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Comparative Molecular Docking Of Xanthine Oxidases: In Silico Study of Inhibition of Xanthine Oxidase by Synthetic (2nap1-2nap5) Series As Drug Compound In Parkinson's Disease

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Abstract: ***ROS (Reactive Oxygen Species) production via oxidative stress leads to selective neuronal degeneration and finally results in neurodegenerative diseases. PD (Parkinson's disease) is one of the second most common neurodegenerative disease caused by overproduction of ROS due to the activity of xanthine oxidase. XO activity inhibitors may prove to be promising antiparkinson agents. Present investigation describes the synthesis and characterization of fluoro substituted pyrazolyl pyrazoline derivatives using spectrometer and 1HNMR spectra. Molecular docking studies of NAP1-NAP5 derivatives were performed in silico using molecular docking software. The resulting dock score in terms of D score has been compared with the standard XO inhibitor i.e. Allopurinol and Oxypurinol.***

The in silico approach used to study the Drug and Enzyme interaction using docking mechanism. Results indicated that the NAP derivatives accounted for better region specificity towards XO. It shows the strong binding interaction of ligand and receptor calculated on the basis of D score; hence the D score shows highest affinity. Overall score tabulation for D score as 2NAP1: -101.78; 2NAP2: -118.32; 2NAP3: -99.921; 2NAP4: -105.22; 2NAP5: -101.56; accordingly with the comparison of standard ligand molecule i.e. Allopurinol: -37.016 and Oxypurinol: -37.233.

Keywords: ROS, PTP, FT-IR spectrometer, 1HNMR, NAP, PEG-400, TLC etc.

Abbreviations: XO- Xanthine Oxidase; NAP- N Acetyl Pyrazole; HNMR- Nuclear Magnetic Resonance.

I. INTRODUCTION

Parkinson's disease is the second most common neurodegenerative disease characterized by progressive loss of dopaminergic neurons in the substantia nigra and aggregation of protein α- synuclein. Recently it has observed that, increase in deletions of mitochondrial DNA, results in formation of oxidative stress by producing ROS (Reactive oxygen species) [1].A combination of ROS production and mitochondrial Ca2+ initiates the opening of mitochondrial permeability transition pore (PTP), which allows translocation of proapoptotic molecules from the mitochondria to the cytosol, in order to trigger apoptotic cell death. [2, 3]. One of the major factor contributing overproduction of ROS is Xanthine oxidase catalysis. Xanthine oxidase (XO) is complex metalloflavoprotein that catalyses the conversion of hypoxanthine to xanthine and xanthine to uric acid with concomitant production of hydrogen peroxide and superoxide anions. The overproduction of ROS or superoxide anion generation by XO (Xanthine oxidase) results in cancer generation, ischemic reperfusion injury, aging and mutagenesis. Therefore, XO inhibitors along with free radical scavenging activity may prove to be promising agents to treat Parkinson's disease and hyperuricemia associated side effects. There are number of purine analogs which are used as XO inhibitor such as Allopurinol, 2-amino-4 hydroxy -6-hydoxymethyl pteridine [4], 6- amino purine [5], 2-chloromethyl amino purine and 3-hydroxy -1-nitrophenyl-1H pyrazolo [4, 3- C] pyridines [6] etc. The inhibitors of xanthine oxidase (oxidoreductase, EC:1:17.1.4, MMDB ID:58680, PDB ID:2E1Q) significantly suppressed *OH generation in rat striatum toxic model of Parkinson's Disease, induced by MPP+ suggesting a potential for xo in the oxidative stress associated with Parkinson's Disease [7]. In this Parkinson's disease treatment the use of purine analogs it leads to development of symptoms. Therefore there is need for the development of novel compounds with the better safety profiles that could be used to relive associated side effects. The formation of fluoro substituted pyrazolyl pyrazoline (2NAP1-2NAP5) (heterocyclic compound) possessing a broad spectrum of pharmacological activities [8] including antioxidant [9], anticancer, anti-inflammatory [10], antipyretic and Xanthine oxidase inhibitory [11]. Recently a review by Raj Kumar et al. has also extensively covered survey for Xanthine

oxidase inhibitors including non purine analogs [12]. Pyrazolines are well known and important nitrogen containing five member compounds. Several pyrazoline derivatives have been found to possess considerable biological activities which stimulated research activities in this field [13- 17]. Organic synthesis in PEG under low to moderate temperature condition is an area of significance in green organic synthesis. Considering these reports the development of new synthetic strategy for biologically active compounds, herein we report the synthesis of fluoro substituted N-acetyl pyrazoline derivative of pyrazole chalcones in PEG-400 [18-20]. Our consistent work on heterocyclic compound having potent biological activities prompted us to prepare a new series of fluoro substituted pyrazolyl pyrazolines (2NAP1-2NAP5) derivatives bearing different functional moieties and expected to posses potent xo inhibitory activity comparable to standard antioxidant compound. E.g. Allopurinol along with antioxidant enzyme activity. There is number of techniques are routinely used for the docking purposes hence the various docking method as well as the softwares are used including SYBYL X-1.3. Prior to docking it is necessary to consider how the protein and ligand interacted with each other by atomic, surface and grid level of the receptor i.e. SYBYL X-1.3 is commercially available from Tripos, Inc. Hammerhead docking system with a search engine that relies on a surface based molecular similarity method as a means to rapidly generate suitable puttive poses for molecular fragments.

II. MATERIALS AND METHODS

A. Synthesis of Pyrazoles (2NAP1-2NAP5)

A mixture of pyrazole chalcone 1NAP1-1NAP5 (1.0 mmol), hydrazine hydrate (2.0 mmol) in PEG-400 (10 ml) were heated with 1 ml glacial acetic acid for 3-4 hours. Reaction progress was monitored by TLC then reaction mixture was cooled, poured in ice cold water, precipitate formed was filtered off and recrystallized from ethanol, affording compounds (2NAP1 – 2NAP5).

B. Analytical Methods

Melting points were determined in open capillary tubes and were found uncorrected. IR spectra were recorded on FT-IR spectrometer (Brucker) using KBr disc method.1HNMR spectra were recorded on 1HNMR (Brucker 300 MHz and 500 MHz) spectrometer using CDCl3 as solvent. The mass spectra were obtained on a Thermo mass spectrometer. Physical data of the compounds are recorded in table 1.

