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Design, molecular docking, synthesis of some thiazolidinone derivatives and evaluation of its xanthine oxidase inhibitory activity

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ABSTRACT

Structure-based lead optimization approaches are a major role in the drug-discovery process. In the present work, lead compounds that satisfied Lipinski rule of five, drug likeness score and docking studies performed by using Argus lab 4.0. The designed compounds were evaluated for Xanthine oxidase(XO) inhibition binding ability to identify the new lead compounds by molecular docking. XO is the key enzyme that catalyzes the oxidation of hypoxanthine to xanthine and then to uric acid. Hyperuricemia is an underlying cause of gout& cardiovascular diseases. Allopurinol, a widely used XO inhibitor and commonly used drug to treat gout. However, a significant portion of the population suffers from adverse effects of allopurinol like gastrointestinal upset and skin rashes. Therefore use of allopurinol-like drugs with minimum side effects is the ideal drug of choice against gout. In this study, we report the synthesis of a series of thiazolidinone-4-one analogues as effective and a new class of XO inhibitors. TZ2 &TZ4 molecules showed good inhibition against XO, which were more potent than allopurinol based on their respective IC_{50} values. According to the docking study TZ2 and TZ4 identified as the lead moiety and showed best docking score (TZ2=-10.074kcal/mol, TZ4=-9.158kcal/mol) compared to that of standard drug (-6.91kcal/mol). These results highlight the identification of a new class of XO inhibitors that have potential to be more efficacious, than allopurinol, to treat gout and against cardiovascular diseases.

Key words: Molecular docking, Argus lab, Thiazolidinone, Xanthine oxidase, Allopurinol.

INTRODUCTION

The development and implementation of a range of molecular docking algorithms, based on different search methods was observed in the last few years. This approach has had several recent successes in drug discovery. In the field of molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using for example scoring functions. Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs. Given the biological and pharmaceutical significance of molecular docking, considerable efforts have been directed towards improving the methods used to predict docking. Evaluation of existing docking algorithms can assist in the choice of the must suitable docking programs for any particular

study. Effectively, several studies estimating and comparing the accuracies of protein-ligand programs like Dock, ICM, and Gold have been reported.

Xanthine oxidase, localized in vascular endothelium, is an important enzyme in health and disease Hyperuricemia is the underlying cause of gout and other ailments. Xanthine oxidase is the key enzyme which is involved in the oxidation of hypoxanthine to xanthine and then to uric acid. Allopurinol, a widely used xanthine oxidase inhibitor is the most commonly used drug for the treatment of gout. Significant portion of the population suffers from adverse effects of allopurinol that includes gastrointestinal upset, skin rashes and hypersensitivity reactions. Moreover, an elevated level of uric acid is considered as an independent risk factor for cardiovascular diseases.

Therefore use of allopurinol-like drugs with minimum side effects is the ideal drug of choice against gout. In this review, synthesis and design of a series of thiazolidinone-4-ones analogues) as effective xanthine oxidase inhibitors has been reported.

we have designed some new heterocyclic analogues of thiazolidinone-4-ones derivatives, Moreover the designed compounds were evaluated for xanthine oxidase inhibition binding ability to identify the new lead compounds by molecular docking. The most potent compounds selected as lead on which carried out structural modification inorder to obtain new ligands with excellent binding ability.

MATERIALS AND METHODS

MOLECULAR DOCKING:

Preparation of protein molecule

The experimental structure of xanthine oxidase (XO) (PDB ID: 1FIQ) as shown in Figure 1 was retrieved from the RCSB protein data bank as a PDB file. The protein molecules were prepared mainly by using the software Swiss PDB viewer. Active site residues within a range of 4.0 A0 were selected and saved in PDB format.

Preparation of ligand

The ligand compounds thiazolidinone derivatives were drawn using ACD/ Chemsketch (12.0) (Alex, 2009) and saved in mol 2 format. The saved ligand compounds were later imported and minimized in Argus Lab after adding hydrogen bonds. The molecules thus obtained were saved in PDB format.

