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Docking Studies of Potent Xanthine Oxidase Inhibitors-Molecules Patented and Published from 2011-2020

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ABSTRACT

Hyperuricemia is characterized by elevated serum uric (SUA) levels beyond 6.8mg/dl and is a major cause of gout. Clinically, the high uric acid levels are managed using oral xanthine oxidase inhibitors such as febuxostat; however, its long-term use affects liver functions and cannot be used with the person with compromised liver functions.Thus, searching for an alternate xanthine oxidase inhibitor devoid of side effects on the liver is of current interest. Several classes of XO inhibitors have been patented in recent years. Using a docking study, we investigated the binding mode of xanthine oxide inhibitors patented during 2011-2020.The study identified the crucial amino acid residues involved in drug-receptor interactions. The binding side residues and their role in stabilizing the drug-receptor complex is discussed in detail. The information gained from this study will help researchers to design potent and selective xanthine oxidase inhibitors to manage gout and other uric acid-related disorders effectively.

Keywords: Xanthine oxidase inhibitors, Hyperuricemia, Uric acid, Docking, Gout.

INTRODUCTION

Xanthine oxidase is a molybdoflavoprotein mainly found in milk, lung, heart, kidney, and vascular endothelium. XO is involved in purine metabolism, producing uric acid and reactive oxygen species leading to high serum uric acid levels, inflammation, and oxidative damage[1-3]. Xanthine oxidase inhibitors have been used to control elevated uric acid levels, known as hyperuricemia resulting in gout[4,5]. The first drug to inhibit XO and control hyperuricemia is allopurinol (purine-based) (Fig.1) approved by FDA in 1966; however, its associated adverse effects limit its clinical use. A High dose of allopurinol induces hypersensitivity reactions in combination with several other side effects[6]. Another non-purine XO inhibitor for treating hyperuricemia in patients with severe chronic renal impairment is Febuxostat (Fig. 1), approved by the FDA[7]. After the augmented risk of issues related to heart with febuxostat, FDA, in the year 2019, issued a 'Boxed warning' even though the mechanism for cardiac toxicity is unclear. Post-FDA, WHO also issued a worldwide warning for the use of febuxostat only if any other drug cannot soothe a patient condition[8,9]. The recent patent study highlighted that a large number of efforts are made toward the discovery of non-purine and natural compounds. The literature has emphasized on

highly potent molecules acting as XO inhibitors have nanomolar IC_{50} Values. However, they also show off-target activities associated with several adverse effects[10]. Structure-based drug design (SBDD) approach offers a valuable tool to design potent and target selective drug molecules, thus minimizing the side effects of the therapy. SBDD has been used to develop novel therapeutics for treating several disorders[11]. In the present study, we have carried out a docking study on some known XO inhibitors to understand their binding mechanism. The study helped us to propose new XO inhibitors. This will further enable researchers to develop potent and target-specific XO inhibitors. The material methods and results are presented here.

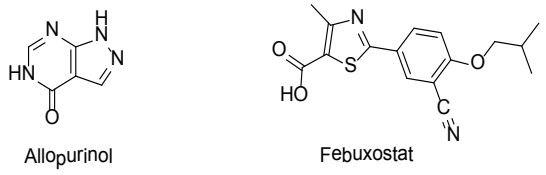

Fig. 1. 2D-Structure of allopurinol and febuxostat

MATERIALS AND METHODS

The study investigates the binding mode of potent XO inhibitors using a docking study. The database of XO inhibitors for docking study was developed considering the biological activity of XO inhibitors available from the public domain. The database was utilized in a systematic docking study, and amino acid residues involved in drug-receptor interactions were analyzed. The details of the material and methods are presented here.

Selection of Compounds for Docking studies

Compounds patented, published, or under clinical trials as potent xanthine oxidase inhibitors from 2011 to 2020 were taken for present research work[10,12]. Compounds were selected based on the IC_{50} value in the nanomolar range (Table 1). Two-dimensional (2D) files of molecules were transformed into three-dimensional (3D) format and optimized using the standard protocol in chem 3D software and MOE software.

