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De Novo Drug Design and Synthesis of certain Indole Derivatives and Screening for their Xanthine Oxidase inhibitory activity

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Abstract: Nearly 300 compounds were screened, out of that compound 6 containing indole was selected as a lead and lead optimization was done to refine the chemical structure in order to improve its drug character. Selected leads were synthesized using indole-2- carboxylic acid. Series S6 $_{(a-e)}$ contains indole attached to triazole moiety. Synthesized compounds were subjected to spectral studies. Newly synthesized compounds were evaluated for their Antioxidant activity using DPPH$^{\circ}$ radical and Xanthine oxidase enzyme. Results were evaluated. Compounds $S6_b$ and S6c which showed highest antioxidant activity were selected as a lead for future study of Xanthine oxidase inhibitor and antioxidant agents.

Keyword: Drug Design, Lipinski's rule, Lead Optimization, Indole, Triazole, Xanthine Oxidase Inhibitor.

INTRODUCTION:

Drug discovery[1] is an extended process that can take as many as 15 years from the first compound synthesized in the laboratory until the therapeutic agents, or drug is brought in to the market. Reducing the research time in the discovery stage is a key priority for pharmaceutical company's world wide. We are trying to achieve this goal through the application and collaboration of advanced technologies such as computational biology, chemistry, computer graphics and high performance computing. Drug design is considered as the process of envisioning the preparation of specific new molecules that can lead to the discovery of more efficient, less toxic and useful drugs. New drug discovery may be considered broadly in terms of two kinds of investigational activities, "exploration" and "exploitation" of leads. In this, the former involves the search for new leads and the latter the assessment, improvement and extension of the lead. Drug designing is also an important tool in the field of medicinal chemistry wherein the synthesis of new medicinal compounds is done by molecular or chemical manipulation of the lead moiety in order to produce highly active compounds with minimum steric effects. Elimination, substitution or introduction of certain groups in the drug molecule and effective combination of two (or) more active moieties are some of the purposeful modifications usually made.

Review of literature reveals the broad spectrum of activity of triazoles[2] like antibacterial, antifungal, anti-inflammatory, antiviral, analgesic, antiulcer, anticonvulsant and anti tubercular activities. Review of literature reveals that indole[3] is a drug like scaffold and is also a core skeleton for the active sites

involved in enzyme inhibition. Indole offers a promising approach for the development of newer drugs for the treatment of gout.

Xanthine oxidase[4, 5] is a source of oxygen-derived free radicals. Both XO and XDH catalyze the removal of hydrogen from a substrate using oxygen as hydrogen acceptor, and it gets reduced. During reoxygenation (ie, reperfusion phase) it reacts with molecular oxygen, thereby releasing super oxide anion radicals. Xanthine oxidase pathway has been implicated as an important route in the oxidative injury to tissue, especially after Ischemia – reperfusion.

Enzyme inhibitors are molecules that bind to enzymes and decrease their activity. Since blocking an enzyme's activity can kill a pathogen or correct a metabolic imbalance, many drugs are enzyme inhibitors. The design of the inhibitors based on the structure of an enzyme active site, is helpful to determine the 3D structure of the enzyme and of enzyme in complex with an inhibitor at high resolution. Precisely all the molecules interactions that are necessary for a drug to bind to its target site based on the architecture of enzymes active site and the interactions that stabilizes the inhibitor with greater binding affinity can be designed with a shape that fits better into the active site and charge distributed suitable for increased interaction energies, also it may involve topographic studies.

In view of the above, the primary objective of the present work was to design the compounds by *de novo* drug designing approach, using mol inspiration software tool[6] and to prepare derivatives of Triazole incorporated with indole ring system and screen the synthesized compounds for their xanthine oxidase inhibitory activity and antioxidant property wherein they scavenge free radicals which are considered to be one of the major causes for cell damage leading to several pathological conditions like gout, cancer etc.,

MATERIALS AND METHODS:

CHEMICALS USED: All the chemicals used were of AR/GR grade**.** Pure samples of Indole-2-carboxylic acid, Phosphorus penta chloride, Carbon tetra chloride, Hydrazine hydrate, Dimethyl formamide, Phenyl acetyl chloride, Triethyl amine, Ethanol, Acetic acid, Ammonium acetate, Sodium bi carbonate, P- Hydroxy benzaldehyde, p- Chloro benzaldehyde, P- N dimethyl benzaldehyde, Anisaldehyde, Vanillin were obtained from sigma Aldrich. 2, 2 – Diphenyl-1-picrylhydrazyl (DPPH), Methanol, Ascorbic acid.