Argus Lab:

ArgusLab4.0 has fast become a favorite introductory molecular modeling package with academics mainly because of its user-friendly interface and intuitive calculation menus (Thompson, 2004). The ArgusDock docking engine, implemented in ArgusLab, approximates an exhaustive search methods. Flexible ligand docking is possible with ArgusLab, where the ligand is described as a torsion tree and grids are constructed that overlay the binding site. Ligand's root node (group of bonded atoms that do not have rotatable bonds) is placed on a search point in the binding site and a set of diverse and energetically favorable rotations is created. For each rotation, torsions in breadth-first order are constructed and those poses that survive the torsion search are scored. The N-lowest energy poses are retained and the final set of poses undergoes coarse minimization, re-clustering and ranking

Docking of thiazolidinone derivatives to xanthine oxidase:

Docking of thiazolidinone derivatives(TZ1-TZ5) with xanthine oxidase was performed using ARGUS LAB4.0. The algorithm exhaustively searches the entire rotational and translational space of the ligand with respect to the receptors. The various solutions evaluated by a score, which is equivalent to the absolute value of the total energy of the ligand in the protein environment. The best docking solutions ARGUS LAB score for each compound was considered. It was noted that ARGUS LAB scores of comp TZ1 and TZ4 was -10.074 and -9.158 respectively, which is greater than allopurinol drug score value -6.91. as shown in Table 1, Figures 2 and 3.

The drug like activity of the ligand molecules are characterized using ADME properties. Allopurinol and thiazolidinone derivatives satisfy Lipinski rule of 5 and ADME properties results are shown in Table 2.

Table 1. ARGUS LAB scores and interactions of Allopurinol drug and thiazolidinone derivatives.

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S.No	Compound	Argus- Lab (Kcal/mol)
1	**TZ_1**	-9.126
2	**TZ_2**	-10.074
3	**TZ_3**	-8.519
4	**TZ_4**	-9.158
5	**TZ_5**	-8.433
6	Alloporinol	-6.91

Table 2. Lipinski rule of thiazolidinone derivatives and allopurinol drug.

Compound Code	Log P	MW	"H" Donor	"H" Acceptor	Violation	GPCR Ligands
TZ_1	1.939	353.40	5	2	0	0.20
TZ_2	3.096	371.84	2	5	0	0.17
TZ_3	2.521	380.47	4	3	0	0.17
TZ_4	2.475	367.43	2	6	0	0.15
TZ_5	1.758	383.42	3	7	0	0.15
Allopurinol	0.534	152.00	2	5	0	-0.18

Fig. 1 Crystal structure of bovine milk XO (1FIQ) from Protein Data Bank (entry code: 1FIQ)