Table 1: 2D-structure of xanthine oxidase inhibitors selected for docking studies

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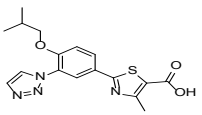
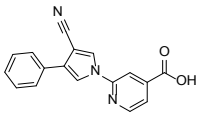
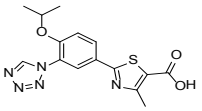
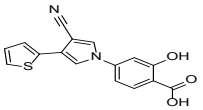
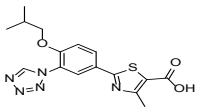
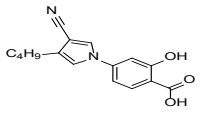
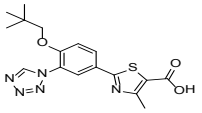
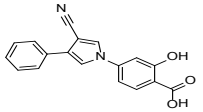
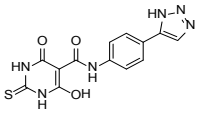
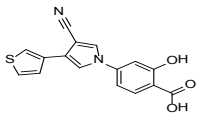
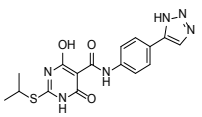
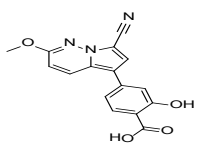
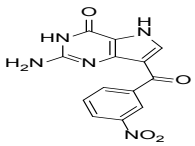
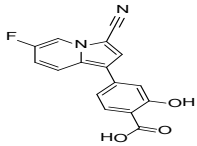
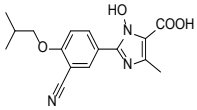
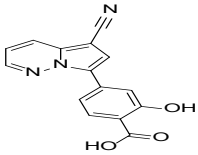
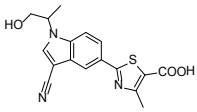
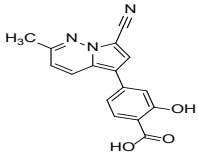
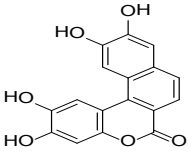
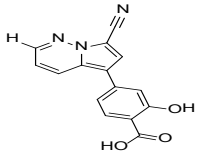
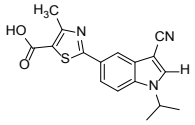
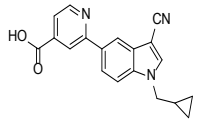
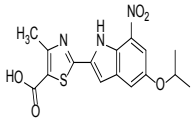
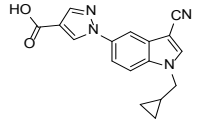
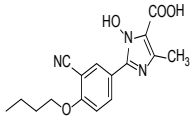
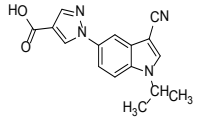
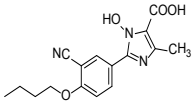
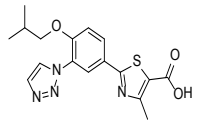
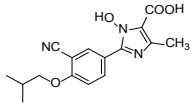
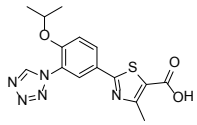
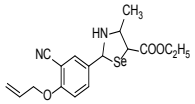
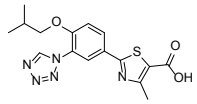
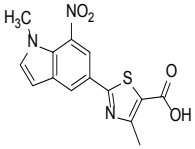
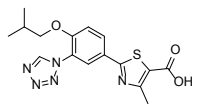
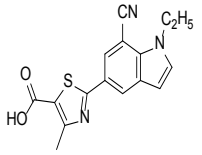
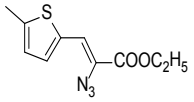
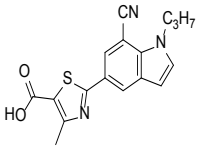
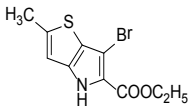
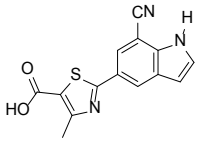
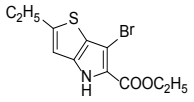
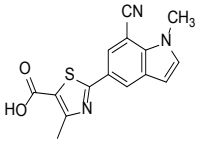
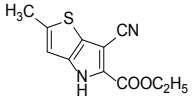
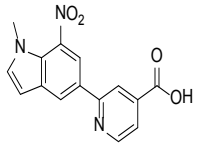
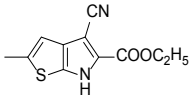
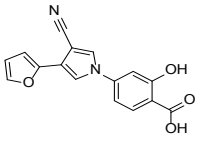
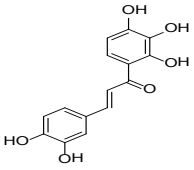
1		<5	24		52.1
2		<5	25		7.8
3		<5	26		3.3
4		<5	27		6.2
5		60	28		5.2
6		25	29		3