ANALYTICAL WORK: Melting points were determined by using melting point apparatus MP-DS TID 2000 V. Scientific and were uncorrected. Reactions were monitored by thin layer chromatography (TLC) on a precoated silica gel G plates using iodine vapours as visualizing agents. UV spectra were recorded on JASCO V-530 UV/Vis Spectrophotometer. IR spectra were recorded on JASCO FT/IR-140 spectrophotometer.

EXPERIMENTAL WORK:

SELECTION OF LEAD AND LEAD OPTIMIZATION

The selection of lead was done by screening various nucleuses such as benzthiazole, imidazole, indole, pyridazine, pyrimidine, naphthyridine etc by using mol inspiration software. Mol inspiration is a cheminformatic software tool, which gives the molecular properties of any chemical structure as well as prediction of bioactivity score for the most important drug targets and possible molecular toxicity. Nearly 300 compounds were screened; the data obtained for few compounds were listed in table: 1.

Table: 1 Bio activity score for some of the leads

Compound code	GPCR	Ion channel Modulator	Kinase Inhibitor	Nuclear Receptor
1	-0.76	-1.38	-0.96	-1.35
2	-0.64	-1.06	-0.88	-0.84
3	-0.85	-0.73	-1.12	-1.37
4	-0.3	-0.6	-1.16	-1.04
5	-0.56	-0.59	-0.67	-0.71
6	0.01	-0.23	-0.79	-1.28
7	-0.91	-1.09	-0.83	-1.30
8	-0.41	-0.70	-0.13	-0.55
9	-0.65	-0.65	-0.98	-0.94
10	-0.43	-0.67	-0.11	-0.66
11	-0.99	-1.69	-1.32	2.29
12	-0.65	-1.54	-0.99	-0.65
13	-0.12	-0.43	-0.34	-0.06
14	-0.76	-0.16	-0.32	-0.56
15	-0.45	-0.43	-0.65	-0.32

>0.00- Active, -0.50- 0.00-Moderately active, < 0.50-Inactive

Compounds were filtered from the huge numbers of screened compounds based on the following criteria;

- Positive value for any one of drug targets like (GPCR, Ion channel blocker, Kinase receptor, Nuclear receptor)
- It should satisfy the Lipinski's Rule of Five.

From the filtered compounds, Compound 6[7], containing Indole was selected as a lead, which optimizes the complex 3 dimensional descriptions of a binding pocket of the target protein.

Lead optimization was made by screening different heterocyclic ring system incorporated with indole lead. Triazole showed significant bioactive score when incorporated with indole. These desired compounds were synthesized by simple scheme starting from Indole-2-Carboxylic acid. Due to wide range of biological activities exhibited by Triazole and Indole an attempt was made to investigate the Anti oxidant and Xanthine oxidase inhibitory activities of above designed compounds. (Values are displayed in table: 2)

Structure of compound 6

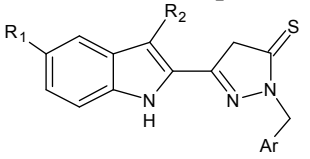
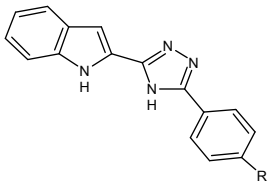


TABLE 2: Molecular Properties and Bio active score of compounds $S_{6\,(a\text{-}e)}$

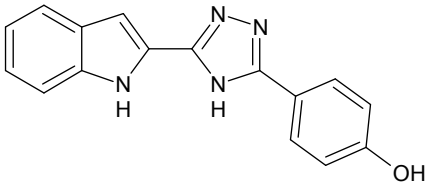
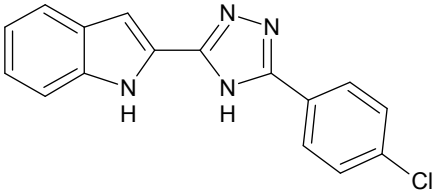
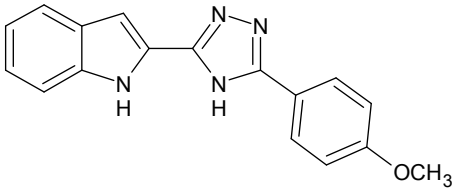
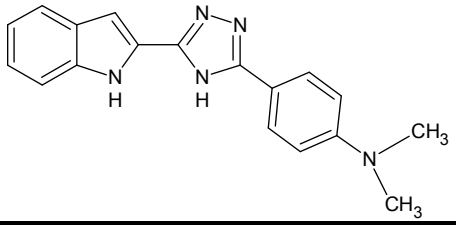
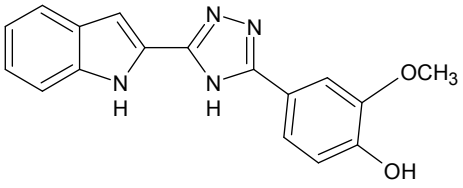