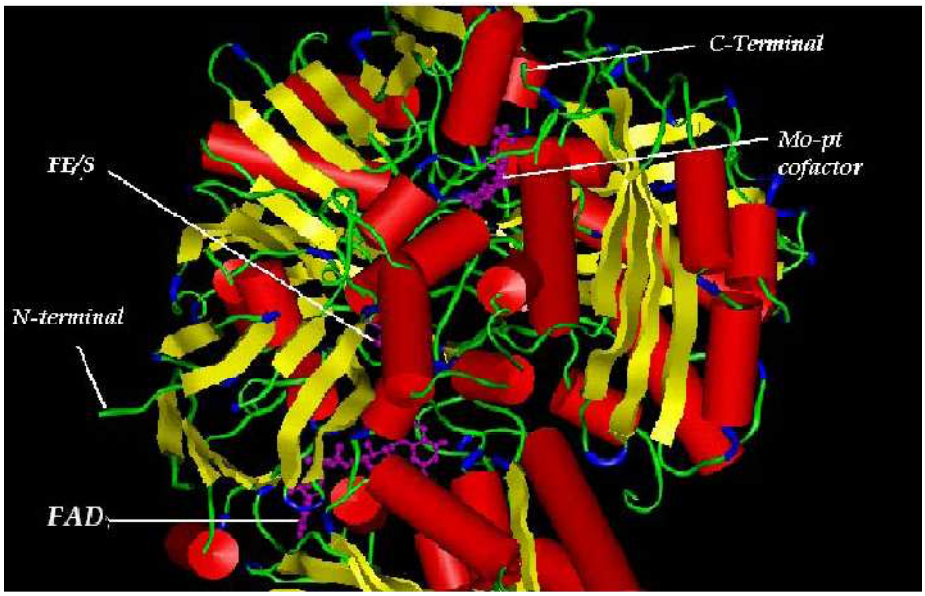


Fig. 2 Amino acid residues that contribute in the catalytic reaction in the active site

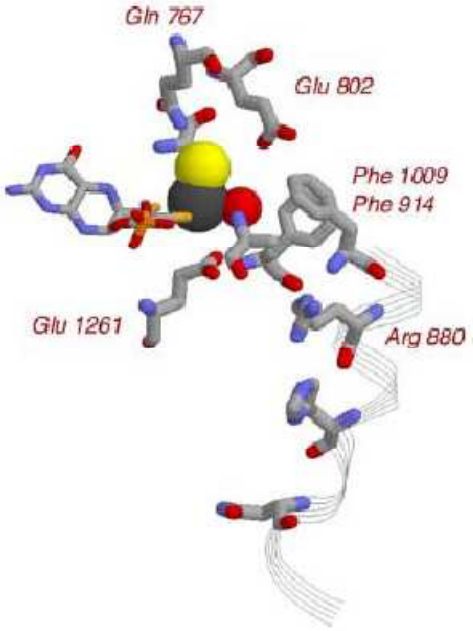


Fig. 3 Molecular docking in Argus Software(allopurinol)

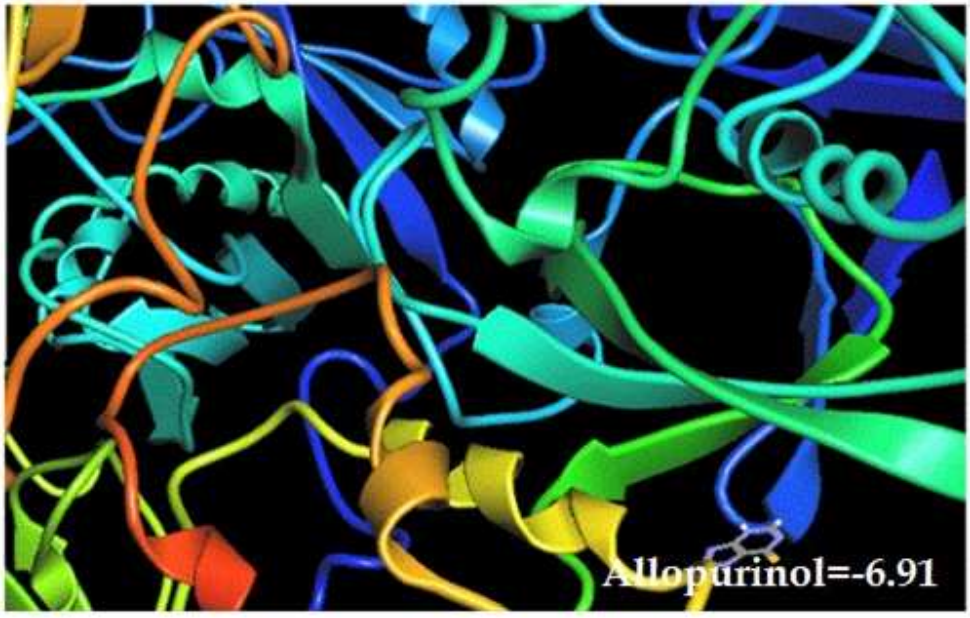


Fig. 4 Molecular docking in Argus Software(TZ2)