7		55.1	30		4
8		6	31		4
9		3	32		5
10		60	33		5
11		3.5	34		2.1
12		5.1	35		2.8
13		3	36		3.4
14		3	37		<5
15		6	38		<5
16		5.5	39		<5
17		8	40		<5

18		5	41		13
19		5	42		10
20		7	43		10
21		8	44		14
22		48	45		13
23		1.7	46		74

[1]Compounds no. 1-9 are from reference 10, and 10-46 are from reference11

Development of docking model for structure-based virtual screening

The coordinates of bovine milk XO complexed with salicylic acid were retrieved from the protein data bank (PDB entry: 1fiq). XO is a metalloenzyme that contains molybdenum as a cofactor at the catalytic site. Here, molybdenum (VI) plays a crucial role in electron transfer reactions, consequently leading to substrate oxidation. In the present study, we have considered the catalytic site of XO as a target-binding site for inhibitors. Any compound that binds at the XO's catalytic site may inhibit the natural substrate from interacting with the molybdenum cofactor and thus suppresses the XO activity [15]. Chemdraw software was used to draw ligands, and energy minimization of the ligand was done in the MOPAC module, using the AM1 method for closed-shell system, available in the CS Chem3D Ultra. The ligands were drawn in ChemDraw and subjected to energy minimization in the MOPAC module, using the AM1 procedure for closed-shell systems, implemented in the CS Chem3D Ultra. Using GOLD 5.3.0, ligands were docked into the catalytic site of xanthine oxidase[15]. Genetic algorithm-based ligand docking is enacted by Gold to enhance the contour of the ligand at the receptor-binding site. It employs the GoldScore fitness function to appraise the several conformations of ligand at the binding site. It consists of four components: protein-ligand hydrogen bond energy, protein-ligand van der Waals (vdw) energy, ligand internal vdw energy, and ligand torsional strain energy measured by the Goldscore fitness function[13]. Docking was replicated ten times for each isomer, and the Goldscore fitness function was used to rank each

pose. The highest scored conformations were carefully chosen for discussion.

Docking validation

The Docking procedure was confirmed by reproducing the crystal structure conformation of the co-crystallized ligand using Pymol version 1.3. The validation was carried out by re-docking and calculating the RMSD value between the docked ligand and the reference ligand to evaluate the performance of the docking protocol. Protein-ligand docking is an instrument to envisage binding poses and evaluate the binding affinity.

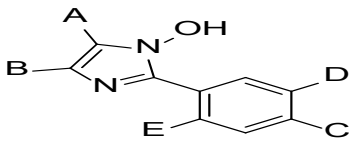
Docking of selected compounds

Docking studies for molecules with activity in a nanomolar range from patented and published molecules from 2011 to 2020 were carried out (Table 1). A Docking study was performed using GOLD 5.3.0 (Cambridge Crystallographic Data Center, Cambridge, UK). Based on binding affinity, Goldscore is used as a scoring function. The binding site was defined based on salicylic acid as a reference ligand. The cavity was set within 6 Å of the bound molecule affinity. Docking was replicated ten times for each isomer, and the Goldscore fitness function was used to rank each pose. Interactions with amino acids were checked using GOLD software images were prepared. Based on the interactions with amino acid residues Phe914, Phe1009, Arg880, Thr1010, docking results of hit compounds were assessed visually to select the best interaction pose.