$S_{6\,(a\text{-}e)}$

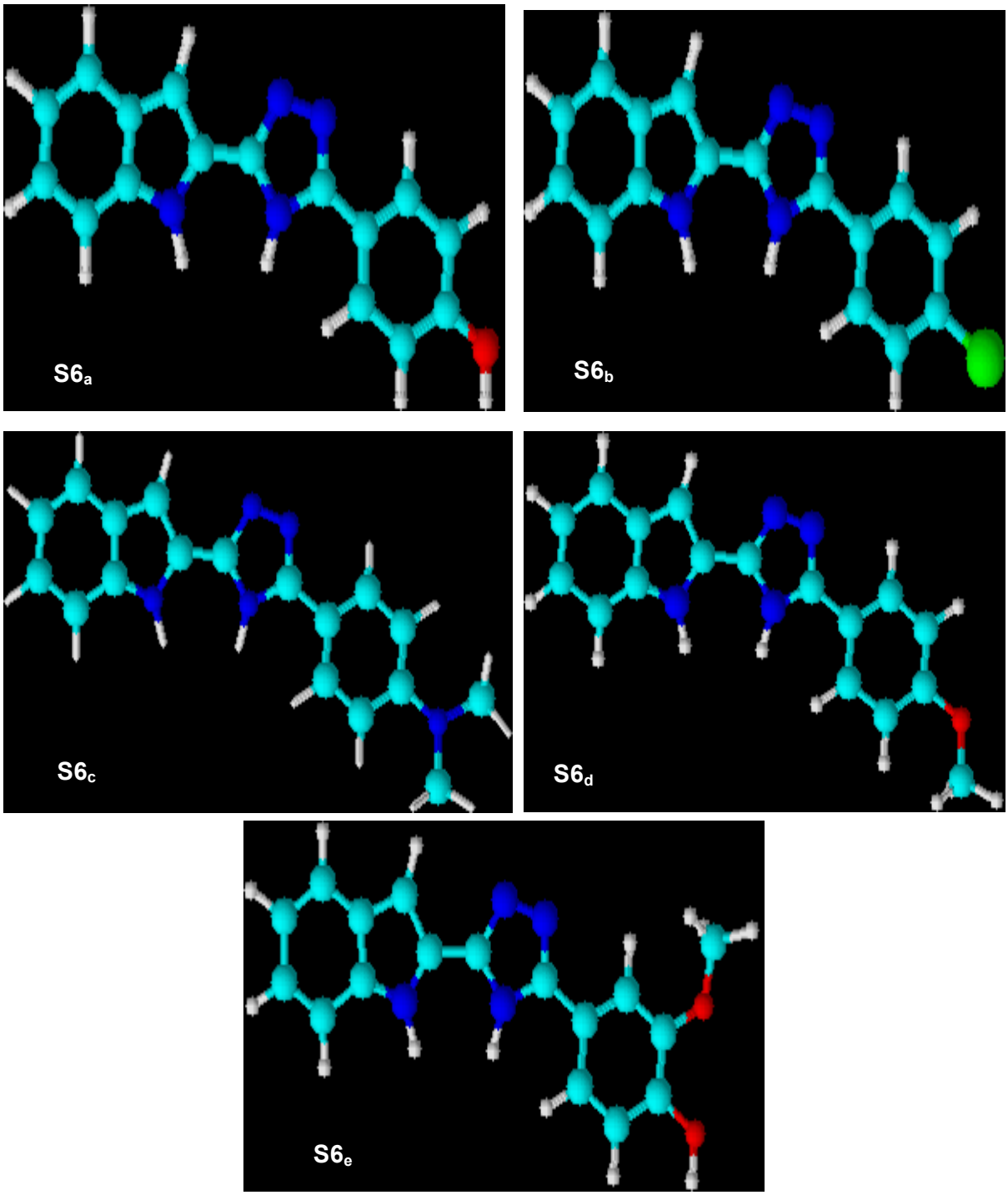
COMPD	Log P	MW	"H" Acceptor	"H" Donor	Violation	GPCR Ligands	Kinase Inhibitor
S_{6a}	3.723	260.3	2	4	0	0.12	0.46
S_{6b}	4.401	294.745	2	4	0	0.17	0.46
S_{6c}	3.244	276.299	3	5	0	0.11	0.44
S_{6d}	3.78	290.326	2	5	0	0.13	0.44
S_{6e}	3.825	303.369	2	5	0	0.11	0.40