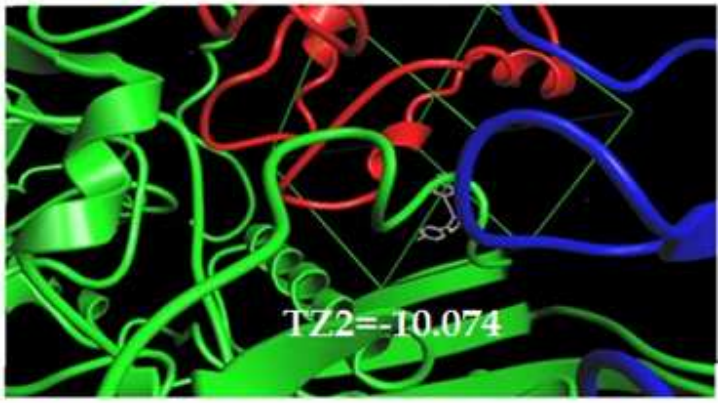
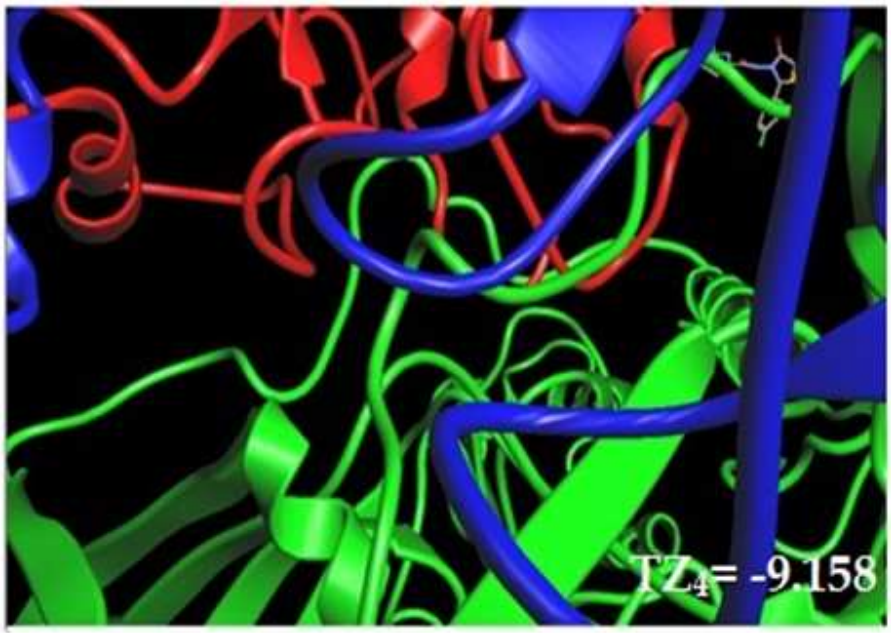


Fig. 5 Molecular docking in Argus Software(TZ4)



SYNTHESIS:

Step I

Synthesis of Indole-2-carboxylic acid ester12

A mixture of 20.4 gm, (0.1 mole) of indole-2- carboxylic acid,6.4mlof methanol, 100 ml of dichloromethane and 5drops of concentrated sulphuric acid was refluxed for 5 hrs and cooled to 5°C. The contents were poured into 100ml of ice cold water. The organic layer at the bottom was separated anddichloromethane was distilled off to get the crude product. The high vacuum distillation of this crude product afforded the pure compound which is crystallized from methanol. The purity of the ester was established by single spot on the TLC plate. The solvent system used was methanol : chloroform (3:1).

Step II

Synthesis of Indole-2-carbohydrazide13

To 21.7g,(0.1mol)of Indole-2-carboxylicacidesterin 20 ml ethanol, 2 ml of 99% hydrazine hydrate was added in drops with constant stirring and themixturewasrefluxed for 4 hrs . After cooling, the solution was poured on to crushed ice. The solid separated was filtered, dried and recrystallised from methanol. The purity of the compound was established by single spot on the TLC plate. The solvent system used was methanol : chloroform (3:1).

Step III
Synthesis of Schiff's Base14
Indole-2-acid hydrazide (1mol) and arylaldhyde (1mol) were dissolved in 15 ml of ethanol in a 100 ml beaker and the mixture was refluxed for 2 hrs. The reaction mixture was cooled and the solid formed was separated by filtration, washed with cold ethanol and recrystalised from ethanol. Purity of the product was established by single spot on the TLC plate. The solvent system used was methanol : chloroform (3:1).

Step IV
Synthesis of substituted thiazolidinone 15
A mixture of Schiff base (1mM) in DMF and 0.92 ml of thioglycolic acid with a pinch of Zinc chloride was taken in a 100 ml beaker and the reaction mixture was zapped inside a microwave oven at 20% for 3 min. The solution was then diluted with ice cold water and solid formed was separated and recrystallized from ethanol.

Compound. TZ1
IR (KBr,cm-1): 3343.96 (N-H str),1716.34 (C=O str),667.25 (C-S-C str),1187.94(C-N str), 1H NMR(DMSO):11.92 (s,1H,indole NH),10.2(s,1H,Ar OH), 8.22 (s, 1H, CONH), 7.84 (s, 1H, N-CH, cyclic), 7.23-7.59(m, 7H, ArH), 2.50 (s, 2H, N-CH2). lmax: 286.

Compound. TZ2
IR (KBr,cm-1): 3340.10 (N-H str),1716.23 (C=O str),663.39 (C-S-C str),1189.94(C-N str), 740.53(C-Cl str). 1H NMR(DMSO):11.54 (s,1H,indole NH), 8.32 (s, 1H, CONH), 7.8 (s, 1H, N-CH, cyclic), 7.2-7.6(m, 7H, ArH), 2.50 (s, 2H, N-CH2). lmax: 312.

Compound. TZ3
IR (KBr,cm-1): 3337.10 (N-H str),1718.3 (C=O str),668.39 (C-S-C str),1186.94(C-N str), 2850.56, (N-CH3 str). MS(m/z+)- 381.(M+1), 222.178,161. lmax: 306.

Compound. TZ4
IR (KBr,cm-1): 3343.10 (N-H str),1718.23 (C=O str),673.39 (C-S-C str),1191.94(C-N str), 1098.53 (0- CH3 str). lmax: 321

Compound. TZ5
IR (KBr,cm-1): 3345.6 (N-H str),1719.4 (C=O str),668.25 (C-S-C str),1188.94(C-N str), 1099.3(0-CH3 str). lmax: 36

***in vitro* Xanthine oxidase inhibitory Activity**
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 a little volume of DMSO (not exceeding more than 5% of total volume) initially and then made up to the required volume with KH2PO4 buffer of pH 7.5. The assay mixture consisted of 1 ml of test solution (5-100 mg/mg), 2.9 ml of KH2PO4 buffer [pH 7.5; adjusted with 1 M KOH] and 0.1 ml of xanthine oxidase (XO) enzyme solution (0.1/ml in KH2 PO4 buffer, pH 7.5) prepared immediately before use. After preincubation at 25°C for 15 min, the reaction was initiated by the addition of 2ml of the substrate solution (150 mM xanthine in phosphate buffer of pH 7.5). The assay mixture was incubated at 37°C for 10 min and 0.5 ml of 0.58M HCl was added to stop the reaction. The absorbance was measured at 290 nm against blank (buffer solution) and percentage of inhibition was calculated using the following formula.16,17,18,19