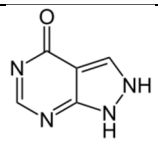
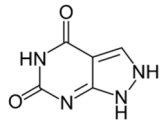
C. Spectroscopic Data of Selected Compounds:

1) *(2NAP1):*1-[3'-(4-Chloro-phenyl)-5-(4-fluoro-phenyl)-1'-phenyl-3,4-dihydro-1'H-[3,4']bipyrazolyl-2-yl]-ethanone.Light yellow solid, mp: 228-2300C, IR (KBr): νmax :3059, 1656, 1600, 1505, 1414, 1229, 836, 7601H NMR (500 MHz, CDCl3): δ 2.46 (s, 3H, N-acetyl), 3.05-3.10 (dd, 1H, Ha), 3.61-3.65 (dd, 1H, Hb), 5.85-5.88 (dd, 1H, Hx), 7.1-7.13 (t, 2H, N-Ar), 7.42-7.45 (t, 2H , N-Ar), 7.28-7.31 (t, 1H, N-Ar), 7.4-7.42 (d, 2H , H-Ar-Cl), 7.66-7.70 (m, 4H, H-Ar-F), 7.72-7.73 (d, 2H , H-Ar-Cl), 7.80 (s, 1H, H-5 of pyrazole)MS: m/z = 459 (M+1)
2) *(2NAP2):*1-[3'-(4-Bromo-phenyl)-5-(4-fluoro-phenyl)-1'-phenyl-3,4-dihydro-'H[3,4']bipyrazolyl-2-yl]-ethanone.Light yellow solid, mp: 2140C, IR (KBr): νmax : 3062, 1657, 1599, 1505, 1415, 1229, 835, 758 1H NMR (300 MHz, CDCl3): δ 2.44 (s, 3H, N-acetyl), 3.01-3.08 (dd, 1H, Ha), 3.61-3.65 (dd, 1H, Hb), 5.82-5.87(dd, 1H, Hx), 7.07-7.12 (t, 2H, N-Ar), 7.39-7.44 (t, 2H , N-Ar), 7.26-7.29 (t, 1H, N-Ar), 7.45-7.56 (d, 2H , H-Ar-Cl), 7.66-7.68 (m, 4H, H-Ar-F), 7.63-7.65 (d, 2H , H-Ar-Cl), 7.78 (s, 1H, H-5 of pyrazole) MS : m/z = 505 (M+2)
3) *(2NAP3):*1-[3'-(4-Methyl-phenyl)-5-(4-fluoro-phenyl)-1'-phenyl-3,4-dihydro-1'H-[3,4']bipyrazolyl-2-yl]-ethanone.Light yellow solid, mp: 1980C, IR (KBr): νmax : 3192, 2915, 2839, 1648,1595,1501, 1407, 1222, 824, 7461H NMR (300 MHz, CDCl3): δ 2.36 (s, 3H, -CH3) 2.44 (s, 3H, N-acetyl), 3.02-3.09 (dd, 1H, Ha), 3.54-3.64 (dd, 1H, Hb), 5.87-5.92 (dd, 1H, Hx), 7.04-7.10 (t, 2H, N-Ar), 7.20-7.23 (m, 3H, N-Ar), 7.38-7.43(d, 2H , H-Ar-Cl), 7.61-7.66 (m, 4H, H-Ar-F), 7.66-7.69 (d, 2H , H-Ar-Cl), 7.76 (s, 1H, H-5 of pyrazole) MS: m/z = 439 (M+1)
4) *(2NAP4):1-*[3'-(4-Methoxy-phenyl)-5-(4-fluoro-phenyl)-1'-phenyl-3,4-dihydro-1'H-[3,4']bipyrazolyl-2-yl]-ethanone.Light yellow solid, mp: 162-1640C, IR (KBr): νmax : 2995, 2892,1660, 1601, 1507,1359, 1239, 1154, 1052, 833,753 1H NMR (500 MHz, CDCl3): δ 2.46 (s, 3H, N-acetyl), 3.83 (s, 3H, -OCH3), 3.06-3.1 (dd, 1H, Ha), 3.58-3.63 (dd, 1H, Hb), 5.87-5.91 (dd, 1H, Hx), 6.95-6.97 (t, 2H, N-Ar),7.08-7.12 (t, 2H, N-Ar), 7.27-7.28 (t, 1H, N-Ar), 7.41-7.44 (d, 2H , H-Ar-Cl), 7.69-7.71 (m, 4H, H-Ar-F), 7.65-7.69 (d, 2H , H-Ar-Cl), 7.79 (s, 1H, H-5 of pyrazole) MS : m/z = 455 (M+1)
5) *(2NAP5):*1-[1',3'–diphenyl-5-(4-fluoro-phenyl)-3,4-dihydro-1'H-[3,4']bipyrazolyl-2-yl]-ethanone.Light yellow solid, mp: 1740C, IR (KBr): νmax : 3010, 1654, 1600, 1503, 1240, 838, 750 1H NMR (300 MHz, CDCl3): δ 2.44 (s, 3H, N-acetyl), 3.03-3.10 (dd, 1H, Ha), 3.56-3.66 (dd, 1H, Hb), 5.87-5.92 (dd, 1H, Hx), 7.05-7.11 (t, 2H, N-Ar),7.39-7.44 (t, 2H, N-Ar), 7.28-

7.31(t, 1H, N-Ar), 7.32-7.44 (m, 3H , H-Ar), 7.62-7.70 (m, 4H, H-Ar-F), 7.74-7.76 (d, 2H , H-Ar), 7.78 (s, 1H, H-5 of pyrazole) MS : m/z = 425 (M+1).

Table 1: Physical Data of Pyrazolyl pyrazoline Compounds 2NAP1-2NAP5:

Entry	Mol. Wt.	MP (0^0C)	Yield	R1	R2	Structure of NAP
2NAP1	458.5	228-230	90	Cl	H	
2NAP2	503	214	85	Br	H	
2NAP3	438	198	90	Me	H	
2NAP4	454	164	92	OMe	H	
2NAP5	424	174	94	H	H	

Drug Compound (Standard)	Formulae	Molecular weight	IUPAC Name	Inhibition	Structure
Allopurinol	$C_5H_4N_4O$	136.11	1*H*pyrazolo[3,4d]pyrimidin-4(2H)-one	(XO)	
Oxypurinol	$C_5H_4N_4O_2$	152.11	1,2-Dihydropyrazolo[4,3-e]pyrimidine-4,6-dione	(XO)	

D. Tools and Materials

1) *Hardware and Software*: The number of software and hardware are used for the docking and viewing purpose but here for the present study we used (*in silico*) bioinformatics software's such as Discovery Studio 2.5, SYBYL-X 1.3, Marvin Sketch, and Chimera1.6.1.
2) *Prediction of Activity Spectra for Biologically Active Substances (PASS) for (2NAP1-2NAP5)* compounds The concept of the biological activity spectrum was introduced to describe the properties of biologically active substances. The PASS (prediction of activity spectra for substances) software product, which predicts more than 300 pharmacological effects and biochemical mechanisms on the basis of the structural formula of a substance, may be efficiently used to find new targets (mechanisms) for some ligands and, conversely, to reveal new ligands for some biological targets. We have developed a WWW interface for the PASS software. A WWW server for the on-line prediction of the biological activity spectra of substances has been constructed. SOURCE: http://www.ibmh.msk.su/PASS/

III. METHODOLOGY

A. Prediction of activity spectra for biologically active substances (PASS)

A web server PASS, which predicts more than 300 pharmacological effects and biochemical mechanisms on the basis of the structural formula of a substance, was efficiently used to find new targets (mechanisms) for all ligands (NAP series). The biological activity spectrums of all ligands were obtained one by one proceeding via the internet by submitting a standard .mol file. The prediction result is returned in the form of a table containing the list of biological activity with appropriate probability values i.e. probable activity (Pa) and probable inactivity (Pi). Source: http://www.ibmh.msk.su/PASS/

B. Docking Methods

The molecular docking was performed using SYBYL-X 1.3 and Discovery studio 1.5 molecular docking software. To evaluate various docking methods, it is important to consider how the protein and ligand are represented. There are three basic representations of the receptor: i.e. atomic, surface, and grid [21].

1) *Molecular Docking with SYBYL-X 1.3:*The chemically synthesized compounds N-acetyl pyrazolylpyrazolines (NAP) were first drawn in the Marvin Sketch software and saved in sdf file format. As well as the standard structures of XO (Xanthine Oxidases) were downloaded in sdf file format from pubChem databases i.e. NCBI or PDB.

The docking protein i.e. Xanthine Oxidase [PDB ID: 2E1Q] from human (Homo sapiens) were retrived from NCBI (http://www.ncbi.nlm.nih.gov/structure) online site in PDB format. Then these are prepared for docking purpose by using SYBYL X 1.3 software. The water molecules, other ligands were removed, Hydrogen atoms were added to the receptors, energy minimization step is carried out and protomol generation step is carried out in which ligand binding site is determined in the receptor. Also the ligands (2NAP1-2NAP5 & Standard oxidase inhibitors) were prepared by using SYBYL software caption i.e. prepared ligand. Finally, the Docking was carried out between above prepared ligand-receptor by SYBYL software. SYBYL automates the docking of ligand into the active site using a Surflex Algorithm. And then obtained results were analysed for protein-ligand interaction.

2) *Discovery* Studio 2.5: The structures of human xanthine oxidase (Oxidoreductase, EC: 1:17.1.4, MMDB ID: 58680, PDB ID: 2E1Q) were retrieved from NCBI (http://www.ncbi.nlm.nih.gov/structure) online site in PDB format. All retrieved structures

were refined and prepared by using prepare protein protocol in Discovery Studio which uses CHARMm force field for energy minimization of receptors. Also, ligands (NAP series & Standard XO inhibitors) were also prepared by using SYBYL software. As well as ligands drawn in Marvin sketch were taken and they were prepared by using prepare ligand protocol in Discovery Studio® (http: www. accelrys.com/ product/ dstudio/) software (Accelrys® software corporation, San Diego, USA) in which Hydrogen bonds were added and the energy was minimized using CHARMm force field.

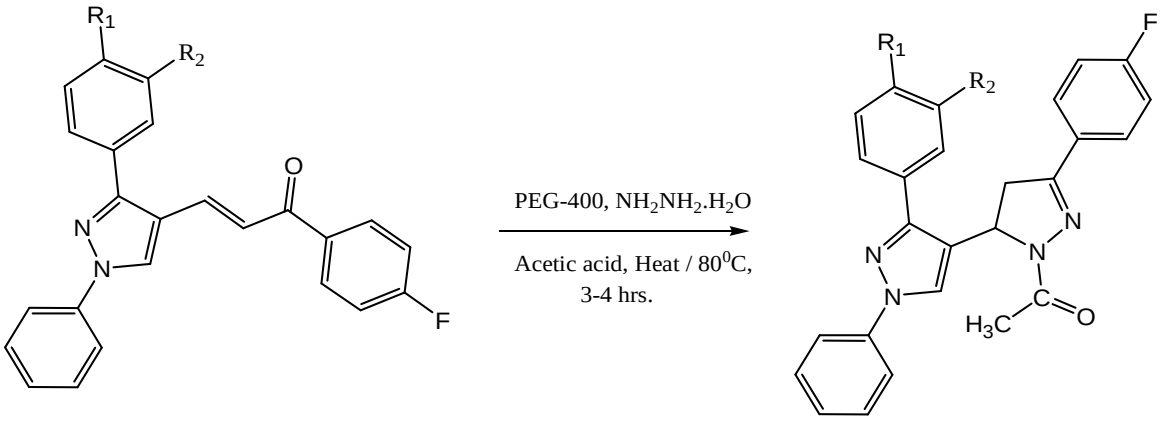
1-acetyl-5-[3-(4-substitutedphenyl)-1-phenyl-1H-pyrazol-4yl]-3-(4-fluorophenyl)-4,5dihydro-1H-pyrazoles (2NAP1-NAP5)derivatives (ligand) were docked into active site of XO enzymes using Discovery Studio. The docking method used in this study was CDOCKER which uses a CHARMm-based docking algorithm. To perform docking process the modeled protein, a protocol called "Dockligands" (CDOCKER) was selected among those listed under receptor-ligand interaction protocol cluster in Discovery Studio® (http: www. accelrys.com/ product/ dstudio/) software (Accelrys® software corporation, San Diego, USA). Each ligand compound was given as input in the parameter meant for "input ligands" and the protocol was run against the prepared receptors and ligand-receptor interaction study was carried out.

IV. RESULTS

A. Synthesis of Pyrazolyl pyrazoline (2NAP1-2NAP5)

Synthesis of Pyrazolyl pyrazolines were successfully carried out by making mixture of pyrazole chalcone 1NAP1-1NAP5 (1.0 mmol),and hydrazine hydrate (2.0 mmol) in PEG-400 (10 ml) were heated with 1 ml glacial acetic acid for 3-4 hours. Reaction mixture was cooled, poured in ice cold water; precipitate formed was filtered off and recrystallized from ethanol, affording compounds 2NAP1-2NAP5. IR spectra were recorded on FT-IR spectrometer (Brucker) using KBr disc method as well as the ^{1}HNMR spectra also recorded on ^{1}HNMR spectrometer using CDCl3 as solvent. Mass spectra and melting points were also found. Physical data of the compounds are recorded in Table 1.

Scheme 1:



1NAP1-1NAP5 2NAP1-2NAP5

R_1 = F, Cl, Br, Me, OMeH, H

R_2 = H, H, H, H, H, NO2, H

B. Rendering of 3D atomic coordinates of ligand

The chemically synthesized fluoro substituted pyrazolyl pyrazolines compound were drawn in Marwin sketch and Export in the form of SD format (2NAP1-2NAP5).This exported structure is successfully used in SYBYL X-1.3 by optimizing and minimizing energy in prepare ligand caption of SYBYL X-1.3 and saved in SD format.

C. Prediction of Activity Spectra for Biologically Active Surfaces (Pass)

Predictions of activity spectra of NAP compound were carried out using online PASS Site i.e.: http://www.ibmh.msk.su/PASS/.All the compounds of NAP series show an antioxidant activity, anti-inflammatory, anticancer, radio protective, anticonvulsant, and antidepressant activity. But our current study shows the antioxidant effect of NAP series on XO only.

D. Molecular Docking Analysis

The 3D structure of Xanthinne Oxidase (XO) [Oxidoreductase, EC: 1:17.1.4, MMDB ID: 58680, PDB ID: 2E1Q] were retrieved in PDB format from the online website of NCBI (http://www.ncbi.nlm.nih.gov/structure). Retrived PDB structures of XO were imported in SYBYL X-1.3 and prepare for structure and ligand docking in stepwise manner. Taking into consideration that the interaction between (XO) receptor protein and NAP as ligand molecules as well as the active site of protein carried out *in silico.*. Molecular docking of (2NAP1-2NAP5) NAP with Xanthine oxidases was performed successfully. i.e. for SYBYL-X C_Score and for Discovery studio CDOCKER score. Using these two software tools the synthesized NAP compounds were screened for their inhibitory activity against Xanthine oxidase. In case of NAP4 and shows significant dock score, whereas for NAP1 NAP2 NAP3 & NAP5 as well as standard compound Allopurinol, Oxypurinol shows significant enzyme inhibitory activity. Enzyme and ligand interaction by considering the obtained score value i.e. C score in SYBYL X-1.3.With the help of these two softwares the chemically synthesized NAP molecules screened for inhibitory effect against Xanthine Oxidase.

Allopurinol (1*H*-pyrazolo [3, 4-d] pyrimidin-4(2H)-one, PubChem Id: CID 2094) which shows greatest similarity score with NAP series. Therefore significant inhibition of Xanthine oxidase by derivatives 2NAP1, 2NAP2, 2NAP3, and 2NAP5 may prove interesting as use of antiparkinson's agent.

Table 3: Docking Score of 2NAP1-2NAP5 on Xanthine Oxidase:

Docking Ligand	Docking Receptor	Total score	D_Score	PMF_Score	G_Score	CHEM Score	C_Score	Global Score
2NAP1	(Human) Xanthine Oxidase	5.0646	-101.78	-39.800	-165.64	-29.18	5	5
2NAP2		4.1668	-118.32	-23.567	-225.13	-22.87	5	5
2NAP3		3.9593	-99.921	-2.9009	-172.37	-23.70	5	5
2NAP4		5.6568	-105.22	-21.656	-155.63	-45.26	4	4
2NAP5		3.5645	-101.56	-19.586	-125.69	-21.56	5	5
Allopurinol (Standard)		3.3627	-37.016	-33.8785	-85.619	-17.16	4	4
Oxypurinol (Standard)		2.7406	-37.233	-18.620	-75.140	-13.60	5	5

E. Crystal Structure of Target Oxidase

Crystal structure of xanthine oxidase retrieved from NCBI and prepared for protein preparation captions and finally ligand (NAP) has docked into the active site of receptor protein by using the CHAMm force field in discovery studio software (Fig.2).

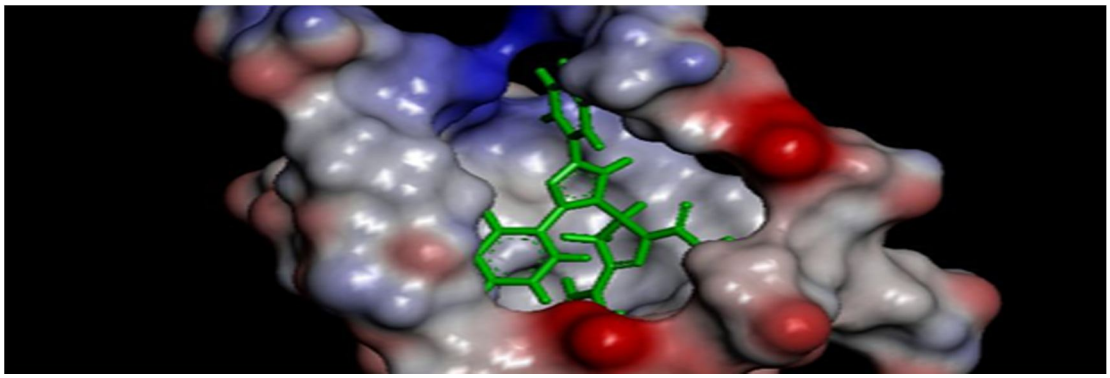


Fig.2. Focused view of Xanthine Oxidase protein, the binding site (Multicolor) and the N-acetyl Pyrazolyl pyrazoline docked (A screen shot of Discovery Studio).

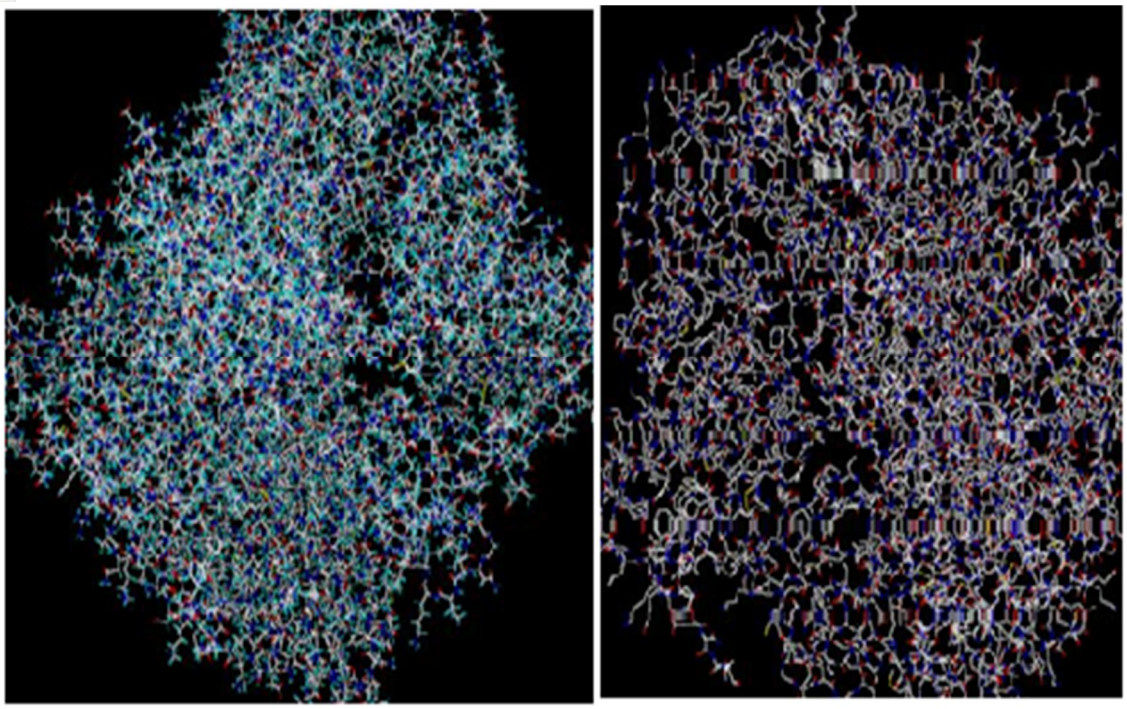


Fig.3 (A) Fig.3 (B)

Fig.3 (A) and 3 (B) showing retrieved and prepared structure of Xanthine Oxidase respectively from SYBYL X-1.3 Software.

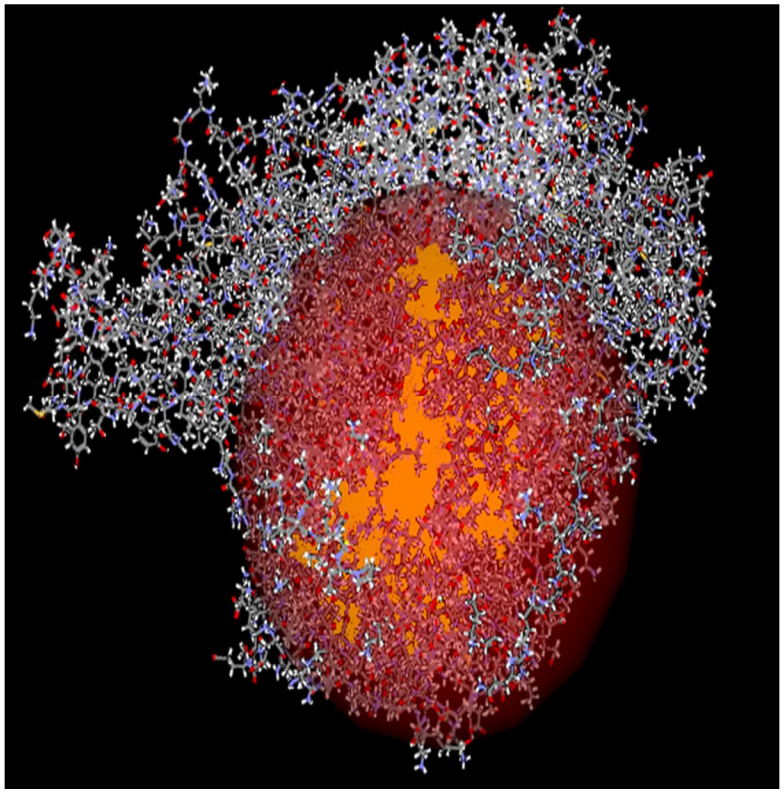


Fig.4 (A)

Fig.4 (A) Showing a crystal structure of Xanthine Oxidase having interaction (Active site) with Allopurinol indicated by Orange patches inside circle in Discovery Studio 2.5.

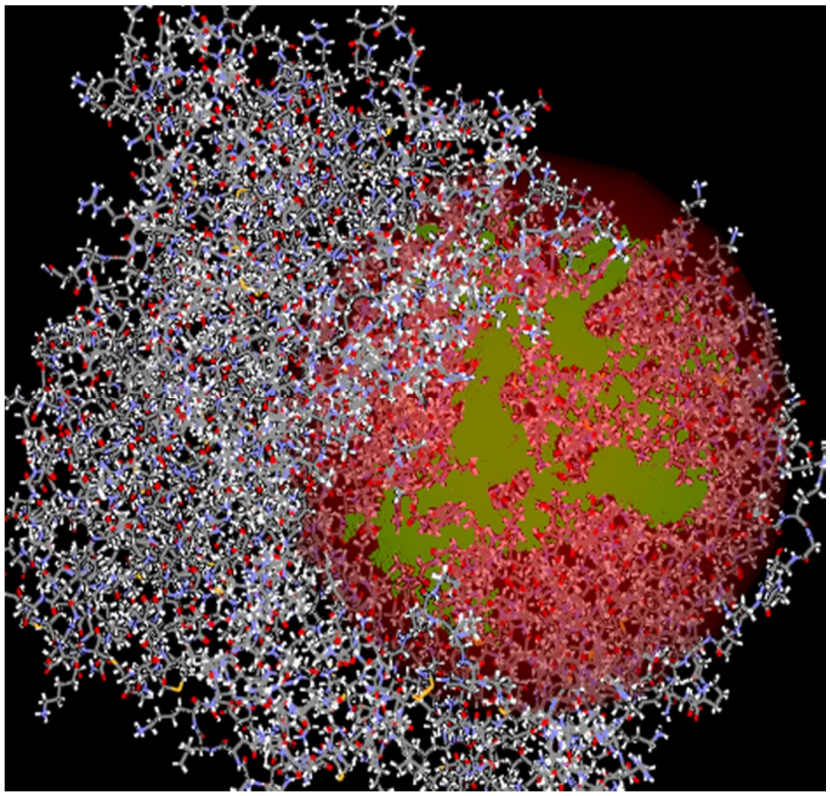


Fig.5 (A)

Fig.5 (A) Showing a crystal structure of Xanthine Oxidase having interaction (Active site) with oxypurinol indicated by Green patches inside circle in Discovery Studio 2.5.

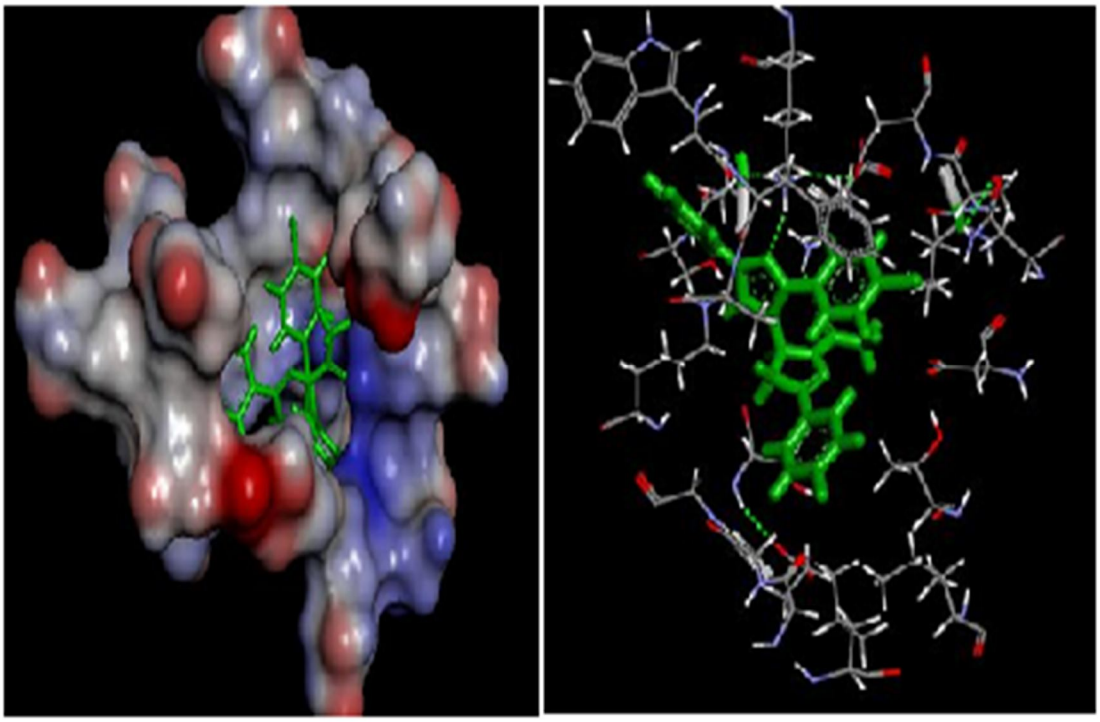


Fig.6 (A) Fig.6 (B)

Fig.6 (A) and (B) showing a crystal structure of Xanthine Oxidase having interaction with N-acetyl Pyrazolyl pyrazolines (2NAP1) indicated by Green dotted lines in SYBYL X-1.3 (Fig.6B).

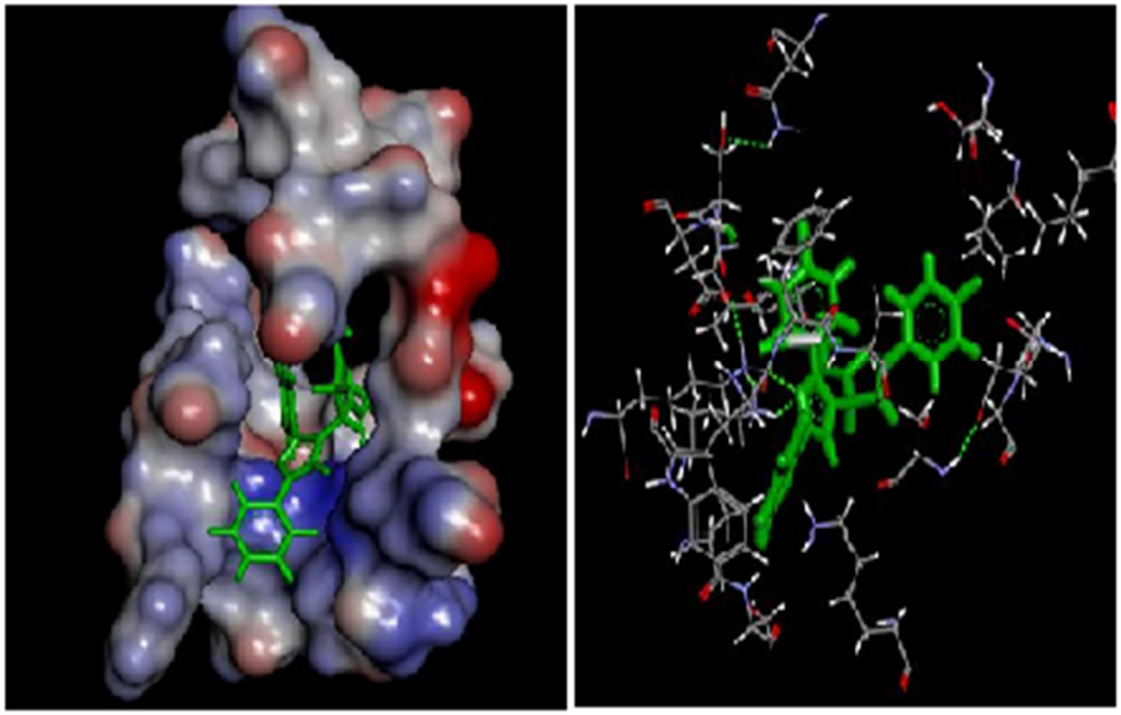


Fig. 7 (A) Fig. 7 (B)

Fig.7 (A) and (B) showing a crystal structure of Xanthine Oxidase having interaction with N-acetyl Pyrazolyl pyrazolines (2NAP2) indicated by Green dotted lines in SYBYL X-1.3 (Fig.7B).

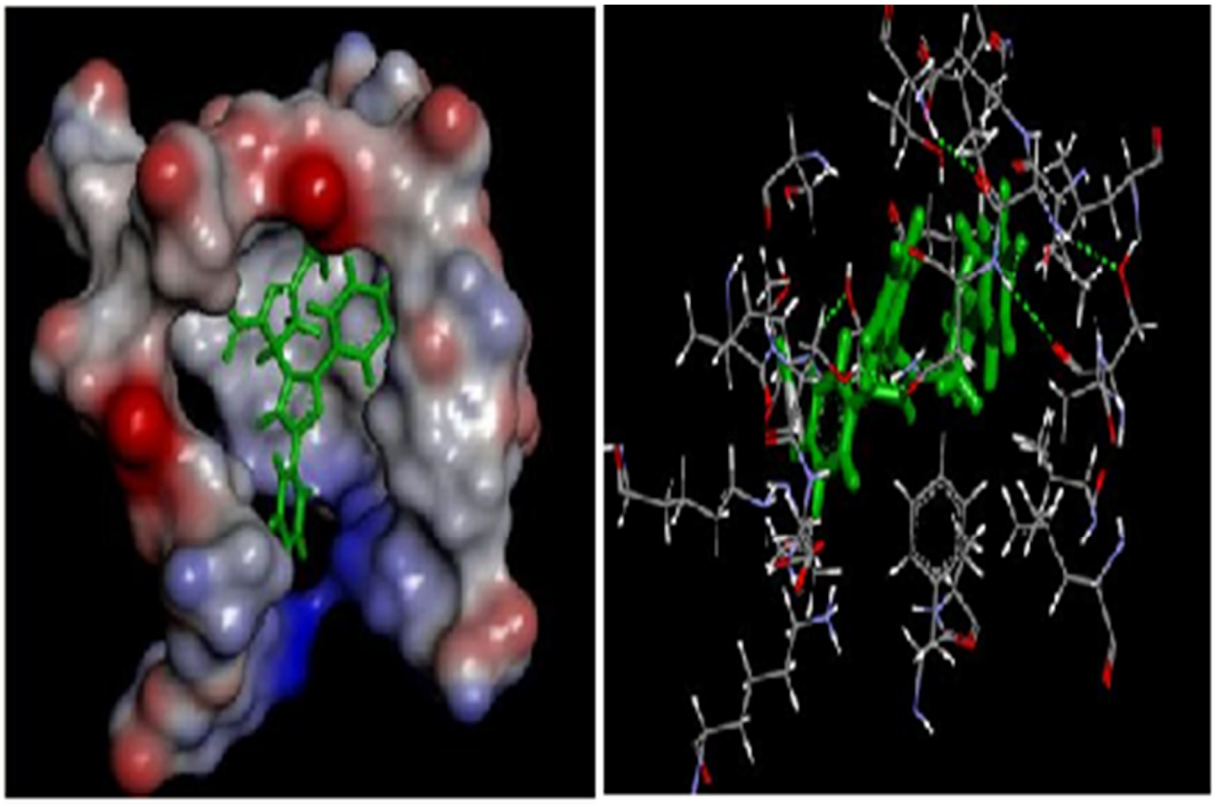


Fig.8 (A) Fig.8 (B)

Fig.8 (A) and (B) showing a crystal structure of Xanthine Oxidase having interaction with N-acetyl Pyrazolyl pyrazolines (2NAP3) indicated by Green dotted lines in SYBYL X-1.3 (Fig.8B).