>0.00- Active, -0.50- 0.00-Moderately active, < 0.50-Inactive

TABLE: 3 LIST OF NEWLY DESIGNED COMPOUNDS

Code No	Compound Name	Structure
$S6_a$	4-[5-(1*H*-indol-2-yl)-4*H*-1,2,4-triazol-3-yl]phenol	
$S6_b$	2-[5-(4-chlorophenyl)-4*H*-1,2,4-triazol-3-yl]-1*H*-indole	
$S6_c$	4-[5-(1*H*-indol-2-yl)-4*H*-1,2,4-triazol-3-yl]-*N*,*N*-dimethylaniline	
$S6_d$	2-[5-(4-methoxyphenyl)-4*H*-1,2,4-triazol-3-yl]-1*H*-indole	
$S6_e$	4-[5-(1*H*-indol-2-yl)-4*H*-1,2,4-triazol-3-yl]-2-methoxyphenol	

3D optimization view of newly designed compounds
Fig: 1 COMPOUNDS S6 $_{(a\text{-}e)}$



Step1: SYNTHESIS OF INDOLE -2- CARBONYL CHLORIDE (2)[8]

Indole-2-carboxylic acid (1 mmole) and phosphorous penta chloride (1 mmole) were suspended on 10ml of dry carbon tetrachloride and the mixture was stirred on a heating mantle at 50°C till the evolution of hydrochloric acid ceases. The solvent was evaporated in vacuum and the residue was recrystallised from carbon tetra chloride to obtain the acid chloride. The compound was established by single spot on TLC plate.

Step2: SYNTHESIS OF INDOLE -2- CARBOHYDRAZIDE (3)[9]

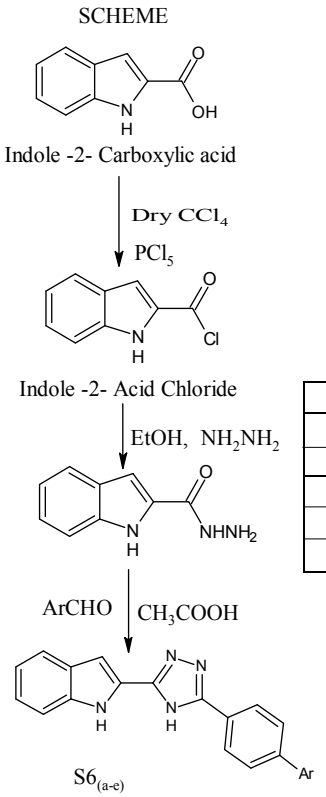
Indole -2- carbonyl chloride (1 mmol) in ethanol (20ml), hydrazine hydrate (4 mmol) was added in drops with constant stirring and irradiated under microwave for 3min at 20% power. After cooling, the solution was poured into crushed ice. The separated

solid was filtered, dried and recrystallised from ethanol. Melting point: 184-186°C

Step3: SYNTHESIS OF SUBSTITUTED TRIAZOLE DERIVATIVE (4) S6 $_{(a-e)}$ [10]

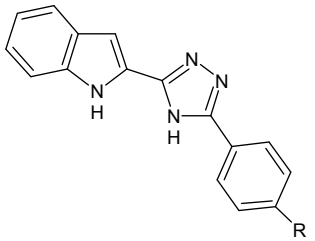
Indole -2- acid hydrazide (0.1 mmol) in acetic acid (20ml), a pinch of ammonium acetate was added followed by the addition of aromatic aldehyde (0.1mol). The mixture was stirred for 24hr at room temperature. The mother liquor on neutralization with sodium bi carbonate solution gave a solid, which was filtered and recrystallized from ethanol.