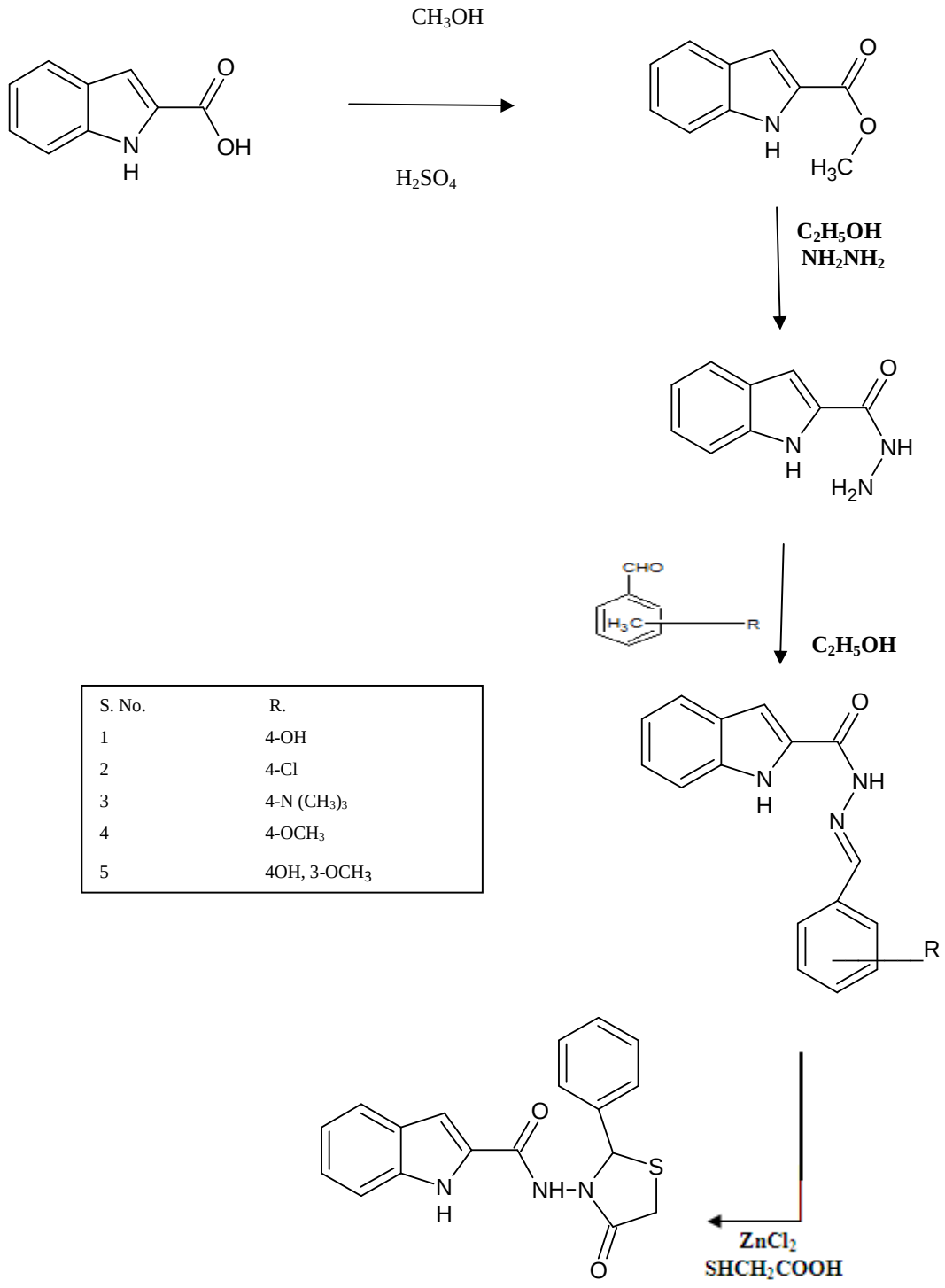
% of inhibition = [1 - B/A) x 100

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 mg/ml) was used as a positive control). The details of the activity of the compounds were shown in table:4



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

TABLE NO: 3: PHYSICAL CHARACTERIZATION OF NEWLY SYNTHESIZED COMPOUNDS

S.No.	Compound Code	R	Molecular Formula	Molecular Weight	Melting Point	R_f value	% yield
1	TZ_1	4-hydroxy	$C_{18}H_{15}N_3O_3S$	353.40	79.9°C	0.69	82%
2	TZ_2	4-chloro	$C_{18}H_{14}ClN_3O_2S$	371.84	75.7°C	0.68	87%
3	TZ_3	4-dimethyl amino	$C_{20}H_{20}N_4O_2S$	380.47	82°C	0.72	84%
4	TZ_4	4-methoxy	$C_{19}H_{17}N_3O_3S$	367.43	78.9°C	0.78	73%
5	TZ_5	4-hydroxy-3-methoxy	$C_{19}H_{17}N_{30}O_4S$	383.42	77.9°C	0.74	72%

Solvent System = Methanol : Chloroform

TABLE NO:4 IN VITRO XANTHINE OXIDASE INHIBITORY ACTIVITY

Compound Code	5µg/ ml	10 µg/ml	25µg/ml	50µg/ml	100µg/ml	IC_{50} (µg/ml)
TZ_1	43.48 ±0.32	56.30±0.22	65.75±0.21	73.52±0.11	91.16±0.18	15
TZ_2	45.03±0.18	65.51±0.27	71.13±0.06	84.87±0.19	95.19±0.21	12
TZ_3	38.34±0.15	43.18±0.18	45.21±0.45	49.04±0.73	52.40±0.27	27
TZ_4	43.18±0.26	64.74±0.32	71.75±0.36	81.77±0.34	94.09±0.07	13
TZ_5	36.44±0.17	39.51±0.38	44.14±0.28	46.75±0.18	50.15±0.32	29
Standard (Allopurinol)	48.20±0.07	59.75±0.04	75.76±0.18	85.19±0.06	89.95±0.05	25

RESULTS AND DISCUSSION

All the synthesized compounds were characterized by recrystallization, TLC, Melting point, UV, IR, 1HNMR analysis,and Mass fragmentation pattern. All the synthesized structures showed satisfactory result. The chemical shift values of the synthesized compounds were full agreement with the number of protons present in it.

All the newly synthesized thiazolidinone derivatives of indole were evaluated for their in vitro 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 5mg/ml to 100 mg/ml was calculated with the percentage of inhibition of the standard (Allopurinol) was also calculated and it was found to be 89.95% at the concentration 100mg/ml.

Among the newly synthesized compounds (TZ1 - TZ5) TZ2 and TZ4 showed high Xanthine inhibitory activity at 10 mg / ml, 50 mg/ml and 100 mg/ml concentrations. The IC50 value for these compounds were found and to be 12 mg/ ml and 13 mg/ml which is less than the IC50 value for Allopurinol, the standard (25 mg/ml) showing that these compounds exhibits high Xanthine inhibitory activity.

Compound TZ_2 and TZ_4 showed maximum enzyme inhibitory activity compared to the standard drug. Their bioactive score were comparatively greater than the standard. Hence, compounds TZ_2 and TZ_4 which satisfy Lipinski's rule, drug likeness property and showing low energy level in docking study can be taken as a lead for Xanthine oxidase inhibitors. From the research work we found the indole ring incorporated with a thiazolidinone moiety have significant role in the enzyme inhibitory activity. The invitro activity also suggest that the derivatives obtained from the presence of chloride and methoxyl group in the para substituted aldehyde having appreciable activity.

These results highlight the identification of a new class of XO inhibitors that have potential to be more efficacious, than allopurinol, to treat gout and against cardiovascular diseases.

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