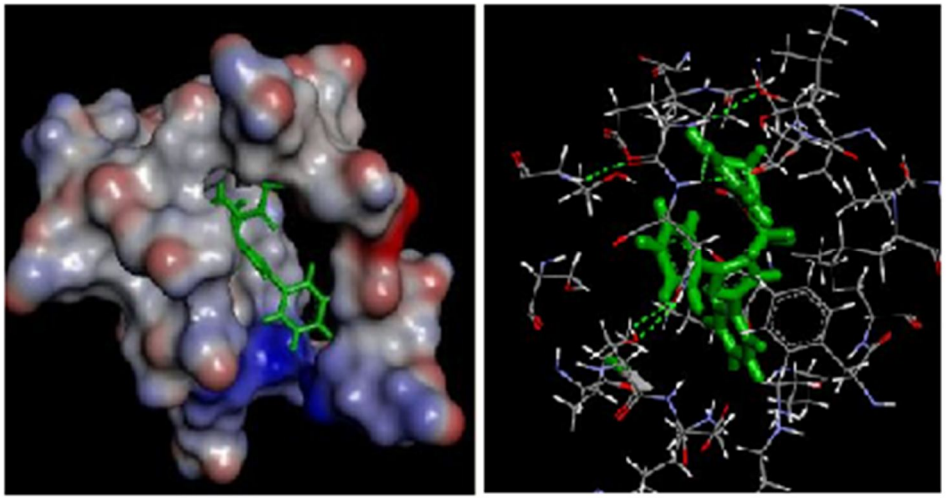


Fig.9 (A) Fig.9 (B)

Fig.9 (A) and (B) showing a crystal structure of Xanthine Oxidase having interaction with N-acetyl Pyrazolyl pyrazolines(2NAP4) indicated by Green dotted lines in SYBYL X-1.3 (Fig.9B).

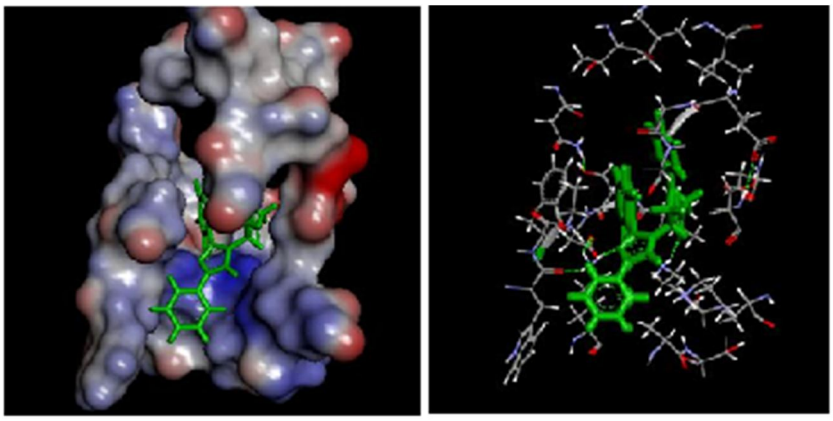


Fig.10 (A) Fig.10 (B)

Fig.10 (A) and (B) showing a crystal structure of Xanthine Oxidase having interaction with N-acetyl Pyrazolyl pyrazolines (2NAP5) indicated by Green dotted lines in SYBYL X-1.3 (Fig.10B).

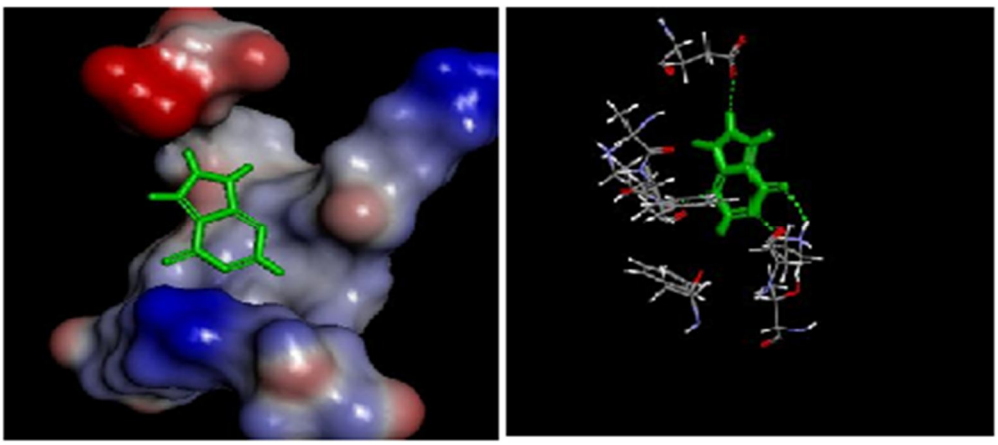


Fig.11 (A) Fig.11 (B)

Fig.11 (A) and (B) showing a crystal structure of Xanthine Oxidase having interaction with Allopurinol (Standard compound) indicated by Green dotted lines in SYBYL X-1.3 (Fig.11B).

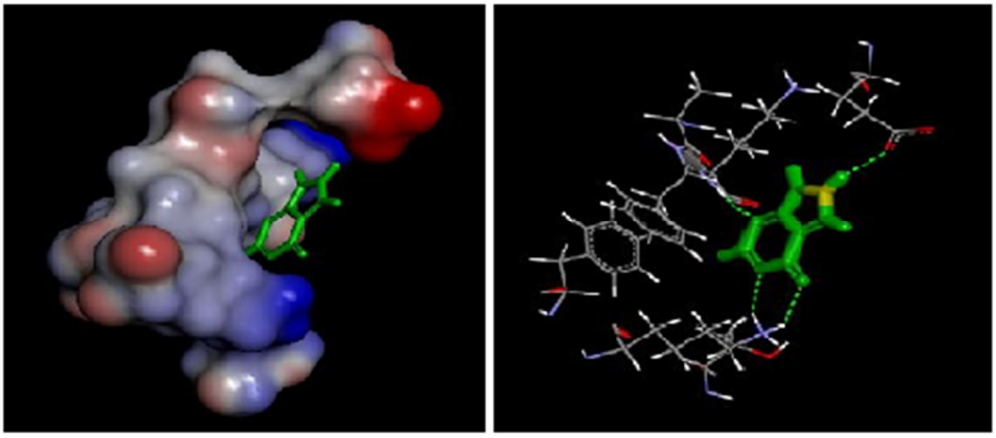


Fig.12 (A) Fig.12 (B)

Fig.12 (A) and (B) showing a crystal structure of Xanthine Oxidase having interaction with Oxypurinol (Standard compound) indicated by Green dotted lines in SYBYL X-1.3 (Fig.12B).

V. DISCUSSION

Our research of interest gives importance to the synthesis of NAP series compound i.e 1-acetyl5-[3-(4-substitutedphenyl)-1-phenyl-1H-pyrazol-4yl]-3-(4-fluorophenyl)-4, 5-dihydro-1H-pyrazole, in PEG 400 [18-20] and docking it with xanthine oxidase. As we introduce about P.D is the second most common neurodegenerative disease. This affects both adult and younger as well as the male and female. Parkinson's disease symptomises the disabling condition of brain which shows the slowness of movement, loss of balance, stiffness and shaking etc.In view of this observation, herein we report the synthesis and antioxidant activity of series of 1-acetyl 5-[3-(4-substituted phenyl)-1-phenyl-1H-pyrazol-4yl]-3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazole by using in vitro and in silico approach. Our in silico approach on antioxidant activity of NAP series revaled that this series of compound were docked in the active site of xanthine oxidase by inhibiting the production of ATP and hence the series of ROS (Reactive Oxygen Species) pathway simultaneously. ROS pathway are involve in the production of free radical therefore we might be also called it as NAP series of compound shows free radical scavenging activity. From the above result of SYBYL X, the compound mentioned in the result i.e. 2NAP1, 2NAP2, 2NAP3, 2NAP5 shows better binding value (C_Score) with XO. Also in comparison with standard drugs molecules Allopurinol & Oxypurinol shows most confirmative inhibitory effect, which are also observed in the NAP series of compound by strong binding with active binding site of XO. Thus the inhibitory effect of NAP compound was evaluated in these work revealing 2NAP1, 2NAP2, 2NAP3, 2NAP5 as potent inhibitor of XO.

VI. CONCLUSION

From the above mentioned observation and results we can concluded that, the series of fluro substituted N-acetyl pyrazoline derivatives were synthesized. Also the spectral analysis of these compounds were carried out by using (IR, 1HNMR, LCMS) method. Here the derivatives of NAP such as 2NAP1, 2NAP2, 2NAP3, 2NAP5 shows the exact binding with XO and acts as potent inhibitor. The inhibitory activity of NAP is carried out by using SYBYL- X and Discovery Studio as a viewer software. Therefore results indicate that they act as antioxidant drugs by inhibiting XO of ROS pathway.

A. Conflict of Interest

No conflict of interest

VII. ACKNOWLEDGEMENT

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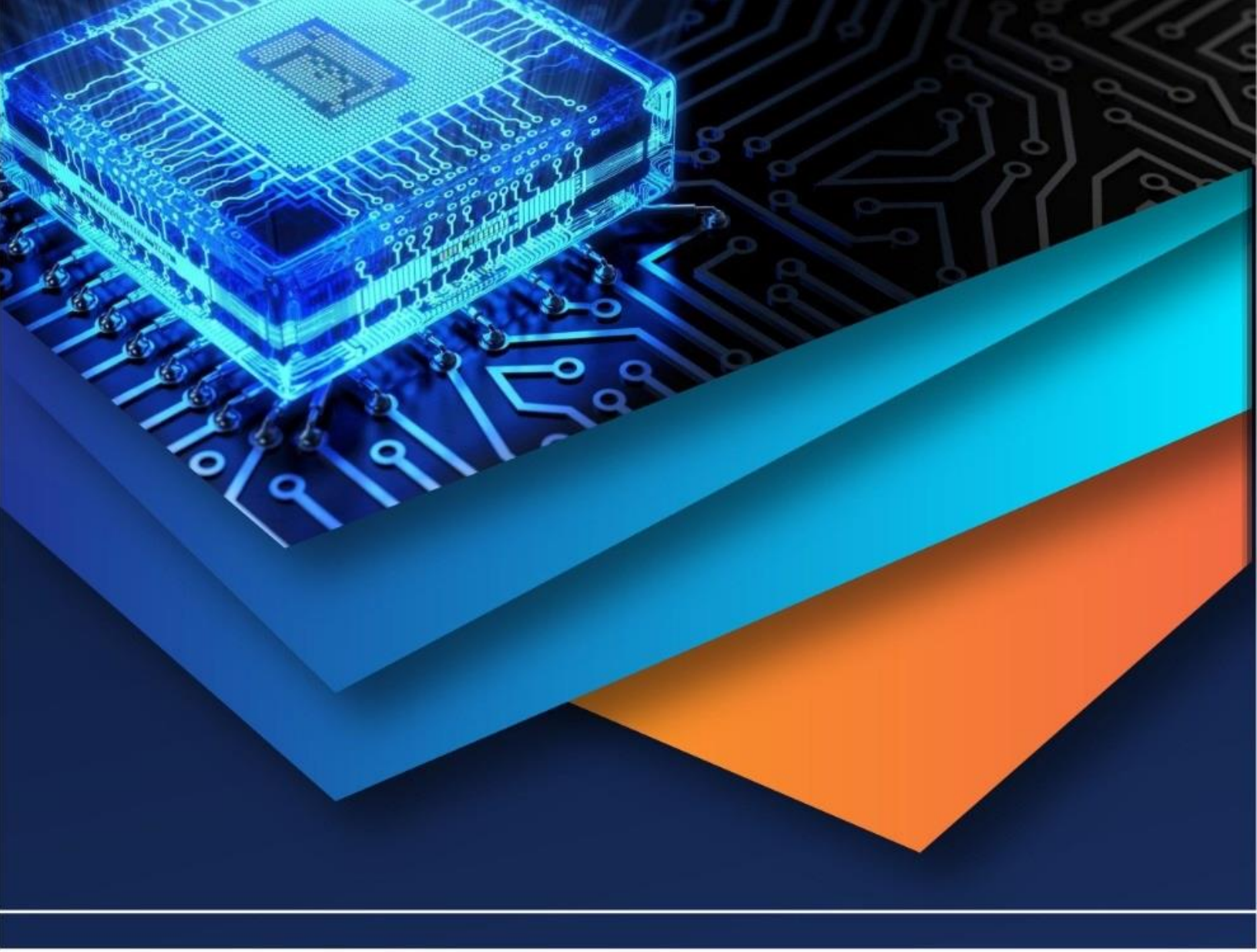

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