Design of Derivatives and their docking study

Based on the docking score of compounds 8 and 23, two series of compounds A and B, were prepared for further docking study. Hydrophobic and hydrophilic groups were used to prepare derivatives. Docking was performed for the derivatives (Table 2 and Table 3).

Table 2: Structure of derivatives of scaffold 8 and their Goldscore

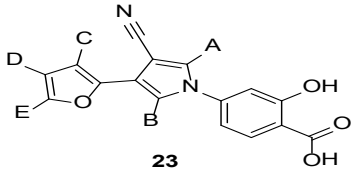


8

S.No.	Compound	A	B	C	D	E	Goldscore
1	8a	-COOH	$-CH_3$	H	H	$-CH_3$	57.06
2	8b	-COOH	$-CH_3$	H	H	-OH	60.20
3	8c	-COOH	$-CH_3$	H	H	$-NH_2$	59.56
4	8d	-COOH	$-CH_3$	H	H	-Cl	59.22
5	8e	-COOH	$-CH_3$	H	H	-F	57.02
6	8f	-COOH	$-CH_3$	H	H	$-NO_2$	73.45
7	8g	-COOH	$-CH_3$	H	H	$-NHCH_3$	56.61
8	8h	-COOH	$-CH_3$	H	H	$-OCH_3$	58.02
9	8i	-COOH	$-CH_3$	H	H	$-COCH_3$	59.09
10	8j	-COOH	$-CH_3$	H	H	-COOH	66.86
11	8k	-COOH	$-CH_3$	H	H	$-OCOCH_3$	56.47
12	8l	-COOH	$-CH_3$	H	H	$-C_2H_5$	56.20
13	8m	-COOH	$-CH_3$	$-CH_3$	H	H	61.25
14	8n	-COOH	$-CH_3$	-OH	H	H	59.94
15	8o	-COOH	$-CH_3$	$-NH_2$	H	H	61.09
16	8p	-COOH	$-CH_3$	-Cl	H	H	60.16
17	8q	-COOH	$-CH_3$	-F	H	H	57.03
18	8r	-COOH	$-CH_3$	$-NO_2$	H	H	56.65
19	8s	-COOH	$-CH_3$	$-NHCH_3$	H	H	60.74
20	8t	-COOH	$-CH_3$	$-OCH_3$	H	H	59.35
21	8u	-COOH	$-CH_3$	$-COCH_3$	H	H	62.33
22	8v	-COOH	$-CH_3$	-COOH	H	H	60.13
23	8w	-COOH	$-CH_3$	$-OCOCH_3$	H	H	66.64
24	8x	-COOH	$-CH_3$	$-C_2H_5$	H	H	67.35
25	8y	-COOH	$-CH_3$	H	$-CH_3$	H	58.20

26	8z	-COOH	$-CH_3$	H	-OH	H	59.54
27	8aa	-COOH	$-CH_3$	H	$-NH_2$	H	60.32
28	8ab	-COOH	$-CH_3$	H	-Cl	H	58.20
29	8ac	-COOH	$-CH_3$	H	-F	H	57.72
30	8ad	-COOH	$-CH_3$	H	$-NO_2$	H	49.43
31	8ae	-COOH	$-CH_3$	H	$-NHCH_3$	H	57.24
32	8af	-COOH	$-CH_3$	H	$-OCH_3$	H	61.59
33	8ag	-COOH	$-CH_3$	H	$-COCH_3$	H	61.29
34	8ah	-COOH	$-CH_3$	H	-COOH	H	59.95
35	8ai	-COOH	$-CH_3$	H	$-OCOCH_3$	H	63.84
36	8aj	-COOH	$-CH_3$	H	$-C_2H_5$	H	60.96
37	8ak	-COOH	$-CH_3$	$-OCH_3$	H	-NO2	76.11
38	8al	-COOH	$-CH_3$	$-OC_2H_5$	H	-NO2	76.01
39	8am	-COOH	$-CH_3$	c-propyl methyl	H	-NO2	68.92
40	8an	-COOH	$-CH_3$	isopropyl	H	-NO2	65.44
41	8ao	-COOH	$-CH_3$	isobutyl	H	-NO2	62.86
42	8ap	-COOH	$-CH_3$	neopentyl	H	-NO2	62.82
43	8aq	-COOH	$-CH_3$	$-CH_3$	H	-NO2	69.66
44	8ar	-COOH	$-CH_3$	$-C_2H_5$	H	-NO2	69.00
45	8as	-COOH	$-CH_3$	$-C_3H_7$	H	-NO2	71.91
46	8at	-COOH	$-CH_3$	$-CH_2Cl$	H	-NO2	69.90
47	8au	-COOH	$-CH_3$	$-CCl_3$	H	-NO2	63.61

Table 3: Structure of derivatives of scaffold 23 and their Goldscore



23

S. no.	Compound	A	B	C	D	E	Goldscore
1	23a	-OH	-H	-H	-H	-H	69.73
2	23b	-F	-H	-H	-H	-H	68.49
3	23c	-Cl	-H	-H	-H	-H	68.30
4	23d	-H	-OH	-H	-H	-H	69.30
5	23e	-H	$-NH_2$	-H	-H	-H	68.59
6	23f	-H	a*	-H	-H	-H	60.57
7	23g	-H	-H	CH_2OH	-H	-H	69.58
8	23h	-H	-H	$-NH_2$	-H	-H	71.50
9	23i	-H	-H	-SH	-H	-H	72.39
10	23j	-H	-H	-H	$-CF_3$	-H	69.41
11	23k	-H	-H	-H	$-CH_3$	-H	68.10
12	23l	-H	-H	-H	$-CCl_3$	-H	72.74
13	23m	-H	-H	-H	$-OCH_3$	-H	71.48
14	23n	-H	-H	-H	$-C_2H_5$	-H	70.85
15	23o	-H	-H	-H	-H	$-CF_3$	70.75
16	23p	-H	-H	-H	-H	$-CH_3$	70.07
17	23q	-H	-H	-H	-H	$-CH_2F$	70.10
18	23r	-H	-H	-H	-H	$-CCl_3$	73.97
19	23s	-H	-H	-H	-H	isopropyl	74.01
20	23t	-H	-H	-H	-H	$-C_2H_5$	71.31
21	23u	-H	a**	-H	-H	-H	69.72

a*- C replaced with N in the ring without double bond, a**- C replaced with N in the ring with double bond

RESULT AND DISCUSSION

Docking validation

Docking validation was done using Pymol 1.3 by giving the function "align docked ligand, reference ligand". The RMSD was obtained as 0.00 Å as shown in Figure 2.

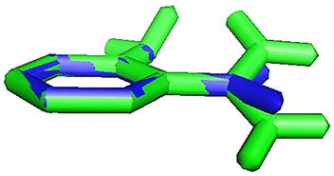


Fig. 2. Overlay of co-crystallized and docked conformation of salicylic acid. (Blue: co-crystallized conformation; Green: docked conformation)

Molecular Docking

The docking score of the compounds, along with the docked conformation and interactions with the amino acids, are shown in Table 4. Interaction Diagrams of the suitable docking compounds are shown in Fig. 3. It was observed that most of the compounds had shown interactions with the Phe914 and Arg880, which play an essential role in xanthine oxidase inhibition.

Design of Derivatives and their docking study

The docking score of the promising derivative compounds, along with the docked conformation and interactions with the amino acids, are shown in Table 5. Interaction Diagrams of the suitable docked compounds are shown in Fig. 3a and 3b. It was observed that the docking score of some of the derivatives increased by 5-10% in comparison to the parent compound.

Table 4: Docking score of high-ranked xanthine inhibitors (Table 1) and amino acid residues involved in D-R interactions

S. no.	Compound	Docked Conformation	Gold score	Amino acid residues involved in D-R interactions
1	4	4	75.84	Phe914, Arg880, Asn768
2	40	3	75.39	Phe914, