than the R-isomer. The former is now marketed as escitalopram. SSRIs appear to exhibit fewer detrimental side effects than the earlier tricyclic antidepressants (TCAs), such as amitryptiline, imipramine and doxepin. However, TCAs are more effective in treating severe depression.(Wilson and gisvold's,2015)



Talopram



Citalopram



Fluoxetin

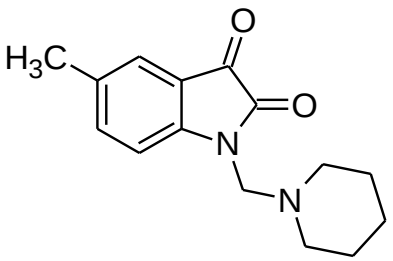
The drug design and development involves various stages including identification of target disease, selection of potent lead for inhibiting the enzyme involved in the mechanistic action and enzyme inhibitory activity of the potential scaffold. As many drugs have been failed even after marketing due to toxic effects on administration, their pharmacokinetic activity was predicted before synthesizing. Computational tools are available which eliminates the time utilized for trial and error in selecting the lead instead providing a potential scaffold with better binding interaction. The enzyme inhibitory activity of the predicted potent moiety will be analyzed using invitro or invivo studies. The structure activity relationship was also studied to understand which part of the lead is responsible for the activity. Extensive studies like QSAR was done to design a molecule with very potent activity based on their physical or chemical descriptors. The current study was emphasized to

design an antidepressant drug which should be safe and effective by inhibiting the MAO-A enzyme pathway. The drug likeness was studied using the Lipinski's rule of five and preAdmet was used to analyze the toxicity of the drug. The literature survey showed Isatin moieties to be the bioisosteres of the marketed antidepressants and hence selected as the lead moiety. The molecular docking studies will be used to predict the binding energy of the lead with the target enzyme. The compounds with best binding energy will be selected for the *invitro* enzyme inhibitory studies.The *insilico,* synthesis of novel isatin derivatives to inhibit MAO-A enzyme as antidepressants was planned to study.

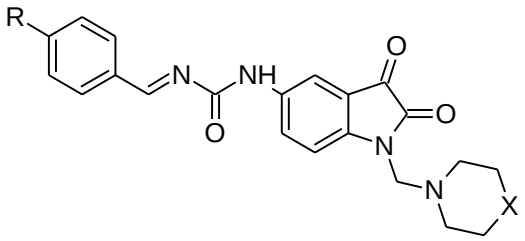
REVIEW OF LITERATURE

Synthesis

- Synthesis of Isatin- N-Mannich Bases Rojendro S. Vorma ond W. Lewis Nobles The synthesis of a series of isatin-N-Mannich bases derived from isatin, 5-methyl and 5-bromoisatins a s the active hydrogen component and dimethylamine, diethylamine, morpholine, piperidine, N-ethylcyclohexylamine, 3 azabicyclo[3,2,2]nonane and 3-azabicyclo[3,2,]loctane a s the secondary amine moiety .



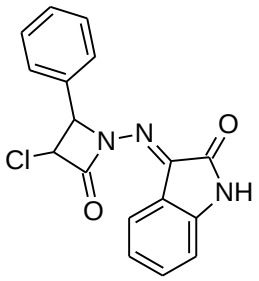
- Chinnasamy Rajaram Prakash , Sundararajan Raja Design, synthesis and antiepileptic properties of novel 1-(substituted benzylidene)-3-(1 (morpholino/piperidino methyl)-2,3-dioxoindolin-5-yl) urea derivatives these compounds 5c revealed protection in MES at a dose of 30 mg/kg and100 mg/kg 0.5 h and 4 h after i.p. administration.



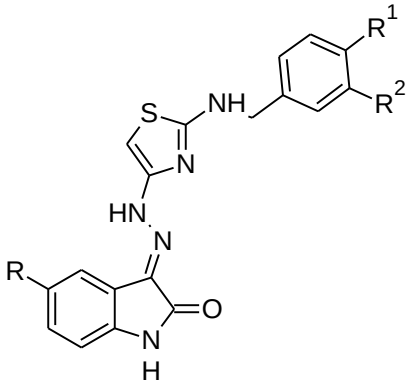
R=H, X=O

Anti depressant

- Deweshri Rajendrakumar Kerzare, Sunil Sugnomal Menghani, Pramod Bhujangrao Khedekar Synthesis, Characterization, Antidepressant Activity and Docking Studies of Some Novel Indole Bearing Azetidinone Derivatives an attempt has been made to generate new molecular template by linking two pharmacophores (indole and azetidinone), which are likely to exhibit antidepressant-like action in animal models by force swim test. (1)

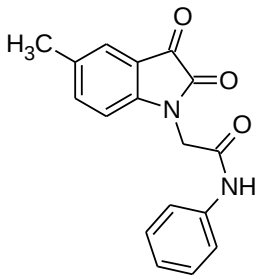


- Venkateshwar Rao Jupallya, Venkateshwarlu Eggadib, Sharavana Bhava Bandaru Sheshagirib, Umasankar Kulandaivelub**,** Synthesis and evaluation of neuropharmacological profile of isatin- 3-[N 2-(2-benzalaminothiazol-4-yl)] hydrazones was investigated by gross behavioural profile, hole board, locomotor activity, hypnotic activity, forced swim test, tail suspension test and rota rod test in mice (2)



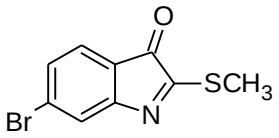
R=5-Cl, R_1=OH, R_2 =OCH_3

- Xinghua Zhena,y, ZhouPenga,y, ShuilianZhaoa, YanHana, Qinghao Jinb,n, LipingGuana, Synthesis, potential anti convulsant and antidepressant effects of 2-(5-methyl-2, 3-dioxoindolin-1-yl)acetamide derivatives evaluated for their anti-convulsant activityin a pentylenetetrazole(PTZ) evoked convulsion model and anti-depressant activity in the forced swimming test**(3)**

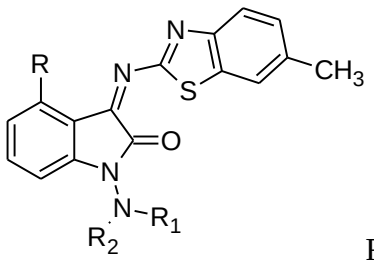


Anti cancer activity

- Kara L. Vine, Julie M. Locke,b,c Marie Ranson,Stephen G. Pyneb, and John B. Bremner In vitro cytotoxicity evaluation of some substituted isatin derivatives were synthesized and their cytotoxicity evaluated against the human monocyte-like histiocytic lymphoma (U937) cell line in vitro. SAR studies identified C5, C6, and C7 substitution greatly enhanced activity with some di- and tri-halogenated isatins giving IC50 values <10 µM **(4)**



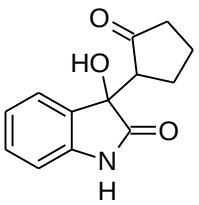
- V. Raja Solomon , Changkun Hua, Hoyun Lee Hybrid pharmacophore design and synthesis of isatin–benzothiazole analogs for their anti-breast cancer activity were determined using three different human breast tumor cell lines, MDA-MB231, MDA-MB468, MCF7, and two non-cancer breast epithelial cell lines, 184B5 and MCF10A. by SRB assay**(5)**



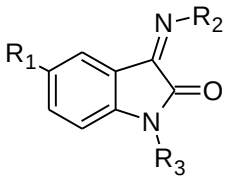
R=H, R_1R_2 =morpholinyl

Anticonvulsants

- Monika Raj, Nagarathanam Veerasamy, Vinod K. Singh Highly enantioselective synthesis of 3-cycloalkanone-3-hydroxy-2 oxindoles, potential anticonvulsants activity was achieved by using primary-tertiary diamine-Brønsted acid catalyst in both organic medium and aqueous medium **(6)**



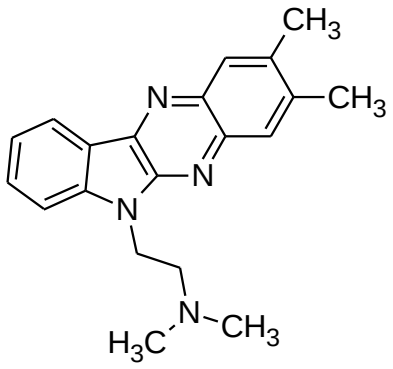
- Seshaiah Krishnan Sridhara, Surendra N. Pandeyab, James P. Stablesc, Atmakuru Ramesha , Anticonvulsant activity of hydrazones, Schiff and Mannich bases of isatin derivatives 3-(4-chloro-phenylimino)-5-methyl-1,3-dihydro-indol-2-one was found to be the most potent compound of the series with 87% protection at 100 mg/kg and an ED of 53.61 mg/kg metrazol-induced convulsions(MET). **(7)**



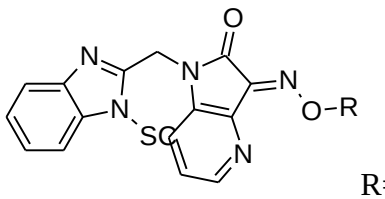
R_1=NO_2, R_2=4-bromo phenyl, R_3=H

Anti viral activity

- Marina O. Shibinskaya, Sergey A. Lyakhov , Alexander V. Mazepa , Sergey A. Andronati ,Alexander V. Turov , Nadezhda M. Zholobak , Nikolay Ya. Spivak Synthesis, cytotoxicity, antiviral activity and interferon inducing ability of 6-(2-aminoethyl)-indolo[2,3-b]quinoxalines Morpholine and 4-methyl-piperidine derivatives appeared as the most active antivirals and the least cytotoxic when compared with tilorone standard.



- Ny Sin , Brian L. Venables , Keith D. Combrink , H. Belgin Gulgeze , Kuo-Long Yu , Rita L. Civiello , Jan Thuring , X. Alan Wang , Zheng Yang , Lisa Zadjura ,Anthony Marino , Kathleen F. Kadow , Christopher W. Cianci Junius Clarke ,Eugene V. Genovesi , Ivette Medina , Lucinda Lamb , Mark Krystal , Nicholas A. Meanwell Respiratory syncytial virus fusion inhibitors. Part 7: Structure–activity relationships associated with a series of isatin oximes that demonstrate antiviral activity in vivo in which 18i, 18j and 18n that demonstrated antiviral activity in the BALB/c mouse model of RSV infection following oral dosing.



R=CH_2CH_2F

Enzyme activity

- R. 0. KOBES, R. f. WYATT, AND L. M. NECKERS
  A Sensitive and Rapid Fluorimetric Assay for Monoamine Oxidase Utilizing High Pressure liquid chromatography.

- This method utilizes high pressure liquid chromatography with fluorescence excitation at 280 nm and detection at 330 nm at pH 5.0 of indoieacetic acid and 5-hydroxyindoleacetic acid, the deaminated products of two substrates for MAO, tryptamine, and serotonin. The ease and rapidity of this assay make it suitable for use where routine enzyme determination**. (9).**
- Tomio Takeuchi, Keiji Ogawa, Hironobu Iinuma, Hiroyuki Suda, Katsuko Ukita, Toshiharu Nagatsu, Masaaki Kato And Hamaoumezaw Monoamine oxidase inhibitors isolatedfrom fermented broths Cultured broths were screened by measuring the inhibition of monoamine oxidase. Using serotonin as the substrate, two active agents were isolated from Actinomycetes and shown to be pimprinine and trans-cinnamimc acid amide, and a compound isolated from a mushroom was identified using benzylamine as the substrate**(10)**
- Soheila Kashanian, Mohammad Mehdi Khodaei, and Parvaneh Pakravan Spectroscopic Studies on the Interaction of Isatin with Calf Thymus DNA. The interaction of native calf thymus DNA (CT-DNA) with isatin was studied at physiological pH by spectrophotometric, spectrofluorometric, competition experiment, circular dichroism, and viscometric techniques. Spectra of CT-DNA indicated deep conformational changes in the DNA double helix.**(12)**

PURPOSE AND PLAN OF WORK

The development of drug for the target disease depression is essential as chronic medical conditions and depressive disorders frequently co-occur. Depression occurring concomitantly with other chronic diseases incrementally worsened health compared with depression alone. Also depression has been associated with a doubling of the risk for type 2 diabetes and a 64% increase in the risk for coronary artery disease. Depression affects the prevalence, severity, and management of co-occurring chronic medical conditions and vice versa. Hence there is a huge demand to develop a drug for treating depression by which the risk of associated chronic illnesses can be reduced.

The literature survey availed prime motivation for the present work to design a drug which effectively treat the target disease depression which impacts the associated chronic diseases also. The i*n silico* drug discovery tools are playing a great role in drug discovery to design new molecular entities which are safe and effective without consuming much of the research hours. Although various enzymes were responsible for the mechanistic pathway for depression, MAO-A enzyme was selected as target based on the literature survey. The present work focuses on *in silico* optimization of the lead, synthesis of various lead derivatives and *invitro* inhibition of MAO-A enzyme isolated from rat brain.

PLAN OF WORK

The present work has been carried out under the following sections.

Phase I: *In silico* Studies

- Selection of drug target
- Lead Optimization and computation of drug like properties using **Molinspiration server**
- The target enzyme was identified as **MAO-A**
- Docking of the Lead molecules with target enzyme using **AutoDock 4.2**

Phase II: Synthesis of Isatin derivatives

Totally 15 compounds were synthesised using 3 different schemes.

- Scheme 1

The scheme 1 was used to synthesize 5 Isatin derivatives.

- Scheme 2

The scheme 1 was used to synthesize 5 Isatin derivatives.

- Scheme 3

The scheme 1 was used to synthesize 5 Isatin derivatives.

- Spectral Characterization using UV, IR, Mass, NMR was done

Phase III: *Invitro* Enzyme Inhibitory Study

- The *Invitro* MAO-A inhibitory activity was evaluated for the synthesised compounds and the results were recorded.

MATERIALS

Phase I ***INSILICO*** **STUDY**

Softwares and data bases used

- Molinspiration (https://www.molinspiration.com/)
- PreADMET properties (https://preadmet.bmdrc.kr/)
- RCSB protein data bank (https://www.rcsb.org/)
- Online SMILES translator (https://cactus.nci.nih.gov/translate/)
- MGL tools-
  - AutoDock 4.2 (http://autodock.scripps.edu/)
  - Python 2.7 molecule viewer 1.5.6 (http://mgltools.scripps.edu/)
  - Cygwin 64 (https://cygwin.com/install.html)
- ChemSketch (https://www.acdlabs.com/resources/freeware/chemsketch/)

Phase II **SYNTHESIS**

Reagent and chemicals

Isatin, methoxy acetophenone , hydroxy acetophenone, chloro acetophenone, methyl acetophenone, resacetophenone, para formaldehyde, ethanol, trichloroisocyanuric acid (Hi-Media), nitric acid, sulphuric acid

Instruments

- UV visible spectrometer (JASCO V 530) in the department of pharmaceutical analysis, college of pharmacy, Sri Ramakrishna institute of paramedical science Coimbatore.
- IR spectrophotometer (JASCO-410) in the department of pharmaceutical analysis, college of pharmacy, PSG institute of paramedical science Coimbatore.
- Magnetic stirrer (REMI Elektrotechnik Ltd) in the department of pharmaceutical chemistry, college of pharmacy, Sri Ramakrishna institute of paramedical science Coimbatore.

- Stirrer (REMI Elektrotechnik Ltd) in the department of pharmaceutical chemistry, college of pharmacy, Sri Ramakrishna institute of paramedical science Coimbatore.
- Melting point apparatus in the department of pharmaceutical chemistry, college of pharmacy, Sri Ramakrishna institute of paramedical science Coimbatore.

Phase III ENZYME INHIBITORY STUDY

Reagent and chemicals

2M HCL, 5-hydroxy tryptophan purchased in Hi-Media, potassium phosphate buffer, phosphate buffer, 0.25M sucrose, n-butyl acetate

Instruments

- UV-visible spectrometer (JASCO V 530) in the department of pharmaceutical analysis, college of pharmacy, Sri Ramakrishna institute of paramedical science Coimbatore.
- Vortex mixer (Sri Mahalakshmi scientific & co) in the department of pharmaceutical biotechnology, college of pharmacy, Sri Ramakrishna institute of paramedical science, Coimbatore.
- Centrifuge (REMI Elektrotechnik Ltd) in the department of pharmaceutical chemistry, college of pharmacy, Sri Ramakrishna institute of paramedical science Coimbatore.

Drug design approach

Drug designing for MAO-A inhibitor was done in the following phases;

a. Identification of the target enzyme
b. Lead optimization (ADME & Mol inspiration)
c. Docking of the ligand with the MAO-A

A) Identification of the target enzyme

- Dopamine synthesis and its metabolism by MAO-A. The pathway of dopamine synthesis proceeds from tyrosine via tyrosine hydroxylase (TH) catalysis to levodopa (l-DOPA), and subsequent decarboxylation by dopa decarboxylase (DDC) to dopamine. Dopamine is metabolized by intraneuronal monoamine oxidase A (MAO-A), and by glial and astrocyte MAO-A .
- Selective inhibitors of MAO-A (for example, moclobemide) and do not alter the steady-state striatal dopamine levels, although chronic treatment with these drugs does enhance dopamine release, possibly due to the elevation of endogenous brain amines or receptor modulation non-selective MAO-A inhibitors (such as ladostigil) do induce highly significant increases in the levels of dopamine in the striatum and other regions.
- In the present work MAO-A was identified as the target enzyme which is involved in the decrease in the level of dopamine.By blocking this enzyme dopamine level can be increased. The MAO-A is downloaded from the protein database PDB(code:2BXS).(https://www.rcsb.org/)

B) Lead optimization

Lead optimization is a processs in which lead compounds are altered to achieve maximum affinity towards the target. To make the lead moiety safer, effective and to possess good pharmacokinetic properties structural modifications were done.

Evaluation of drug likeness(Molinspirtion)

Pharmacologically active substituents were characterized by calculating steric, hydrophobic, electronic, and hydrogen bonding properties as well as by the

drug-likeness score. The theory of drug-likeness score helps to optimize pharmaceutical and pharmacokinetic properties, for example, chemical stability, solubility, distribution profile and bioavailability. The molecular descriptors have developed as rationally predictive and informative, for example, the Lipinski's Rule-of-Five (Ro5). The better oral absorption of the ligands and drug likeness scores were constructed by getting information about the solubility, diffusion, Log P, molecular weight etc. Molinspiration software was used to evaluate the (Ro5).

Lipinski's Ro5 calculations

1) Open the molinspiration home page (http://www.molinspiration.com/).
2) For calculating using molinspiration, it requires JAVA in the computer.
3) Click calculation of molecular properties of drug likeness.
4) Draw the structure of ligand in the active window.
5) Click calculate properties and predict bioactivity.
6) Save the properties. (https://www.molinspiration.com/)

Evaluation of drug Pre-ADMET

Procedure

- Google search Pre ADMET
- Click on the link ADME Prediction in the home page, when it redirects to login page.
- Login into the server to get redirected to the page for ADME prediction
- One can draw the chemical structure of the drug molecule using the tool bar
- One can also import the structure of a molecule into the tool for predicting ADME. The structure of the molecule should be loaded in "mol" format.
- Once the molecule is open and loaded, click on the calculate button seen at bottom right side of the window.

Calculating the ADME results

Here, one has options like calculate, reset, and clear result. By clicking "calculate" button, it will lead to calculation of ADME properties of a drug molecule. By clicking the reset button it will allow to reset the view of a drug molecule and clear result will clear the result page. It will allow loading of a new molecule without confusion.

There are several methods used in drug selection process to check for intestinal absorbtion. Caco-2 model and MDCK cell are referred to as invitro model for prediction of drug absorbtion, where the drug has been administered orally. PreADMET predicts the permeabilty of Caco-2 cell, MDCK cell, blood brain barrier, Human intestianl absorption, skin permeability and plasma protein binding.

Caco-2 cells are derived from human colon adenocarcinoma which has multiple drug transport pathways.

Caco-2 permeability in PreADMET is classified into three classes.

1. If the permeability value is less than 4, then the molecule is having low permeability.
2. If the permeability value is between 4 to 70, then the molecule is having medium permeability.
3. If the permeability value is greater than 70, then the molecule is having higher permeability.

MDCK cell permeability

MDCK cell refers to Madin-Darby canine kidney cell. Since MDCK cells life span is less than the life span of Caco-2 cells, correlation between them is said to be high. MDCK cell system can be used as a good tool for rapid permeability screening.

MDK cell level of permeability in PreADMET can be classified into three classes,

1. If the permebiality value is less than 25, then the molecule is having low permeability.
2. If the permebiality value is between 25 to 500, then the molecule is having medium permibility
3. If the permebiality value is greater than 500, then the molecule is having higher permeability.

Human intestinal absorption

The intestinal absorption is very important to find the potential candidate and PreADMET finds the absorption in percentage. Poorly absorbed compounds return 0 to 20%, moderately absorbed compounds return 20- 70% and well absorbed compounds return 70-100%.

Skin permeability

Skin permeability factor is very important in case of cosmetics for transdermal delivery of drugs. PreADMET predicts the invitro skin permeability and the result is given as logKp.

Blood Brain Barrier

Predicting whether compounds pass across the blood-brain barrier is crucial in the pharmaceutical sphere because CNS-active compounds are the only substances which must cross the barrier. This happens in order to avoid CNS side effects in the brain. In PreADMET, one can predict rates for BBB penetration in vivo data.

PreADMET explains about the absorbtion to CNS as follows.

Classification	BB	logBB
High absorption to CNS	More than 2.0	More than 0.3
Middle absorption to CNS	2.0 to 0.1	0.3 to -1.0
Low absorption to CNS	Less than 0.1	Less than -1.0

Table1: Absorption level in central nervous system

Plasma protein binding of a drug gives information on not only the drug action but also its efficacy and disposition.

In PreADMET, plasma protein binding capacity is referred to as

Classification	Plasma protein binding
Strongly bound chemicals	More than 90%
Weakly bound chemicals	Less than 90%

Table 2:Binding capacity level interpretation table (value@amirta)

C) DOCKING

For docking study the enzyme was downloaded from the **protein database** where the x-ray crystallographic structure are obtained and the docking study were performed with **Autodock 4.2** version.

Step 1

Ligand file format conversion

- The desire ligand are drawn in ChemSketch.
- Tools→clean structure.
- Tools→generate→SMILES notation.
- Copied the smile notation and uploaded the smiles in online smile translator-cactus.nci.nih.gov/services/translate.
- By choosing the required file format and save the file as pdb format.

Step II

Protein structure refinement

The enzymes monoamino oxidase , were downloaded from RCSB (Research Co-laboratory for Structural Bioinformatics) Protein Data Bank and the protein was refined before use for docking.

- Opened Accelrys discovery studio viewer.
- File→open→RCSBPDB file.
- View→hierarchy→click water molecules→select all water molecules → delete.
- Selected ligand, which was unnecessary and deleted.
- Saved the molecule in a desired location.

Step III

Docking with autodock 4.2

- Opened the refined protein from the location in pdb format.
- Preparation of target and ligand in AutoDock 4.2

Step IV

Preparation of protein

- AutoDock 4.2 →File→Read molecule→Choose refined enzyme file.

Elimination of water molecule carried out by:

- Select→Select from string→Residue (*HOH*) →Add→Dismiss.
- Edit→Hydrogen→Add→Polar only→Ok.
- Edit→charges→Add kollmann charges→Ok.
- File→save→Write pdb→Browse→Save→Ok.
- Edit→Delete all molecules→Continue.

Step V

Preparation of ligand

- Ligand→input→open.
- Ligand→torsion tree→detect root.
- Ligand→ torsion tree→show root expansion.
- Ligand→ torsion tree→choose torsions→done.
- Ligand→ torsion tree→set number of torsions→dismiss.
- Ligand→ torsion tree→hide root expansion.
- Ligand→ torsion tree→show/hide root marker.
- Ligand→output→ save as pdbqt file.
- Edit→delete→delete all molecules→ continue.

Conversion of pdb files of protein in to pdbqt file

- Grid→Macromolecule→Open→Save as pdbqt.

AutoGrid calculation and creating "gpf" file

- Grid→set map types→ open ligand.
- Grid→grid box→set 60 points in XYZ.
- File→close saving current.
- Grid→output→save as gpf.
- Edit→delete→delete all molecules→continue.

Autodock calculation and creating 'dpf' file

- Docking→macromolecule→set rigid file name →open.
- Docking→ligand→open→accept.
- Docking→ search parameters→ genetic algorithm→accept.
- Docking→docking parameters→accept.
- Docking→output→ lamarckian genetic algorithm→save as dpf.

Programming of 'Auto Grid' and 'Auto Dock' execution

Open Cygwin64 and type as given below:

- cd C:
- cd cygwin64
- cd usr
- cd local
- cd bin

Program should list out the pdb, pdbqt, gpf and dpf files of an enzyme and ligand molecule.

Then type as:

- ./autogrid4.exe<space>–p<space>ligand.gpf<space> -l<space>ligand.glg
  If a ligand gets into the spacing of the grid, then the execution of this command was;
- ✓ *'Successful completion'*

Then type as:

- ./autodock4.exe<space> –p<space>ligand.dpf<space> -l<space>ligand.dlg
  If the ligand binds to the amino acids through 10 different conformations, then the execution of this command was;
- ✓ *'Successful completion'*

Step VI

Viewing docking results

Reading the docking log file.dlg

- Toggle the AutoDock Tools button.
- Analyse→Docking.
- Analyse→Conformations → Load.
- Double click on the conformation for to view it.

Visualizing docked conformations

- Analyse→Dockings→Play.
- Load dlg file.
- Choose the suitable conformations.
- Analyse→Docking→Show Interactions.

Obtaining snap shots of docked pose

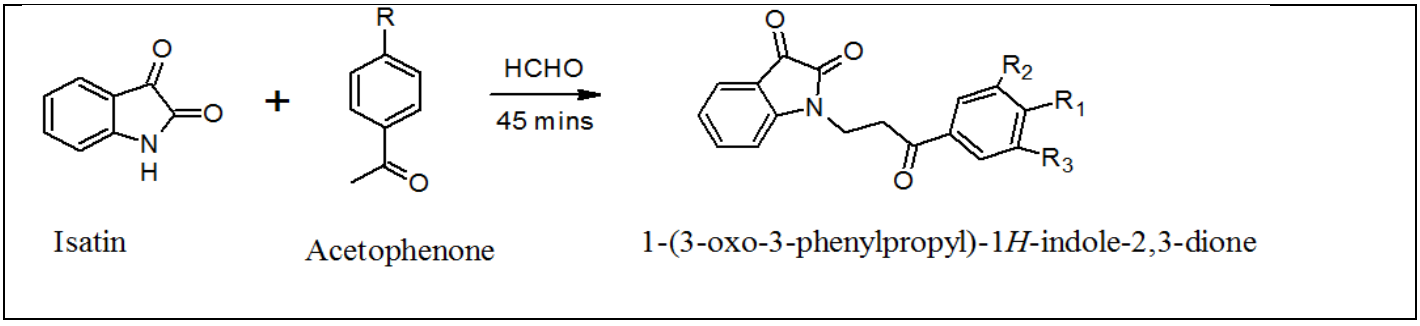
- File→Read Molecule.
- Analyse→Dockings→Open dlg file.
- Analyse→Macromolecule→Choose pdbqt file.
- Analyse→Conformations→Load.
- Double click the desired conformation.
- Analyse→Docking→Show Interactions.

Proteins and ligand interaction was displayed. Zoom it and increase the contrast by holding right key and ctrl. Rapid energy evaluation was attained by pre-calculating the atomic resemblance potentials for each atom in the selected compounds. In the AutoGrid process, the target was enclosed on a three dimensional grid point and the energy of interface of the each atom in the compounds were encountered. The following docking factors were chosen for the Lamarckian genetic algorithm as follows: population size of 150 individuals, 2.5 million energy evaluations, maximum of 27000 generations, and number of top individuals to automatically survive to next generation of 1, mutation rate of 0.02, crossover rate of 0.8, 10 docking runs, and random initial positions and conformations. The probability of performing local search on a single compound in the population was set to 0.06. AutoDock was run various times to obtain various docked conformations, and used to calculate the predicted binding energy.

SYNTHESIS

In the present work the isatin derivatives were synthesized by the ketonicmannich base reaction, with the help of para formaldehyde and acetophenones.

Scheme 1:



compound	R_1	R_2	R_3
IS1	H	OCH_3	H
IS2	H	Cl	H
IS3	H	OH	H
IS4	H	CH_3	H
IS5	OH	H	OH

Synthesis of 1-[3-(4-methoxy phenyl)-3-oxopropyl]-1H-indole-2,3-dione

A mixture of 8.8 g (0.073 mole) of methoxyacetophenone, 3.0 g (0.100 mole) of para formaldehyde, 8.0 g(0.044 mole) of isatin and 10 cc(15ml) of absolute alcohol was heated on a steam-bath for 45 minutes. The mixture was cooled and the precipitated material (4.7 g) recrystallized from ethyl acetate. **(F. F.** BLICKE**AND J. H.** BURCKHAL **vol 64,P-453)**

Synthesis of 1-[3-(4-chlorophenyl)-3-oxopropyl]-1*H*-indole-2,3-dione

A mixture of 8.8 g (0.073 mole) of chloroacetophenone, 3.0 g (0.100 mole) of para formaldehyde, 8.0 g (0.044 mole) of isatin and 10 cc(15ml) of absolute alcohol was heated on a steam-bath for 45 minutes. The mixture was cooled and the precipitated material (4.7 g) recrystallized from ethyl acetate.

Synthesis of 1-[3-(4-hydroxyphenyl)-3-oxopropyl]-1*H*-indole-2,3dione

A mixture of 8.8 g (0.073 mole) of hydroxyacetophenone, 3.0 g (0.100 mole) of paraformaldehyde, 8.0 g. (0.044 mole) of isatin and 10 cc(15ml) of absolute alcohol was heated on a steam-bath for 45 minutes. The mixture was cooled and the precipitated material (4.7 g) recrystallized from ethyl acetate.

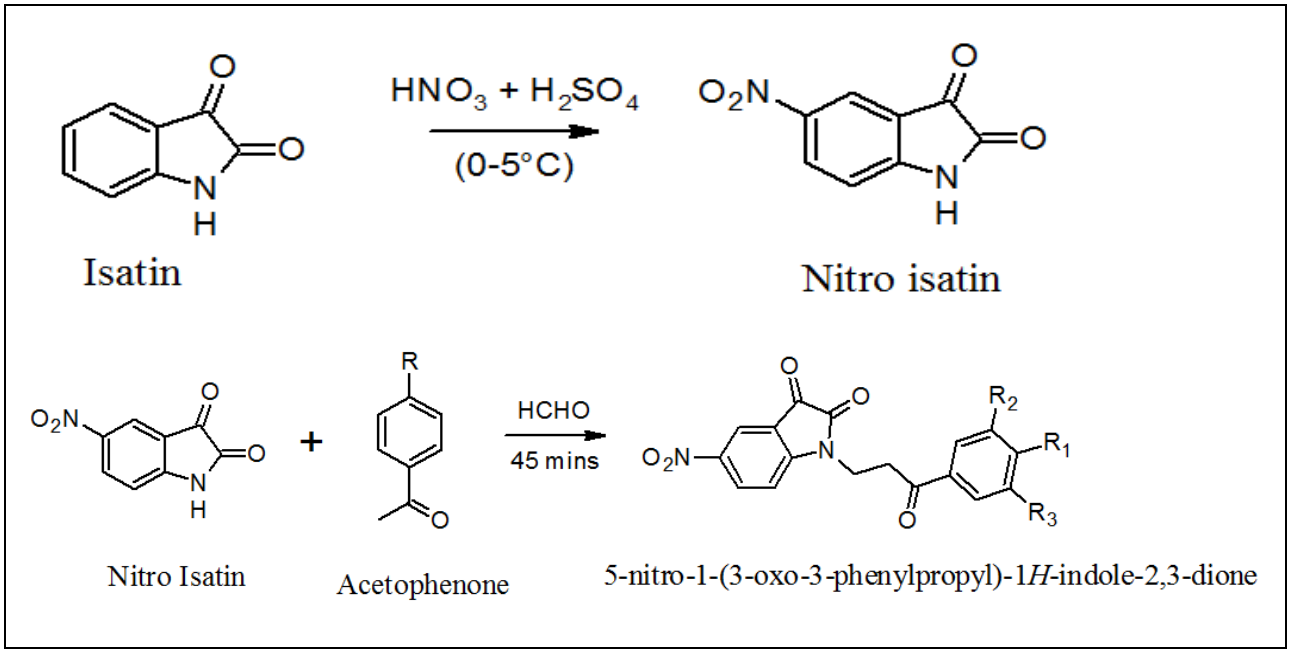
Synthesis of 1-[3-(4-methylphenyl)-3-oxopropyl]-1*H*-indole-2,3-dione

A mixture of 8.8 g(0.073 mole) of methyl acetophenone, 3.0 g (0.100 mole) of para formaldehyde, 8.0 g (0.044 mole) of isatin and 10 cc(15ml)of absolute alcohol was heated on a steam-bath for 45 minutes. The mixture was cooled and the precipitated material (4.7 g) recrystallized from ethyl acetate.

Synthesis of 1-[3-(3,5-dihydroxyphenyl)-3-oxopropyl]-1*H*-indole-2,3-dione

A mixture of 8.8 g (0.073 mole) of resacetophenone, 3.0 g (0.100 mole) of para formaldehyde, 8.0 g (0.044 mole) of isatin and 10 cc(15ml) of absolute alcohol was heated on a steam-bath for 45 minutes. The mixture was cooled and the precipitated material (4.7 g) recrystallized from ethyl acetate.

Scheme 2:



compound	R_1	R_2	R_3
NIS1	H	OCH_3	H
NIS2	H	Cl	H
NIS3	H	OH	H
NIS4	H	CH_3	H
NIS5	OH	H	OH

Synthesis of 5-Nitro Isatin derivative

3.4 mmol solution of isatin was prepared in 3.2 ml of conc. Sulphuric acid and then this solution was added drop wise into 3.4 mmol solution of nitric acid prepared in 3.8 ml of conc. Sulphuric acid over a period of 1 hour whilst the temperature was maintained between 0 to 5°C. The reaction mixture was poured into 25 ml of ice water and precipitates were collected followed by its washing with water. The

crude 5-nitro isatin was purified by re-crystallization with ethyl acetate.(H. 0. CALVERY,' C. R. NOLLERAND ROGER**ADAM vol64 P-3058)**

Synthesis of 1-[3-(4-methoxyphenyl)-3-oxopropyl]-5-nitro-1*H*-indole-2,3-dione

A mixture of 8.8 g(0.073 mole) of methoxyacetophenone, 3.0 g(0.100 mole) of para formaldehyde, 8.0g (0.044 mole) of nitro isatin and 10 cc(15ml) of absolute alcohol was heated on a steam-bath for 45 minutes. The mixture was cooled and the precipitated material (4.7 g) recrystallized from ethyl acetate.

Synthesis of 1-[3-(4-chlorophenyl)-3-oxopropyl]-5-nitro-1*H*-indole-2,3-dione

A mixture of 8.8 g(0.073 mole) of chloroacetophenone, 3.0 g (0.100 mole) of para formaldehyde, 8.0 g (0.044 mole) of nitro isatin and 10 cc(15ml) of absolute alcohol was heated on a steam-bath for 45 minutes. The mixture was cooled and the precipitated material (4.7 g) recrystallized from ethyl acetate.

Synthesis of 1-[3-(4-hydroxy phenyl)-3-oxopropyl]-5-nitro-1*H*-indole-2,3-dione

A mixture of 8.8 g (0.073 mole) of hydroxyacetophenone, 3.0 g(0.100 mole) of para formaldehyde, 8.0 g(0.044 mole) of nitro isatin and 10 cc(15ml) of absolute alcohol was heated on a steam-bath for f45 minutes. The mixture was cooled and the precipitated material (4.7 g) recrystallized from ethyl acetate.

Synthesis of 1-[3-(4-methyl phenyl)-3-oxopropyl]-5-nitro-1*H*-indole-2,3-dione

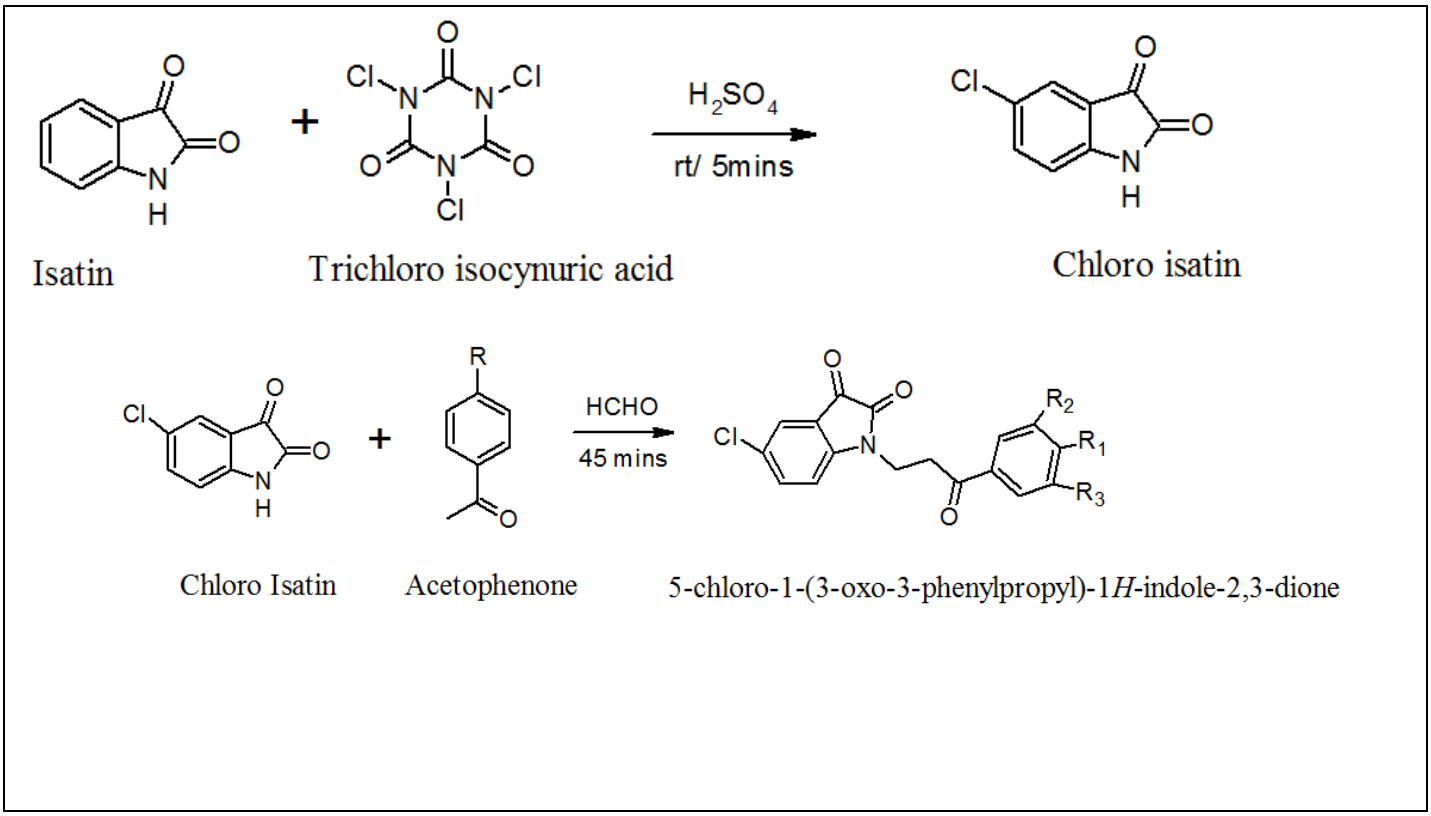
A mixture of 8.8 g(0.073 mole) of methyl acetophenone, 3.0 g(0.100 mole) of para formaldehyde, 8.0 g(0.044 mole) of nitro isatin and 10 cc(15ml)of absolute alcohol was heated on a steam-bath for 45 minutes. The mixture was cooled and the precipitated material (4.7 g) recrystallized from ethyl acetate.

Synthesis of 1-[3-(4-dihydroxy phenyl)-3-oxopropyl]-5-nitro-1*H*-indole-2,3-dione

A mixture of 8.8 g(0.073 mole) of dihydroxyacetophenone, 3.0 g (0.100 mole) of para formaldehyde, 8.0 g(0.044 mole) of nitro isatin and 10 cc(15ml) of absolute

alcohol was heated on a steam-bath for 45 minutes. The mixture was cooled and the precipitated material (4.7 g) recrystallized from ethyl acetate.

SCHEME 3:



compound	R_1	R_2	R_3
CIS1	H	OCH_3	H
CIS2	H	Cl	H
CIS3	H	OH	H
CIS4	H	CH_3	H
CIS5	OH	H	OH

Preparation of 5-Chloro-lH-indole3,3-dione (5-Chloroisatin):

To a mixture of isatin (2.94 g, 20 mmol) and 9 mmol of TCCA (2.09 g, 9 mmol) in an ice bath, 12 mL of H_2SO_4 was added dropwiseover a 5 minute period with magnetic stirring. The mixture was kept in an ice bath under stirring for 15 minutes. The mixture was then poured into cracked ice. The crystals were collected and washed with cold water. (N. M. Ribeiro et al., 2004)

Synthesis of 5-chloro-1-[3-(4-methoxyphenyl)-3-oxopropyl]-1*H*-indole-2,3-dione

A mixture of 8.8 g (0.073 mole) of methoxyacetophenone, 3.0 g(0.100 mole) of para formaldehyde, 8.0 g(0.044 mole) of chloroisatin and 10cc(15ml) of absolute alcohol was heated on a steam-bath for 45minutes. The mixture was cooled and the precipitated material (4.7 g) recrystallized from ethyl acetate.

Synthesis of 5-chloro-1-[3-(4-chlorophenyl)-3-oxopropyl]-1*H*-indole-2,3-dione

A mixture of 8.8 g (0.073 mole) of chloroacetophenone, 3.0 g(0.100 mole) of para formaldehyde, 8.0 g(0.044 mole) of chloroisatin and 10 cc(15ml) of absolute alcohol was heated on a steam-bath for 45 minutes. The mixture was cooled and the precipitated material (4.7 g) recrystallized from ethyl acetate.

Synthesis of 5-chloro-1-[3-(4-hydroxyphenyl)-3-oxopropyl]-1*H*-indole-2,3-dione

A mixture of 8.8 g (0.073 mole) of hydroxyacetophenone, 3.0 g (0.100 mole) of para formaldehyde, 8.0 g(0.044 mole) of chloroisatin and 10 cc(15ml) of absolute alcohol was heated on a steam-bath for 45 minutes. The mixture was cooled and the precipitated material (4.7 g) recrystallized from ethyl acetate.

Synthesis of 5-chloro-1-[3-(4-methylphenyl)-3-oxopropyl]-1*H*-indole-2,3-dione

A mixture of 8.8 g(0.073 mole) of methyl acetophenone, 3.0 g(0.100 mole) of paraformaldehyde, 8.0 g(0.044 mole) of chloroisatin and 10 cc(15ml) of absolute alcohol was heated on a steam-bath for 45 minutes. The mixture was cooled and the precipitated material (4.7 g) recrystallized from ethyl acetate.

Synthesis of 5-chloro-1-[3-(4-di hydroxyphenyl)-3-oxopropyl]-1*H*-indole-2,3-dione

A mixture of 8.8 g (0.073 mole) ofdihydroxyacetophenone, 3.0 g(0.100 mole) of para formaldehyde, 8.0 g(0.044 mole) of chloroisatin and 10 cc(15ml) of absolute alcohol was heated on a steam-bath for 45 minutes. The mixture was cooled and the precipitated material (4.7 g) recrystallizedethylacetatel.

PHASE III

MAO-A enzyme isolation and activity.

MAO a flavoenzyme of the mitochondrial outer membrane, plays an important role in the regulation of the metabolism of exogenous amines, the level of neurotransmitters (such as dopamine, norepinephrine and serotonin) and intracellular amine stores. MAO exists as two isoforms (MAO-A and MAO-B), according to their substrate specificity and sensitivity to specific inhibitors. MAO-A preferentially oxidizes norepinephrine (NE) and serotonin(5-HT).

PROCEDURE

Tissue preparation

Male Wistar rats weighing 150–250 g are sacrificed and the brains rapidly were removed. Whole brain minus cerebellum is homogenized in 9 volumes of ice-cold, phosphate-buffered 0.25 M sucrose, using a Potter-Elvejhem homogenizer. The homogenate is centrifuged at 1000 g for 10 min and the supernatant decanted and recentrifuged at 18000 g for 20 min. The resulting pellet (P2) is resuspended in fresh 0.25 M sucrose and recentrifuged at 18000 g for 20 min. The washed pellet is resuspended in the original volume of 0.25 M sucrose and serves as the tissue source for mitochondrial monoamine oxidase.

Direct spectrophotometry

The activities of MAO-A were determined by UV-Vis spectrophotometry. Briefly, 800 ml of a solution containing potassium phosphate buffer (pH 7.60, 100 mM), 200 ml of MAO protein homogenates 5- HT (for detecting MAO-A) was incubated for 20 min at 37 ^{o}C. The reaction was started by adding 100 mM buffer and 200 ml of 0.02 M 5-HT, and incubated for 60 min. The reaction was stopped by adding 200 ml of 10% perchloric acid or 200 ml of 2MHCl. N-butyl acetate (for detecting MAO-A) was added to the reaction solution; and the tubes were vortexed for 3 min. Following centrifugation at 10,625 g for 6 min, absorbance was determined using a spectrophotometer at 280 nm MAO-A.

RESULT AND DISCUSSION

Phase I

LEAD OPTIMIZATION

Lead optimization is done by evaluating the,

- Drug likeliness score
- Pre ADMET

a) Drug likeliness

For the better oral absorption of the ligands the drug likeliness scores are constructed by getting information about solubility, diffusion, Log P, molecular weight etc. One of the ideal methods for this is using Lipinski's rule of five with the molinspiration server.

The lead optimization of the ligands shows that none of the derivatives had any violations of the rule. These compounds when administered orally will more likely have a good absorption or permeation.

Lipink's rule of five for isatin derivatives

Table 3: Drug likeliness scores of IS1-IS5,NIS1-NIS5,CIS1-CIS5 ligands

SNo.	Compound	miLog P	TPSA	Natom	Mol.wt	nON	nOHNH	n.violation	n.rotb
01	IS1	2.37	65.38	23	309.32	5	0	0	5
02	IS2	2.99	56.15	22	313.74	4	0	0	4
03	IS3	1.84	76.38	22	295.29	5	1	0	5
04	IS4	2.77	56.15	22	293.32	4	0	0	4
05	IS5	1.29	96.60	23	311.29	6	2	0	4
06	NIS1	2.31	111.20	26	354.32	8	0	0	6
07	NIS2	2.93	101.97	25	358.74	7	0	0	5
08	NIS3	1.77	122.20	25	340.29	8	1	0	5
09	NIS4	2.70	101.97	25	338.32	7	0	0	5
10	NIS5	1.22	142.43	26	356.29	9	2	0	5
11	CIS1	3.03	65.38	24	343.77	5	0	0	5
12	CIS2	3.65	56.15	23	348.19	4	0	0	4
13	CIS3	2.49	76.38	23	39.74	5	1	0	4
14	CIS4	3.42	56.15	23	327.77	4	0	0	4
15	CIS5	1.94	96.60	24	345.74	6	2	0	4

Table 4 :Preadmet properties of IS1-IS5

Compounds	IS1	IS2	IS3	1S 4	IS5
BBB	0.689955	1.33882	0.19238	2.16322	0.094
Buffer solubility mg/L	446.448	139.161	37.329	164.218	70.9894
$CaCO_2$	44.889	28.6593	18.817	24.634	10.9042
CYP2C19 inhibition	Non	Non	Non	Non	Non
CYP2C9 Inhibition	Non	Non	Non	Non	Non
HIA	97.58	97.47	93.55	97.840051	93.392033
MDCK	153.6	145.315	9.56087	186.22	4.91328
Plasma protein binding	89.98	93.145421	86.005445	87.203326	83.395846
solubility value	56.469	6.35956	45.8628	13.096	426.259
Skin permeability	-2.5174	-2.6972	-2.9308	-2.5774	-3.0456
SklogP value	3.1334	3.0625	2.217530	2.877480	1.659970

Table 5 :Preadmet properties of NIS1-NIS5

Compounds	NIS1	NIS2	NIS3	NIS4	NIS5
BBB	0.98929	1.33882	0.148974	2.08989	0.0938902
Buffer solubility mg/L	219.537	139.161	277.204	8.75173	70.9894
$CaCO_2$	29.1707	28.6593	19.43	22.1923	10.9042
CYP2C19 inhibition	Non	Non	Non	Inhibitor	Non
CYP2C9 Inhibition	Non	Non	Non	Inhibitor	Non
HIA	98.477173	97.472703	96.507268	97.699004	93.392033
MDCK	45.1166	145.315	78.4936	5.13796	4.91328
Plasma protein binding	86.562362	93.145421	84.267036	88.584353	83.395846
Pure water solubility value mg/L	30.1827	6.35956	127.33	0.430065	426.259
Skin permeability	-2.8067	-2.6972	-2.8283	-3.2687	-3.0456
SklogP value	2.347370	3.062570	1.967280	2.911380	1.659970

Table 6 :Preadmet properties of CIS1-CIS5

Compounds	CIS1	CIS2	CIS3	CIS4	CIS5
BBB	0.98929	1.33882	0.148974	2.16322	0.0938902
Buffer solubility mg/L	219.537	139.161	277.204	164.218	70.9894
$CaCO_2$	29.1707	28.6593	19.43	24.634	10.9042
CYP2C19 inhibition	Non	Non	Non	Non	Non
CYP2C9 Inhibition	Non	Non	Non	Non	Non
HIA	98.477173	97.472703	96.507268	97.840051	93.392033
MDCK	45.1166	145.315	78.4936	186.22	4.91328
Plasma protein binding	86.562362	93.145421	84.267036	87.203326	83.395846
Pure water solubility value mg/L	30.1827	6.35956	127.33	13.096	426.259
Skin permeability	-2.806	-2.697	-2.828	-2.577	-3.045
SklogP value	2.347370	3.062570	1.967280	2.877480	1.659970

DOCKING

The docking results of **2BXS** with ligands **IS1-IS5,NIS1-NIS5,CIS1-CIS5**is reported below. The binding sites and the active sites represented in the snapshots and the binding energy was found to be more when compared to the standard

Table 7:binding energies of **IS1-IS5,NIS1-NIS5,CIS1-CIS5** ligands with **MAO-A (2BXS)**

SL.NO	COMPOUND CODE	BINDING ENERGY (Kcal/mol)ΔG
01.	IS1	-8.76
02.	IS2	-8.15
03.	IS3	-8.52
04.	IS4	-8.17
05.	IS5	-8.76
06.	NIS1	-7.62
07.	NIS2	-8.56
08.	NIS3	-8.35
09.	NIS4	-7.65
10.	NIS5	-8.06
11.	CIS1	-8.91
12.	CIS2	-8.76
13.	CIS3	-7.62
14.	CIS4	-7.87
15.	CIS5	-8.06
16.	SELEGILINE	-6.49

IS1 Binding with MAO A(2BXS.PDB)

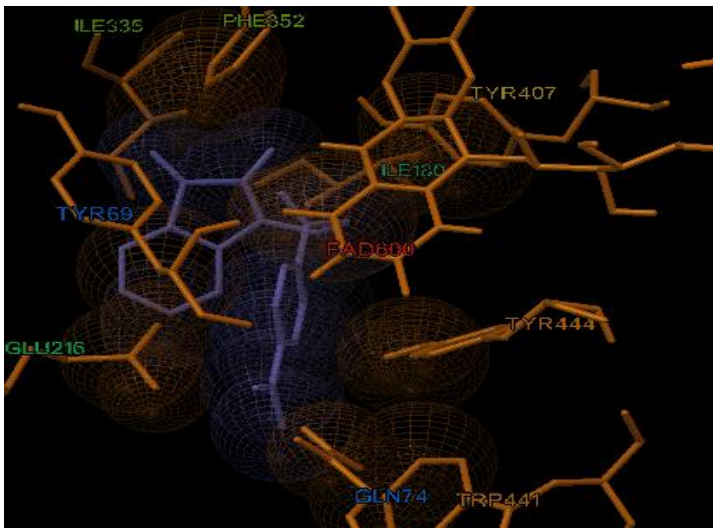


Fig.3: Binding energy = -8.76 kcal/mol

IS2 Binding with MAO A(2BXS.PDB)

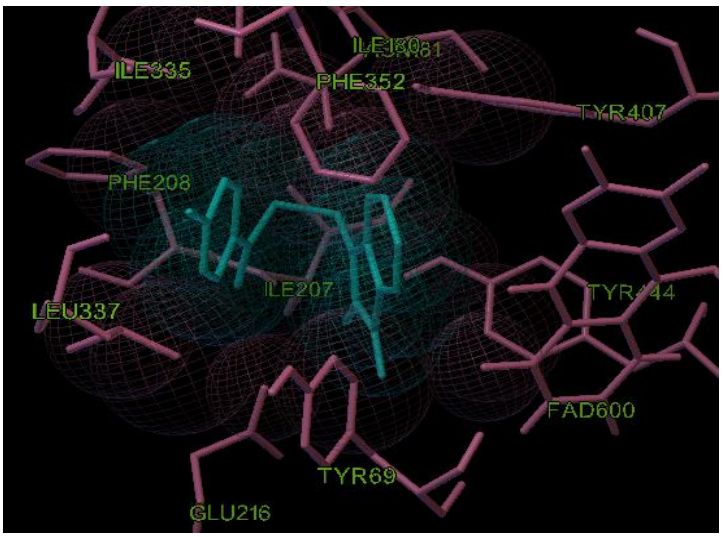


Fig.4: Binding energy = -8.15 kcal/mol

IS3Binding with MAO A(2BXS.PDB)

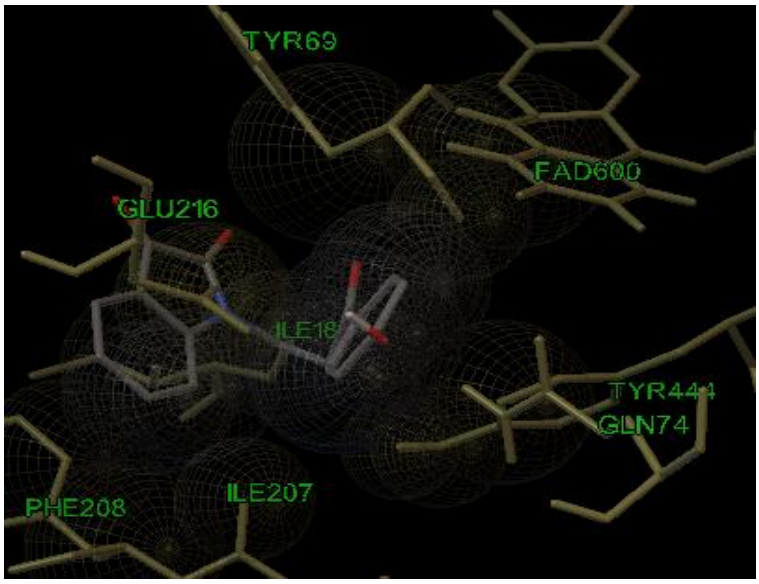


Fig.5: binding energy=-8.52 kcal/mol

IS4Binding with MAO A (2BXS.PDB)

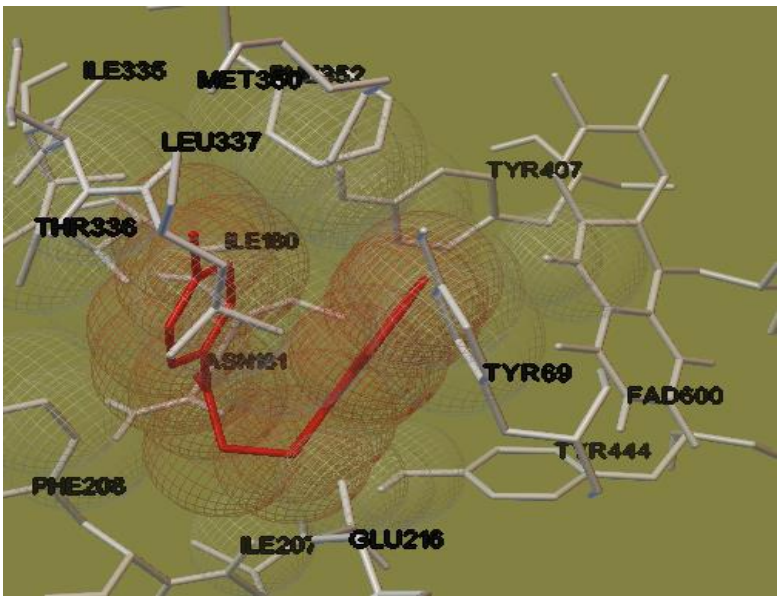


Fig.6: Binding energy= -8.17 kcal/mol

IS5 Binding with MAO A (2BXS.PDB)

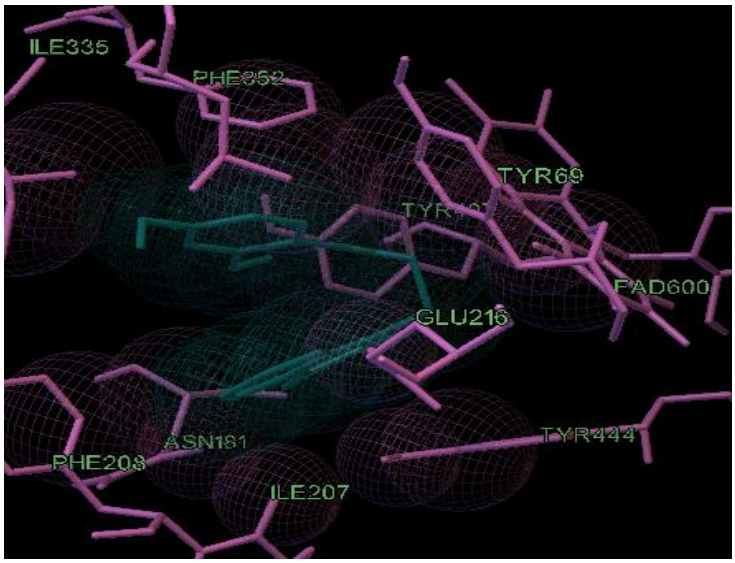


Fig.7: Binding energy= -8.76 kcal/mol

NIS1 Binding with MAO A (2BXS.PDB)

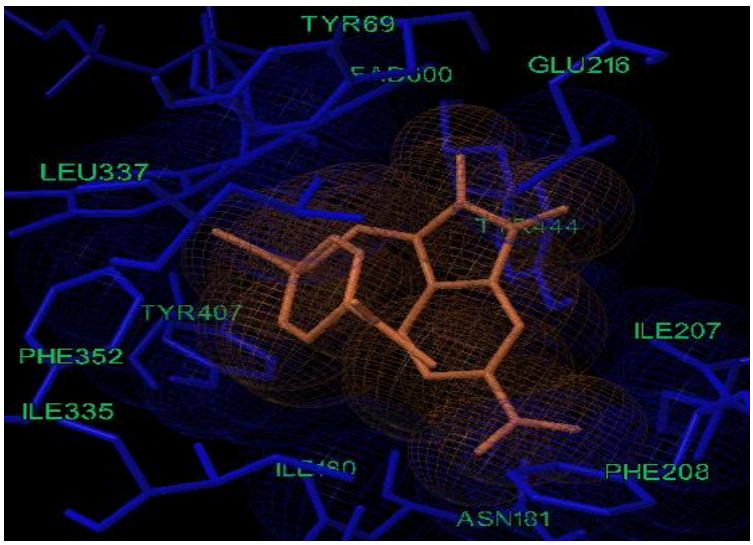


Fig.8: Binding energy = -7.62 kcal/mol

NIS2 Binding with MAO A (2BXS.PDB)

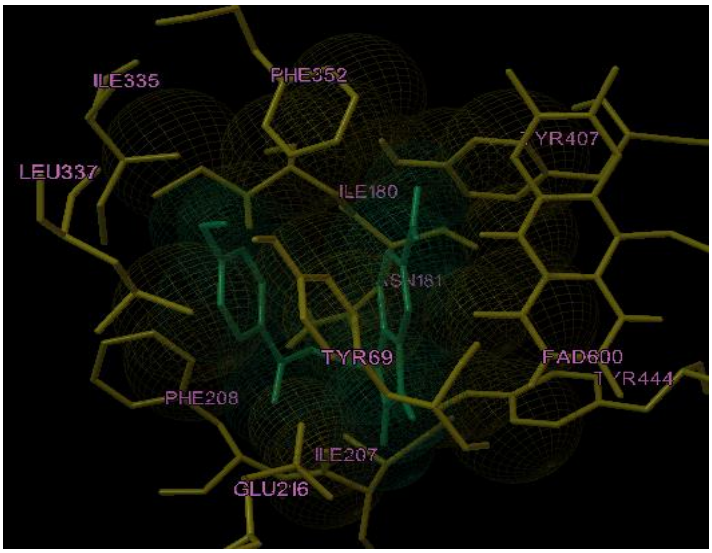


Fig.9: binding energy = -8.56 kcal/mol

NIS3 Binding with MAO (2BXS.PDB)

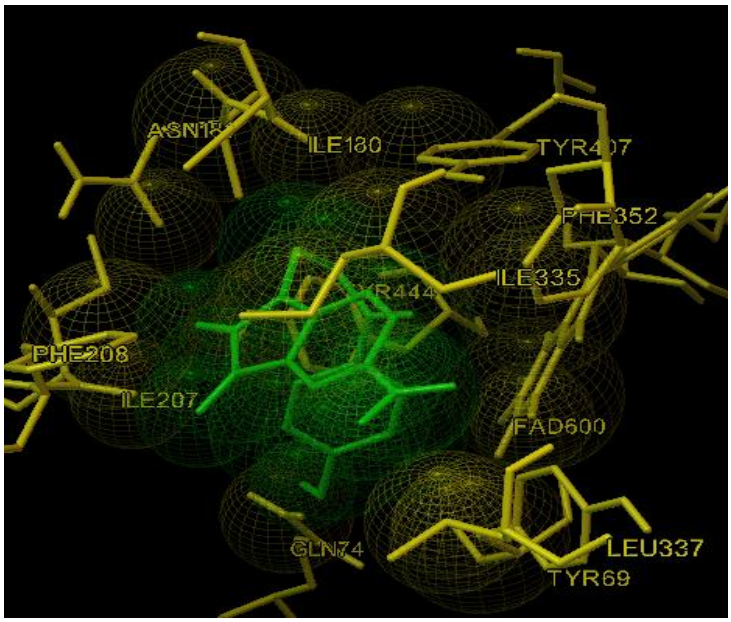


Fig.10: binding energy = -8.35 kcal/mol

NIS4Binding with MAO (2BXS.PDB)

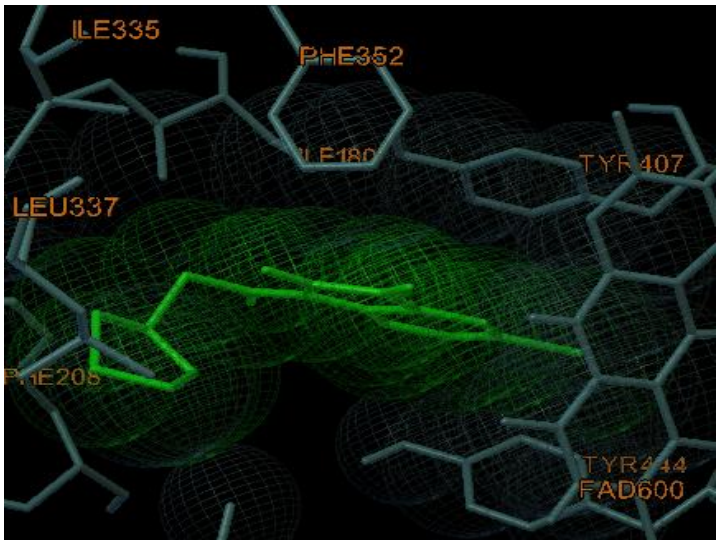


Fig.11: binding energy = -7.65 kcal/mol

NIS5Binding with MAO (2BXS.PDB)

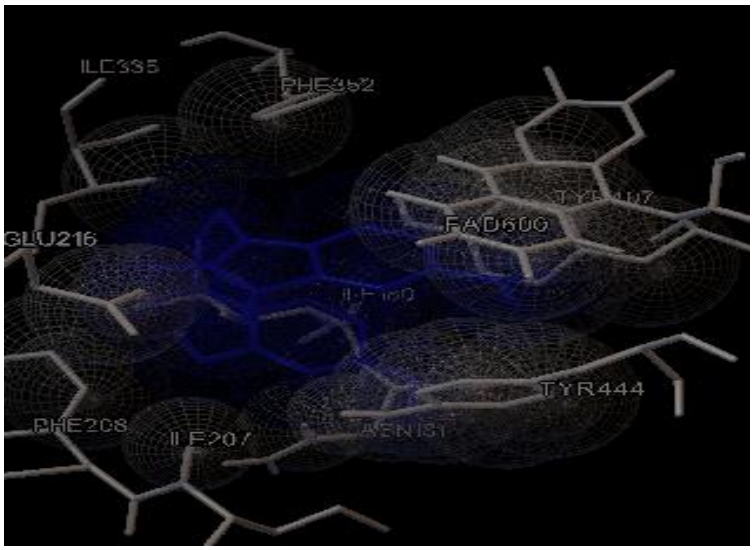


Fig.12: binding energy = -8.06kcal/mol.

CIS1Binding with MAO (2BXS.PDB)

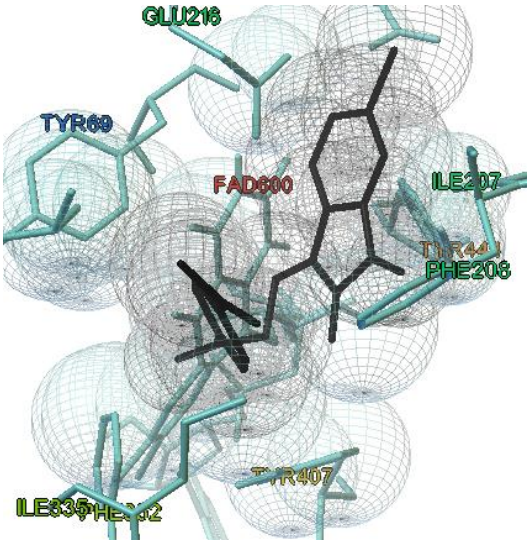


Fig.13: binding energy = -8.91 kcal/mol

CIS2Binding with MAO (2BXS.PDB)

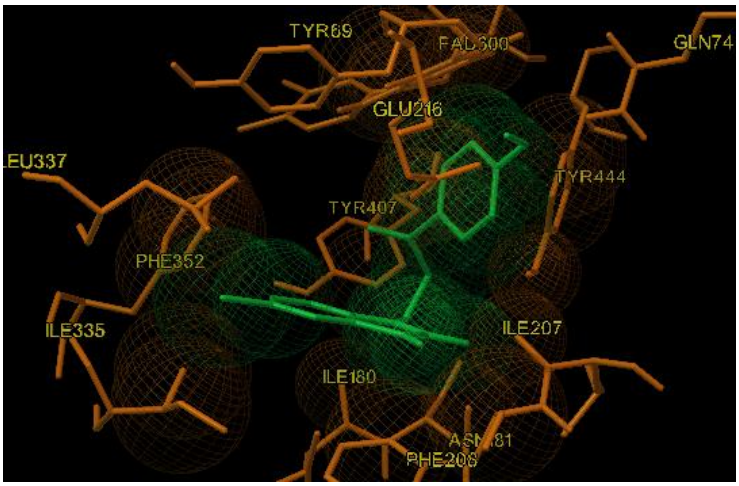


Fig.14: Binding energy= -8.76kcal/mol

CIS3Binding with MAO (2BXS.PDB)

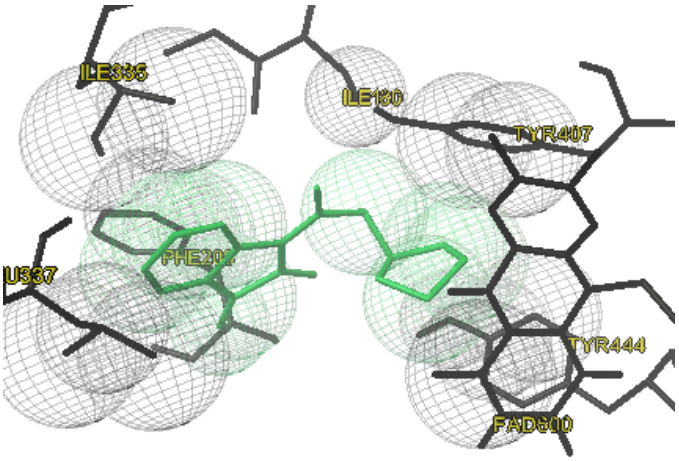


Fig.15: Binding energy= -7.62kcal/mol

CIS4 Bindingwith MAO (2BXS.PDB)

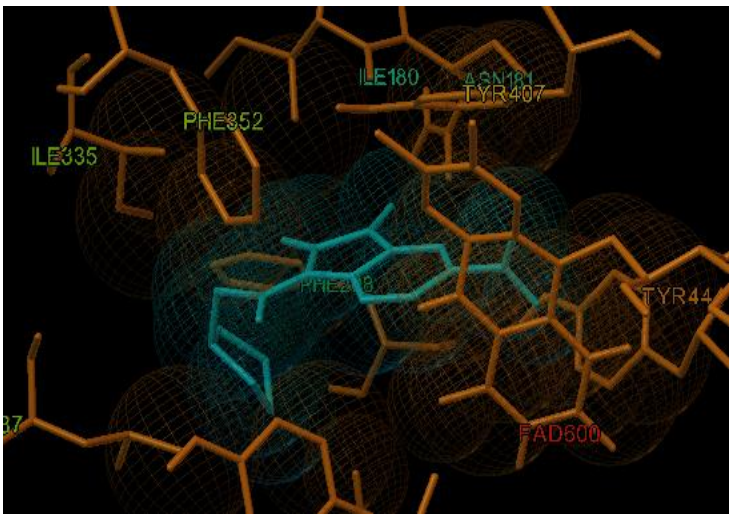


Fig.16: binding energy = -7.87kcal/mol

CIS5Binding with MAO (2BXS.PDB)

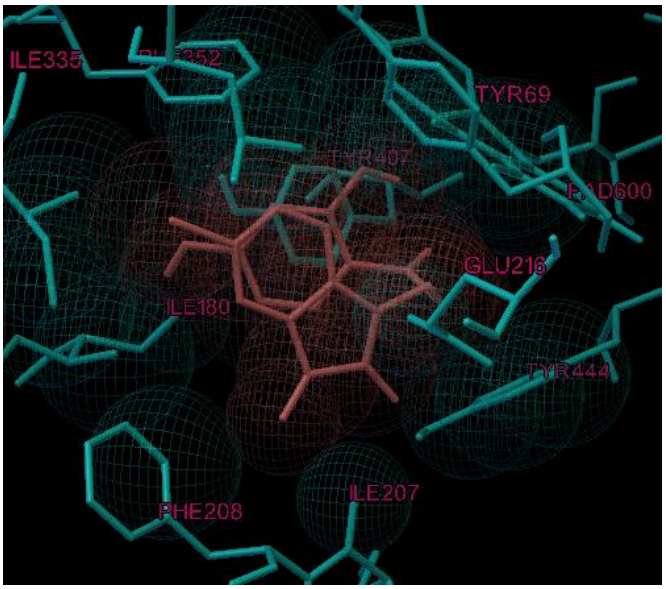


Fig.17:binding energy = -8.06kcal/mol

Selegiline Binding with MAO (2BXS.PDB)

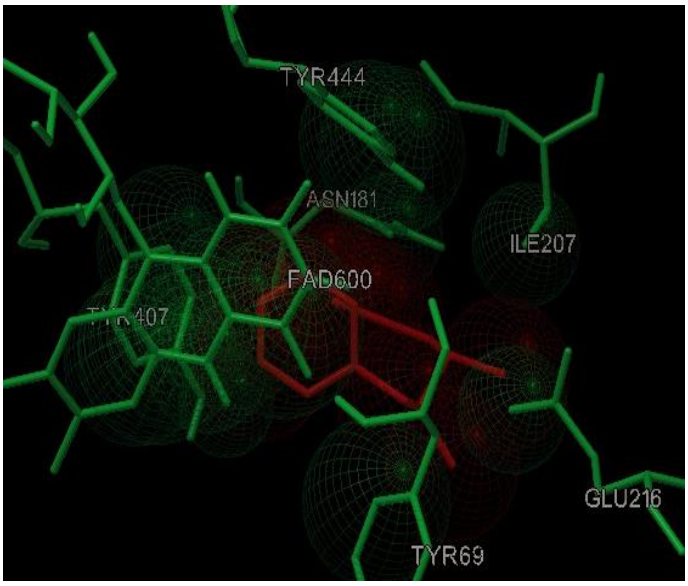


Fig.18: binding energy= -6.49kcal/mol.

All the ligands IS1-IS5, NIS1-NIS5,CIS1-CIS5showed excellent binding interactions with the MAO(2BXS.PDB). Among the isatin derivatives **CIS1,CIS2,IS5,IS1** has shown the highest binding energies **-8.91,-8.79**, **-8.76,-8.76 kcal/mol** when compared to the standard **selegiline**shown the binding energy of **-6.49 kcal/mol.**

RESULTS AND DISCUSSION

All the fifteen new compound(**IS1-IS5, NIS1-NIS5, CIS1-CIS5**) obtained through three schemes were prepared in good yield and their physical (melting point and TLC) and spectral (UV, IR, Mass,NMR)data were evaluated.

Recrystallisationsolvent: Ethyl acetate

Solvent system: chloroform: benzene (1:1)

Visualizing agent: Iodine vapour

Physical characterization

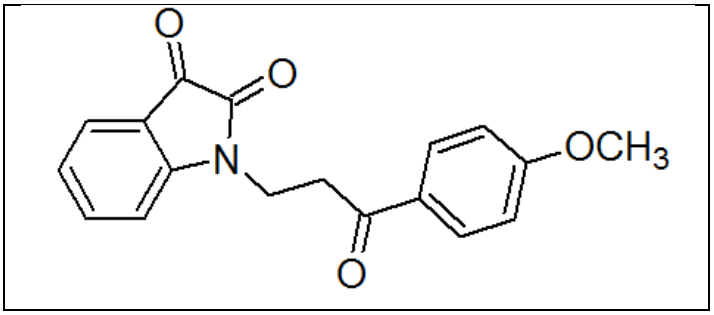
Table 8: Physical characterization of isatin derivatives **IS1-IS5,NIS1-NIS5,CIS1-CIS5**)

Sl no.	Comp Code.	R1	R2	R3	Molecular formula	Molecular weight	% Yield	Melting point °C	Rf value
1	IS1	H	OCH_3	H	$C_{18}H_{15}O_4N$	309.32	79	239.2	0.62
2	IS2	H	Cl	H	$C_{17}H_{12}O_3N_1Cl$	313.74	80	240.6	0.81
3	IS3	H	OH	H	$C_{17}H_{13}O_4N$	295.29	75	241.1	0.79
4	IS4	H	CH_3	H	$C_{18}H_{15}O_3N$	293.32	70	242.5	0.66
5	IS5	OH	H	OH	$C_{17}H_{13}O_4N$	311.29	81	240.4	0.85
6	CIS1	H	OCH_3	H	$C_{18}H_{14}O_4N_1Cl$	354.32	76	238.2	0.65
7	CIS2	H	Cl	H	$C_{17}H_{11}O_3N_1Cl_2$	358.74	73	241.6	0.83
8	CIS3	H	OH	H	$C_{17}H_{12}O_4N_1Cl$	340.29	76	242.8	0.73
9	CIS4	H	CH_3	H	$C_{18}H_{14}O_3N_1Cl$	338.32	71	240.3	0.87
10	CIS5	OH	H	OH	$C_{17}H_{12}O_4N_1Cl$	356.29	83	245.5	0.62
11	NIS1	H	OCH_3	H	$C_{18}H_{14}O_6N_2$	343.77	77	240.4	0.74
12	NIS2	H	Cl	H	$C_{17}H_{11}O_5N_2Cl$	348.19	69	243.4	0.82
13	NIS3	H	OH	H	$C_{17}H_{12}O_6N_2$	39.74	80	241.6	0.80
14	NIS4	H	CH_3	H	$C_{18}H_{15}O_5N_2$	327.77	67	240.8	0.78
15	NIS5	OH	H	OH	$C_{17}H_{13}O_6N_2$	345.74	80	242.6	0.75

SPECTRAL DATA

The structures of compounds synthesized during the present study were established on the basis of the physical data, IR, UV, NMR, and MASS spectral data.The purity of the compounds was established by single spot on TLC plates.

Compound code: IS1



Chemical name : 1-[3-(4-methoxyphenyl)-3-oxopropyl]-1*H*-indole-2,3-dione

UV Spectrum

Solvent used : acetone

λ_{max} : 346nm

IR (KBr, νmax in cm-1) : C=O 1750, benzene 1515, C-H bending 2051, C=C stretching 1620, C-N 1279.

(Align properly)

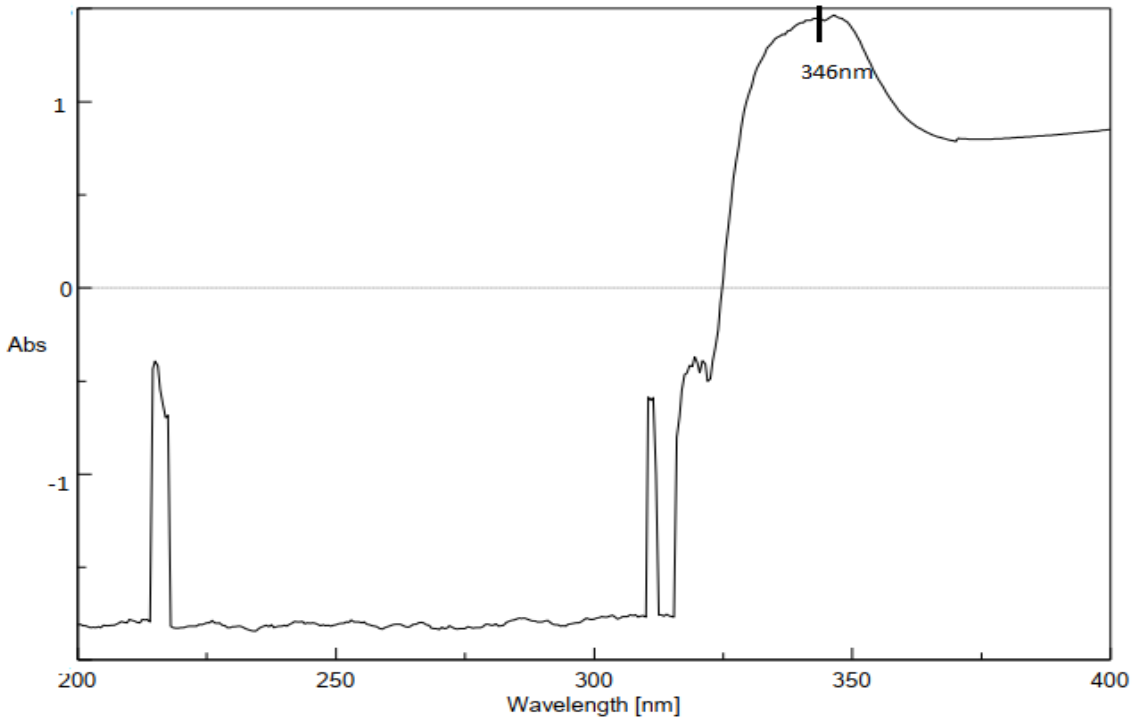


Fig .18: UV spectrum of IS1

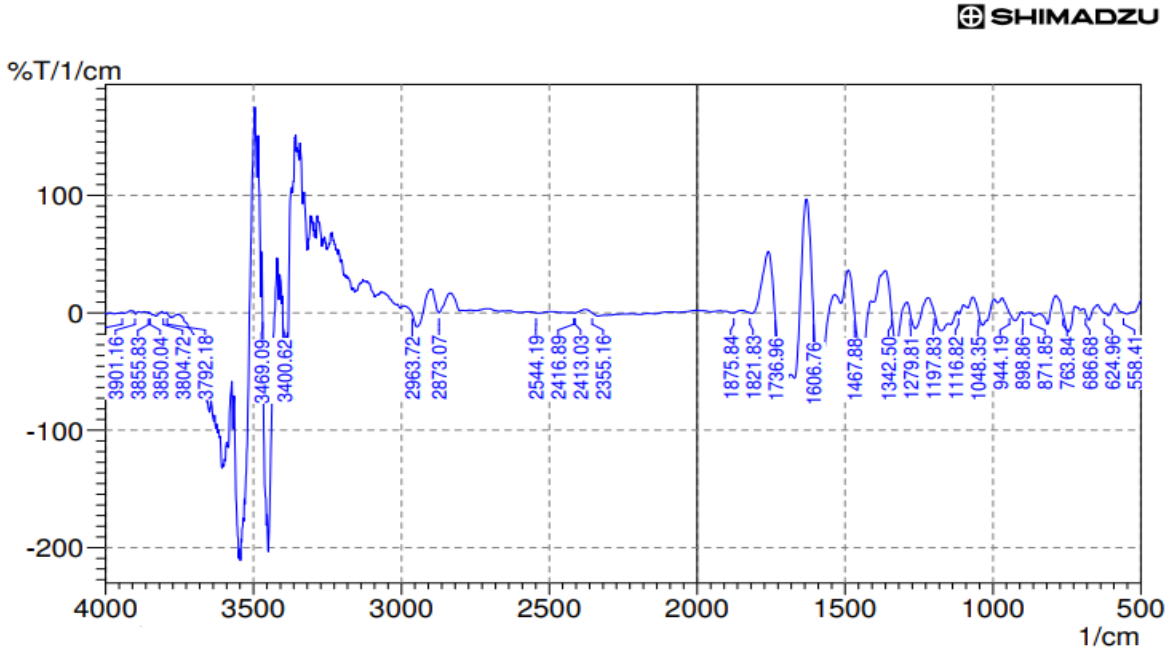
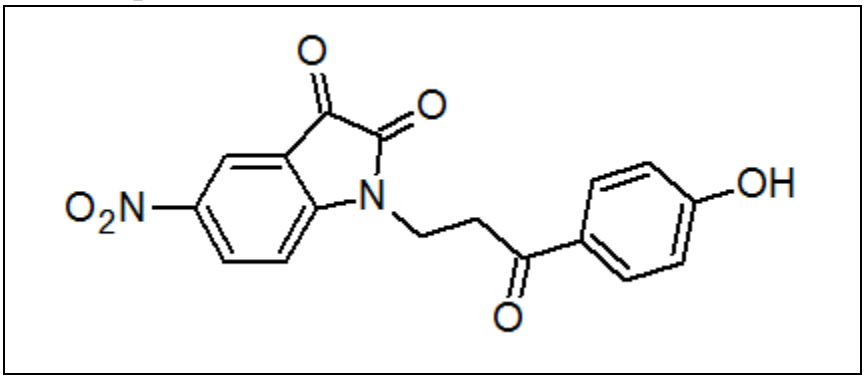


Fig .19: IR spectrum of IS1

Compound code: NIS3



Chemical name : 1-[3-(4-hydroxyphenyl)-3-oxopropyl]-5-nitro-1*H*-indole-2,3-dione

UV Spectrum

Solvent used : acetone

λ_{max} : 330nm

IR (KBr, νmax in cm-1) : NO_2 1331, C=O 1750, benzene 1515, C-H 2051, C=C 1620

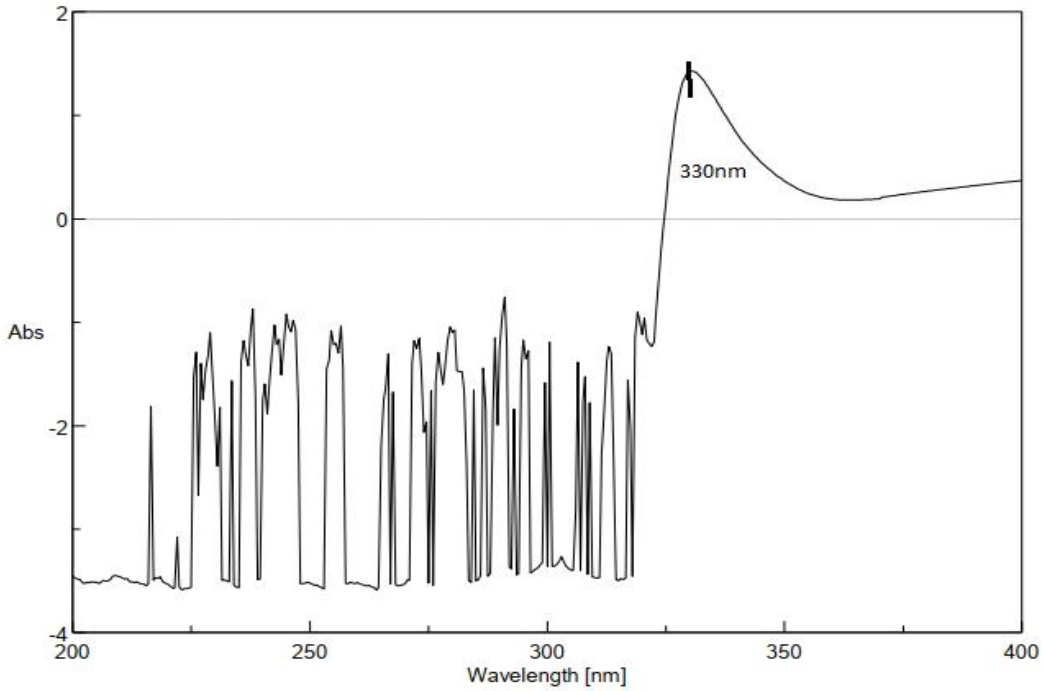


Fig .20: UV spectrum of NIS3

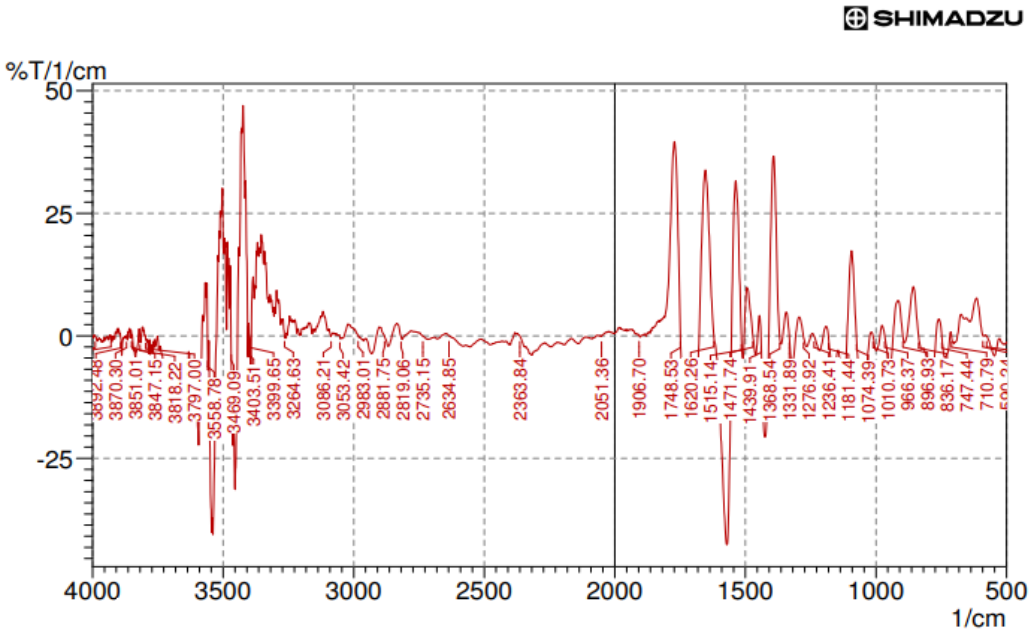
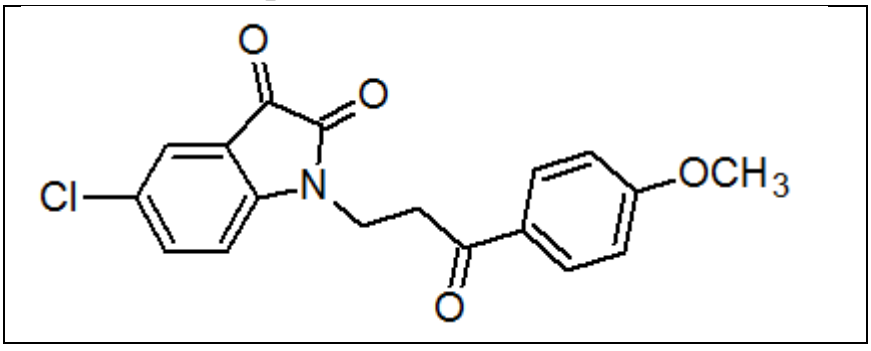


Fig.21: IR spectrum of NIS3

Compound code: CIS3



Chemical name :5-chloro-1-[3-(4-methoxyphenyl)-3-oxopropyl]-1*H*-indole-2,3-dione

UV Spectrum

Solvent used : acetone

λ_{max} : 330nm

IR (KBr, νmax in cm-1) : C=O 1750, benzene 1515, C-H 2051, C=C 1620
C-Cl strecting 765.

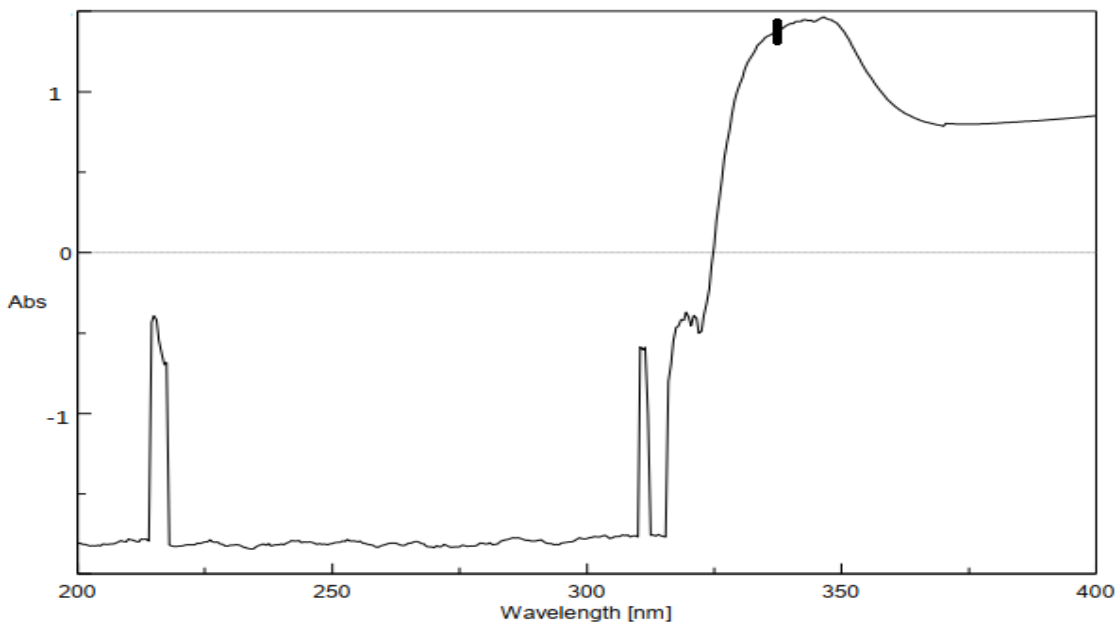


Fig.22: UV spectrum of CIS1

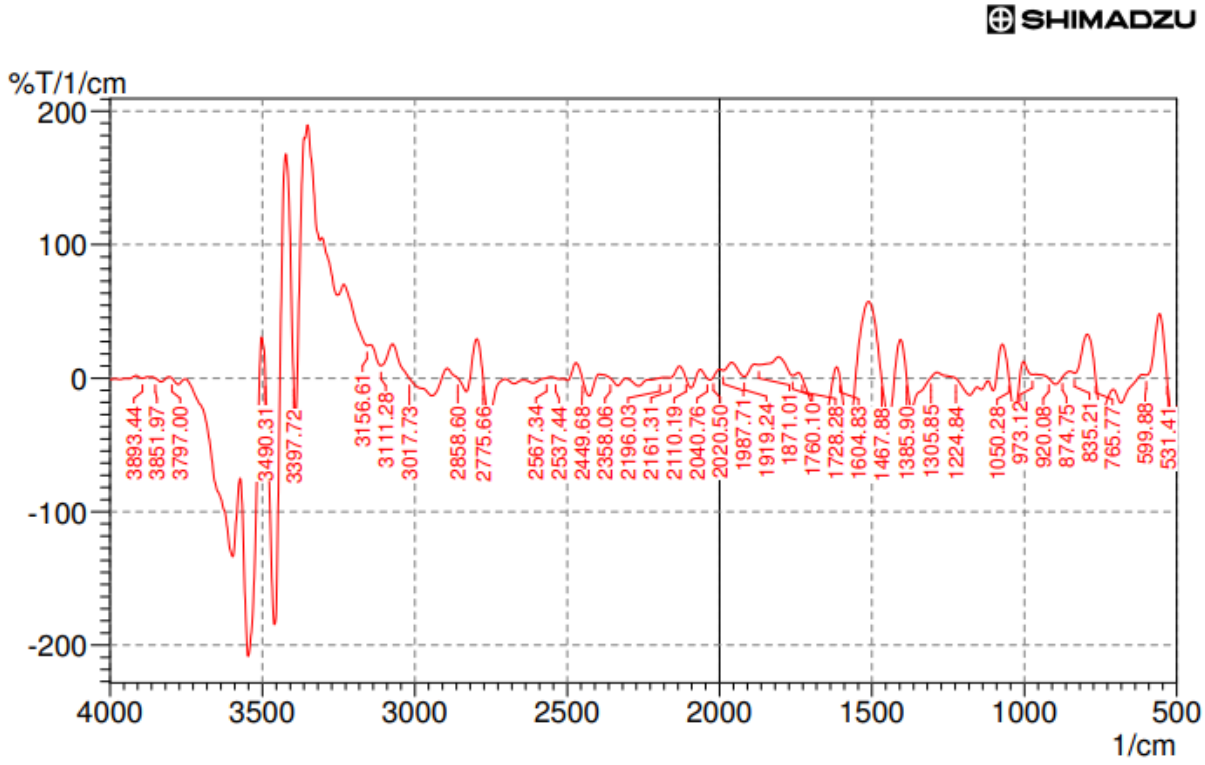


Fig .23:IR spectrum of CIS1

*INVITRO*ENZYME INHIBITORY STUDY

Invitro MAO-A inhibitory activity using isatin derivatives and standard

MAO activity has been determined by measurement of oxygenconsumption and the concentration of hydrogen peroxide (H_2O_2) oran oxidized monoamine product based on their catalytic oxidativedeamination of monoamines.

$$RCH_2NR_1R_2+H_2O+O \longrightarrow RCHO+NR_1R_2+H_2O_2$$

The measurement of oxygen consumption by an oxygensensitive electrode requires specialized equipment, and is notappropriate for the rapid measurement of a large numbers ofsamples. The H_2O_2 method is insensitive and not specific for MAO, because most biological and synthetic compounds alsoabsorb at 230 nm. Additionally, complicated cellular processescould influence the concentration of H_2O_2. Aldehydes,which are oxidized monoamine products, are produced by the incubation of amine substrates with MAO and these can be detectedby spectrophotometry.

The enzyme inhibitory activity of the 9synthesised compound was evaluated by *invitro*procedure using monoamino oxidase A (MAO-A) enzyme extracted from rat brain.The results were tabulated as follows.

Table 9: MAO A inhibitory activity of different concentrations of isatin derivatives and standard selegline

Conc (µg/ml)	100	200	400	800	1600	IC_{50}
Percentage viability						
Selegline	29.26±0.47	33.70±0.36	43.27±0.377	50.13±0.30	65.34±0.73	780 µg/ml
IS1	35.71±0.39	44.03±0.06	54.24±0.93	66.69±0.66	89.17±0.24	330µg/ml
IS3	33.13±0.67	48.13±1.31	. 62.95±3.23	72.36±2.3	88.07±0.41	300µg/ml
IS4	27.54±0.66	40.82±0.66	46.24±0.72	65.21±0.64	73.25±3.37	550µg/ml
CIS1	47.65±0.48	54.54±4.853	62.72±1.71	71.21±2.93	77.17±0.65	160 µg/ml
CIS3	37.43±3.02	42.79±0.78	59.02±1.04	62.59±2.55	76.27±0.73	300 µg/ml
CIS4	27.49±0.29	43.24±0.51	49.23±0.198	67.93±0.64	77.80±0.59	450 µg/ml
NIS1	11.68±0.32	33.14±0.05	41.72±0.07	47.05±0.07	80.25±0.60	830 µg/ml
NIS3	32.25±0.61	45.0±0.66	49±0.69	49.89±0.67	75.47±0.08	450 µg/ml
NIS4	23.05±0.63	31.16±1.64	36.29 ±0.25	53.82±0.45	74.90±0.62	760µg/ml

The synthetic derivatives of isatin shows that MAO-A inhibitory activity at a varying concentrations (100,200,400,800,1600 µg/ml) there was dose dependent increase in the percentage inhibition for all the concentrations.

- The CIS1 at concentration 100 µg/ml showed 47.65 and for 1600 µg/ml it become 77.17 the IC50 value for CIS1 found to be 160 µg/ml.
- The IS3 at concentration 100 µg/ml showed 33.13 and for 1600 µg/ml it become 88.07 the IC50 value for IS3 found to be 300 µg/ml.
- The NIS3 at concentration 100 µg/ml showed 35.25 and for 1600 µg/ml it become75.47±0.08 the IC50 value for NIS3 found to be 450 µg/ml.
- The selegline at concentration 100 µg/ml showed 29.26±0.47and for 1600 µg/ml it become 65.34±0.73 the IC50 value for selegline found to be 780 µg/ml.

SUMMARY AND CONCLUSION

The present work was focused on the design, docking, synthesis and evaluation of anti-depressant activity of isatin derivatives as possible MAO-A inhibitors.

***In-silico* studies**

- **Selection of the target**

The enzyme involved in the anti-depressant was MAO-A was selected as the drug target of the study. The corresponding enzyme were obtained from the protein databank and their accession code were **2BXS.**

- **Lead optimization**

The fifteen modified ligand **IS1-IS5,CIS1-CIS5,NIS1-NIS5** were subjected to *in-silico*lead optimization. The ligand were optimized for evaluating oral bioavailability by utilizing the **Molinspiration server**. Lead optimization revealed that all the

- **Docking**

The optimized leads were subjected to docking studies using **Autodock 4.2** and the interactions of the derivatives with active sites of the enzyme were studied.The derivative were subjected to interactions with MAO-A. Selegline was used as the standard ligand. Most of the derivatives were interacting with the key active sites of the **MAO-A(2BXS)**with superior dock values or binding energies when compared to the **standard (selegline)**.

Bindingsites Arg51A,Cys406A,Tyr407A, Gly66A, Ala44A, Ile273A, Gly67A and FAD.

Compound	BINDING ENERGIES
IS1	-8.76 kcal/mol
NIS3	-8.35 kcal/mol
CIS1	-8.91 kcal/mol
Selegline	-6.46 kcal/mol

Synthesis

In this present work total of fifteen newisatin derivatives were synthesized.

Scheme 1

In this scheme five compounds were synthesized by treating isatin with corresponding acetophenone and with para formaldehyde in the presence of ethanol to give the product

Scheme 2

In this scheme five compounds were synthesized by treating nitro isatin with corresponding acetophenone and with para formaldehyde in the presence of ethanol to give the product

Scheme 3

In this scheme five compounds were synthesized by treating chloroisatin with corresponding acetophenone and with para formaldehyde in the presence of ethanol to give the product

Characterization

Melting point of all the newly synthesized compound were determined. Rf values were determined by fixing various suitable solvent system on pre- coated silicagel-G. The solvent system used was chloroform: benzene (1:1).

Enzyme Inhibitory activity

Anti-depressant activity

The representative compounds from isatin derivatives were screened for anti-depressant activity was performed by in-vitro anti-depressant assay technique by using MAO-A.

Compound	**IC50**
IS1	330 µg/ml
NIS3	450 µg/ml
CIS1	160 µg/ml
Selegline	780 µg/ml

CONCLUSION

- The present studyestablishes that computational tools help in minimizing the tedious process in drug discovery.
- Molinspiration was utilized for filtering the compounds and selecting the lead compounds.
- The binding energy obtained from docking study further confirmed the possibility of the affinity of the selected leads towards the enzyme MAO- A.
- Using the schemes various isatin derivatives were synthesized with good yield.
- Structure of the synthesized compounds were confirmed by Melting point, TLC, UV, IR, NMR and MASS spectra.
- Among the compounds screened for antidepressant activity CIS1 showed the highest activity.

Thus, the present study depicts that the utilization of computer aided drug design is an effective tool in predicting the effectiveness of a series of isatin derivative compounds under study and thus can result in the design of potent antidepressant agents.

BIBLIOGRAPHY

1.) Rajasree G Pai*Et Al.,* 2016. An Endogenous Heterocyclic Compound Isatin, Research Journal Of Pharmaceutical, Biological And Chemical Sciences, Volume 7, Issue 6, November – December, Page Number : 107

2.) SachinA Pishawikar* And Harinath 2013, Synthesis Of Mannish Bases Of Thiosemicarbazide As DNA Polymerase Inhibitors And Novel Antibacterial Agents, International Journal Of Pharma And Bio Sciences, Volume 4, Issue 1, January, Page Number: 547 – 556

3.) R.P. Gupta *, N.L. Narayana1995, Synthesis Of Some MannichBases Of L-Cyclohexylidene-N(1,2-Dihydro-2-Oxo-3H-Indol-3-Ylidene) Thiosemicarbazones And Their Antibacterial Activity, Pharmaceutics Acta Helvetiae, Volume 72, Issue 1997, November, Page Number : 43-45.

4.) Rojendro S. VormaOndW. Lewis Nobles1966, Synthesis OfIsatin- N-Mannich Bases, Volume 3, December, Page Number 462 – 465.

5.) Roy W. Daisleyx, Vasanti K. Shah 1983, Synthesis And Antibacterial Activity Of Some 5-Nitro-3-Phenyliminoindol-2(3H)-Ones And Their N-Mannich Bases, Journal Of Pharmaceutical Sciences I 407 Vol. 73. Issue 3, March ,Page Number : 407.

6.) Anne Beauchard*Et Al.,* 2006, Synthesis Of Novel 5-Substituted Indirubins As Protein Kinases Inhibitors, Bioorganic &Medicinal Chemistry 14 , Page Number : 6434–6443

7.) Razieh Moradi*Et Al.*,2017, Recent Applications OfIsatin In The Synthesis Of Organic Compounds, The Free Internet Journal For Organic Chemistry, Part 1, October, Page Number: 148 – 201

8.) I. P. Ashmarin, 2006, Induction OfAutoimmunity Against Endogenous NeuroregulatorsIsatinAnd Cholecystokinin As A Method Of Modeling And Correction Of Depressive Behavior, Neurochemical Journal, Vol. 1, Issue 2, January, Page Number: 133–137.

9.) Andrei V*Et Al.,* 2016, New N-MannichBases Obtained From Isatin And Piperazine Derivatives: The Synthesis And Evaluation Of Antimicrobial Activity, Volume 52, Issue 1, January, Page Number : 25 – 30.

10.) H. Cecil Caldwellt W.Lewis Nobles, 1955, Mannich Bases From 5-Nitro-2-Acetylfuran And 5 -Nitro-2 -Furfuralacetone, May, Page Number : 273 -276.

11.) Joaquim F. M. Da Silva*Et Al.,* 2001, The Chemistry Of Isatins: A Review From 1975 To 1999, The Journal Of The Brazilian Chemical Society, Volume 12, Issue 3, Page Number : 273 -324.

12.) Gabriela Fonseca Mendonça*Et Al.,* 2005, Trichloroisocyanuric Acid In H2 SO4 : An Efficient Superelectrophilic Reagent For Chlorination Of IsatinAnd Benzene Derivatives, The Journal Of The Brazilian Chemical Society, Volume 16, Issue 4, Page Number :695 -698.

13.) N. M. Ribeiro*Et Al.,* 2013, Organic Preparations AndProcedures International: The New Journal For Organic Synthesis, ORGANIC PREPARATIONS AND PROCEDURES INT, Volume 37, Issue 3, May, Page Number: 265 – 303.

14.) YukioKitagawa, 1994, A Simple Method ForUV Spectrophotometric Assay Of Serotonin Oxidation By Mitochondrial Monoamine Oxidase From Hog Kidney Cortex, Journal Of Taibah University And Medical Sciences, Volume 6, Issue 1, June, Page Number 75 – 83.

15.) Guili Huang*Et Al.,* 2016, A Spectrophotometric Assay For Monoamine Oxidase Activity With 2, 4-Dinitrophenylhydrazine As A Derivatized Reagent, Analytical Biochemistry, August, Page Number : 18 – 25.

16.) SoheilaKashanian*Et Al.,* 2010, Spectroscopic Studies On The Interaction Of IsatinWith Calf Thymus DNA, DNA And Cell Biology, Volume 29, Issue 10, Page Number : 639 – 646.

17.) Sourabh Bhardwaj*Et Al.*, 2010, Synthesis, Characterization And Antimicrobial Activity Of Schiff Bases Of Isatin And Isatin Derivatives, Journal Of Pharmacy Research, Volume 3, Issue 12, September, Page Number : 2983 – 2985.

18.) Kravitz Ford, 2008, Introduction: Chronic Medical Conditions And Depression—The View From Primary Care, The American Journal Of Medicine, Volume 121, Issue 118, November, Page Number : 52 -58.

19.) F. F. Blicke J. H. Burckhalt, 1942, The Preparation Of 0-Keto Amines By The Mannich Reaction, Volume 64, October, Page Number : 451 – 453.

20.) Moussa B. H. Youdim*Et Al.*, 2006, The Therapeutic Potential Of Monoamine Oxidase Inhibitors, Neuroscience, Volume 7, April, Page Number : 295 -309.

21.) Calvery, Noller Adams, 1925, Arsonophenyl-Cinchoninic Acid (Arsonocinchophen) And Derivatives. I1, Volume 47, December, Page Number : 3058 – 3060.

22.) Monika Raj*Et Al.*,2010, Highly Enantioselective Synthesis Of 3-Cycloalkanone-3-Hydroxy-2-Oxindoles, Potential Anticonvulsants, Volume 51, February, Page Number : 2157 – 2159.

23.) Marina O. Shibinskaya*Et Al.*, 2010, Synthesis, Cytotoxicity, Antiviral Activity And Interferon Inducing Ability Of 6-(2-Aminoethyl)-6H-Indolo[2,3-B]Quinoxalines, European Journal Of Medicinal Chemistry, Volume 45, December, Page Number: 1237 – 1243.

24.) Ny Sin*Et Al.*, 2009, Respiratory Syncytial Virus Fusion Inhibitors. Part 7: Structure–Activity Relationships Associated With A Series Of Isatin Oximes That Demonstrate Antiviral Activity *In Vivo*,Bioorganic &Medicinal Chemistry Letters, Volume 19, June, Page Number : 4857 - 4862.

25.) V. Raja Solomon*Et Al.*, 2009, Hybrid Pharmacophore Design And Synthesis Of Isatin–Benzothiazole Analogs For Their Anti-Breast Cancer

Activity, Bioorganic &Medicinal Chemistry, Volume 17, September, Page Number : 7585 -7592.

26.) Seshaiah Krishnan Sridhar*Et Al.*, 2002, A Nticonvulsant Activity Of Hydrazones, Schiff And MannichBases Of Isatin Derivatives, European Journal Of Pharmaceutical Sciences, Volume 16, May, Page Number : 129 – 132.

27.) Kara L. Vine*Et Al.*, 2007, In Vitro Cytotoxicity Evaluation Of Some Substituted Isatin Derivatives, Bioorganic &Medicinal Chemistry, Volume 15, October, Page Number : 931 – 38.

28.) Xinghua Zhen*Et Al.*, 2015, Synthesis, Potential Anticonvulsant And Antidepressant Effects Of 2-(5-Methyl-2, 3-Dioxoindolin-1-Yl)Acetamide Derivatives, Acta PharmaceuticaSinica B, January.

29.) (https://www.molinspiration.com/)

30.) (https://preadmet.bmdrc.kr/)

31.) (https://www.rcsb.org/)

32.) (https://cactus.nci.nih.gov/translate/)

33.) (http://autodock.scripps.edu/)

34.) (http://mgltools.scripps.edu/)

35.) (https://cygwin.com/install.html)

36.) (https://www.acdlabs.com/resources/freeware/chemsketch/)