Fig: 2 SCHEME



S. No.	Ar
1	4-OH
2	4-Cl
3	4-$N(CH_3)_3$
4	4-OCH_3
5	4-OH, 3-OCH_3

TABLE: 3 PHYSICAL CHARACTERIZATION OF NEWLY SYNTHESIZED COMPOUNDS



COMPOUND S $_{(6a-e)}$

Compound code	R	Molecular formula	Molecular weight	Melting Point	% Yield
S_{6a}	4-Hydroxy	$C_{16}H_{12}N_4O$	276.29	230	75%
S_{6b}	4-Chloro	$C_{16}H_{11}N_4Cl$	294.74	206	79%
S_{6c}	4-DimethylAmino	$C_{18}H_{17}N_5$	303.36	172	84%
S_{6d}	4-Methoxy	$C_{17}H_{14}N_4O$	290.32	210	86%
S_{6e}	4-Hydroxy-3-Methoxy	$C_{17}H_{14}N_4O_2$	306.32	196	77%

Solvent system: Methanol: Chloroform (1: 1)

Spectral studies of compounds: The structure of the compounds synthesized during the present investigation was established on the basis of chemical data, IR, UV and Mass spectral data[11, 12]. The purity of the compounds was established by single spot on TLC plates.

ANALYTICAL DATA OF SYNTHESIZED COMPOUND

UV Spectral Data: λ max - 306nm (Solvent: Methanol)

IR Spectral Data:

TABLE: 4

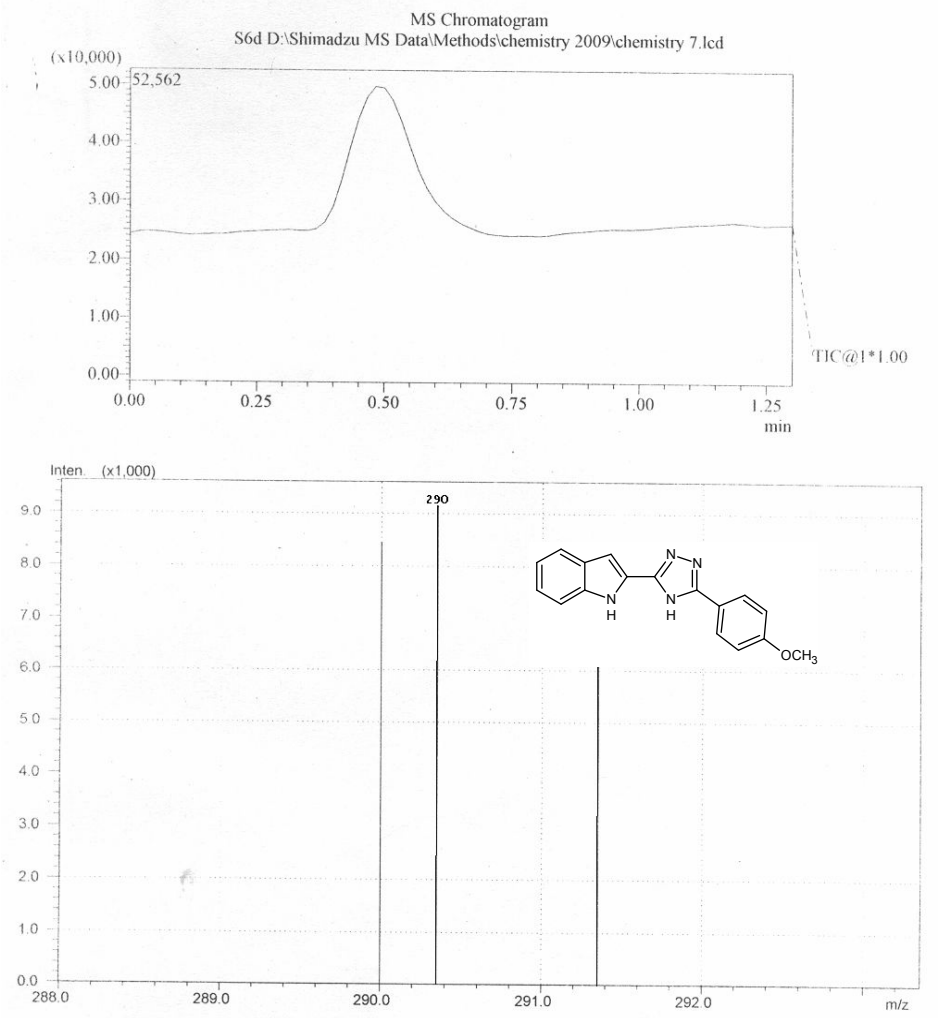
S. No.	Peak No	Type of Vibration	Frequency in cm^{-1}
1	4	N-H stretching	3333.39
2	9	C=N stretching	1588.09
3	14	N-N Hetero aromatic Stretching	1435.74
4	10	C-O-C stretching	1257.36

NMR Spectral Data:

TABLE: 5

S. No.	δValue	Type of protons	No. of. Protons
1	7.7-7.1	Aromatic & -CH protons	9
2	9.1	-NH protons	2
3	3.9	$-OCH_3$	3

MASS Spectral Data:



In vitro Xanthine Oxidase Inhibitory (XOI) activity[13, 14, 15]

The xanthine oxidase inhibitory activity was assayed spectrophotometrically under aerobic conditions. The sample and the standard drug allopurinol (1mg/ml) for in vitro assay were prepared by dissolving the sample in little volume of DMSO (not exceeding more than 5% of total volume) initially and then made up to the required volume with KH_2PO_4 buffer, pH 7.5. The assay mixture consisted of 1ml of test solution (5-100µg/ml), 2.9 ml of KH_2PO_4 buffer (pH 7.5, adjusted with 1M KOH) and 0.1ml of XO enzyme solution (0.1U/ml in KH_2PO_4 buffer, pH 7.5, prepared immediately before use). After Preincubation at 25°C for 15min, the reaction was initiated by the addition of 2ml of substrate solution (150µM xanthine in phosphate buffer, pH 7.5). The assay mixture was incubated at 37°C for 10min and 0.5ml of 0.58M HCl was added to stop the reaction. The absorbance was measured at 290nm against blank (buffer solution) and percentage inhibition was calculated using the following formula,

% I = (1- B/A) * 100

Where,

B = the absorbance with sample (a-b),
a = Absorbance with XO,
b = Absorbance without XO,
A = the absorbance without sample (c-d),
c = Absorbance with XO,
d = Absorbance without XO,

The assay was done in triplicate for each concentration. Allopurinol (1 to 100 µg/ml) was used as a positive control.

FREE RADICAL SCAVENGING ACTIVITY BY DPPH ASSAY METHOD[16, 17, 18]

The model of the scavenging of the stable DPPH radical is extensively used to evaluate antioxidant activity in lesser time than the other methods. DPPH is a stable free radical that can accept an electron or hydrogen radical and thus can be converted into a stable, diamagnetic molecule. DPPH has an odd electron and so has a strong absorption band at 517nm. When this electron becomes paired off, the absorption decreases stoichiometrically with respect to the number of electrons taken up. Such a change in the absorbance produced in this reaction has been widely applied to the capacity of numerous molecules to act as free radical scavengers.

Preparation of solutions

Free radical scavenging activity of the test compounds were determined by DPPH assay method and compared with ascorbic acid as standard.

Preparation of 0.5mM drug solution

10ml of 0.5mM solution was prepared by taking required amount of drug in 1ml of dimethyl sulphoxide to get a clear solution. Then required amount of methanol was added to produce a final volume 10ml. The calculations have been done by using the following formula.

$$\frac{0.5 \times \text{mol wt of drug}}{1000} = \text{x gm in 1000ml}$$

$$= 0.00 \text{ x gm in 10ml}$$

IN VITRO XANTHINE OXIDASE INHIBITORY ACTIVITY

compd	% Xanthine oxidase Inhibition					IC_{50} (µg/ml)
	5µg/ml	10µg/ml	25µg/ml	50µg/ml	100µg/ml	
$S6_a$	23.49 ±0.25	25.69 ± 0.25	29.45± 0.15	31.90 ± 0.23	41.19 ± 0.32	45
$S6_b$	43.03 ± 0.23	55.20 ± 0.27	61.23± 0.18	74.91± 0.19	95.29± 0.121	15
$S6_c$	45.38 ± 0.19	65.74± 0.32	72.75± 0.36	83.27± 0.34	94.09± 0.07	13
$S6_d$	38.34 ± 0.26	44.08± 0.18	44.21± 0.45	46.04± 0.73	52.40± 0.27	29
$S6_e$	18.98 ± 0.15	28.76± 0.15	31.44± 0.22	33.26± 0.24	33.49± 0.33	34
Standard Allopurinol	45.47 ± 0.25	56.30± 0.45	65.78± 0.50	73.62± 0.69	91.26± 0.86	14

Values are mean ± SEM of three parallel measurements.

Preparation of 0.2mM drug solution

From the above solution 4ml quantities were taken in separate 10ml standard flasks and the volume was made up to 10ml using methanol.

Preparation of 0.1mM drug solution

From 0.5mM solution, 2ml lots were taken in separate 10ml standard flasks and diluted to 10ml with methanol.

Preparation of 0.1mM standard solution

0.01gm of ascorbic acid was taken in 100ml standard flask, dissolved in methanol and the volume was adjusted to 100ml with methanol.

Preparation of 0.2mM DPPH solution

0.08gm of DPPH was taken in a 100ml standard flask and dissolved in methanol and the volume was adjusted to 100ml with methanol.

Procedure for evaluations of antioxidant activity

1.5ml of 0.2mM of DPPH solution was added to 1.5ml of different concentrations of the drug solutions. Another series of solutions were prepared by taking 1.5ml of different concentrations of drug solutions and 1.5mlof methanol. The above solutions were allowed to react at room temperature for 30min. After 30min the absorbance values were measured at 517nm and converted to percentage of scavenging activity which was calculated by using the following formula:

$$\% \text{ of Scavenging activity} = \left[\frac{(Ab + As) - Am}{Ab}\right] \times 100$$

Abs=Absorbance of 1.5ml DPPH + 1.5ml methanol

Am=Absorbance of 1.5ml DPPH + 1.5ml drug solution

As=Absorbance of 1.5ml drug solution+ 1.5ml methanol

SCREENING FOR ANTIOXIDANT ACTIVITY BY DPPH (METHOD ($S6_{a-e}$))

Compound Code	Absorbance	Absorbance at 517nm		
		0.5mM	0.2mM	0.1mM
$S6_a$	Drug + DPPH	2.3605	2.7403	3.1968
	Drug + Methanol	0.0656	0.0307	0.0150
	% of Activity	31.69%	19.39%	15.5%
$S6_b$	Drug + DPPH	1.0956	2.7195	3.2701
	Drug + Methanol	0.1069	0.0407	0.0235
	% of Activity	**89.57%**	**79.26%**	**60.36%**
$S6_c$	Drug + DPPH	0.6923	1.0148	1.7322
	Drug + Methanol	0.3584	0.1484	0.0431
	% of Activity	**96.06%**	**84.21%**	**69.72%**
$S6_d$	Drug + DPPH	1.9834	1.3859	1.9397
	Drug + Methanol	0.5734	0.2619	0.1467
	% of Activity	58.03%	66.54%	46.63%
$S6_e$	Drug + DPPH	0.6610	0.8772	1.0178
	Drug + Methanol	0.0119	0.0066	0.0060
	% of Activity	38.93%	18.09%	4.80%
Ascorbic acid	Absorbance	0.9375	0.5862	0.0133
	% of Activity	89%	79.29%	62.28%
Absorbance of Negative control (DPPH + methanol) = 3.3596				

RESULT AND DISCUSSION

RESULT: In Vitro Xanthine Oxidase inhibitory activity

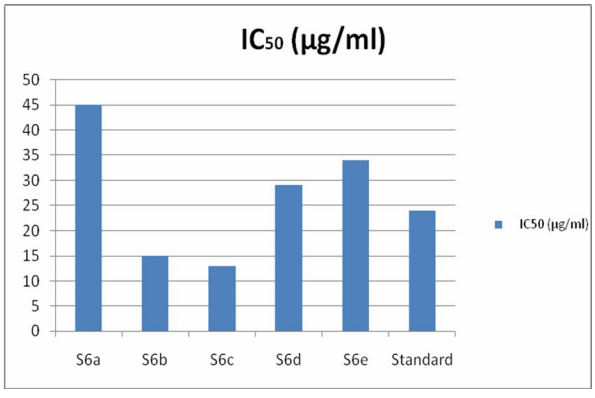
All the newly synthesized triazole derivatives of indole were evaluated for their invitro Xanthine oxidase inhibitory activity. Whereas, Xanthine oxidase is an enzyme responsible for the generation of reactive oxygen species. Evaluation was carried out for all the newly synthesized compounds and the Percentage of inhibition for all the concentration ranging from 5μg/ml to 100μg/ml was calculated with the percentage of inhibition of the standard (Allopurinol) which was found to be 85.39% at the concentration100μg/ml.

- ✓ In S6 $_{(a-e)}$ series compound $S6_b$ and $S6_c$ showed high activity at all the concentration ranging from (5μg/ml to100μg/ml) compared to the standard.
- ✓ Other compounds were found to be less active in all the concentration.

In the newly synthesized compounds $S6_b$ and $S6_c$ showed 95.29% and 94.09% inhibitory activity at the concentration of 100 μg/ml. S6b and $S6_c$ showed 74.91% and 83.27% inhibitory activity at the concentration 50 μg/ml. Among the compounds synthesized, $S6_b$ and $S6_c$ showed maximum inhibitory activity compared to the standard drug Allopurinol. Their values are listed in the table below.

IC_{50} VALUE OF THE NEWLY SYNTHESIZED COMPOUNDS

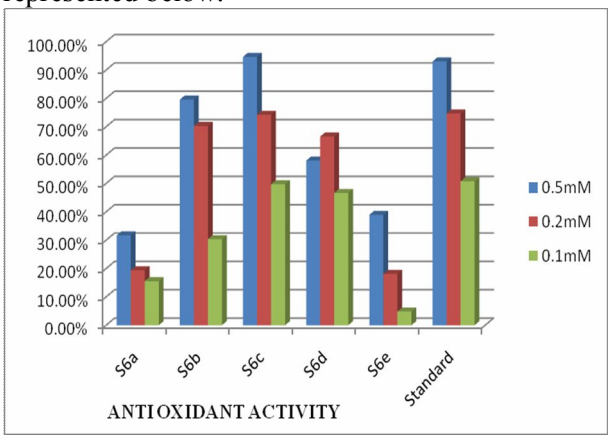
compound	IC_{50} (µg/ml)
$S6_a$	45
$S6_b$	15
$S6_c$	13
$S6_d$	29
$S6_e$	34
Standard (Allopurinol)	24



The IC_{50} values of the synthesized compounds were found by plotting graph of Percentage inhibition Vs Concentration in μg/ml. The values were compared with that of the standard. Among the synthesized compounds, compound $S6_b$ and $S6_c$ showed lowest IC_{50} value of 15 and 13μg/ml respectively compared to the Standard drug Allopurinol which showed IC_{50} value of 24 μg/ml

ANTI OXIDANT STUDIES

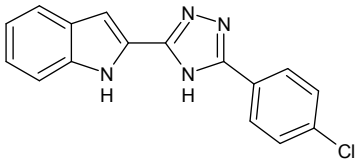
The free radical scavenging activity was carried out for the triazole derivative of Indole and the Percentage of inhibition for all the concentration (0.5mM, 0.2mM and 0.1mM) was calculated. The values are compared with the Percentage of scavenging activity of the standard (Ascorbic acid) which was found to be 93%. Results are graphically represented below.



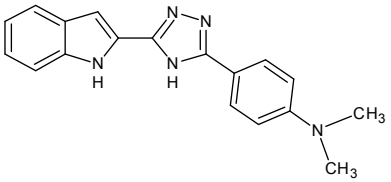
DISCUSSION:-

Among the synthesized compound $S6_a$ and $S6_b$ showed higher anti oxidant property which was determined using Xanthine Oxidase and DPPH$^{\bullet}$. As per the QSAR study, compound synthesized were compared with the standard drug Allopurinol (Bioactive Score of -0.18) and Ascorbic Acid (Bio active score of -1.67) for their GPCR ligand activity. According to the drug likeness rules[19], larger the value of the Bioactive score (>-0.05), higher the probability of the particular molecule to be active. So from the drug likeness data, the highest score of the active compounds were considered. In $S6_{(a-e)}$ series compound $S6_b$ and $S6_c$ showed highest score of 0.18 and 0.19 respectively, likewise compound $S6_a$, $S6_d$ and $S6_e$ showed their highest score of 0.14, 0.14 and 0.13 respectively for GPCR ligand. Compounds of S6 $_{(a-e)}$ series showed highest score ranging from 0.46 to 0.40 for kinase receptor. Compound $S6_b$ and $S6_c$ showed highest Antioxidant and enzyme inhibitory activity. They also showed a highest bioactive score for both GPCR and Kinase receptors. All the newly synthesized

compounds showed higher bioactive score compared to the standard drug Allopurinol which showed bioactive score of -0.18 for GPCR ligand and that of the standard drug ascorbic acid which showed bioactive score of -1.67.Compound $S6_b$ and $S6_c$ showed maximum enzyme inhibitory activity and antioxidant activity compared to the standard drug Allopurinol. Their bioactive score were comparatively greater than the standard. Other compounds showed very less activity even though their bioactive score were greater than the standard. Hence, compound $S6_b$ and $S6_c$ which satisfy Lipinski's rule[20] and Drug likeness property can be taken as a lead for Xanthine Oxidase inhibitors and as antioxidants.



Compound $S6_b$



Compound $S6_c$

CONCLUSION:

According to drug likeness study, Compounds $S6_b$ and $S6_c$ were identified as lead moiety for Xanthine Oxidase inhibition activity and antioxidant activity. They showed high drug likeness score compared to that of standard drug Allopurinol and Ascorbic Acid and it also obeys the Lipinski's rule. Since the compounds $S6_b$ and $S6_c$ posses highest inhibitory activity for DPPH radical and Xanthine oxidase it has been identified as lead moiety for Antioxidant activity.

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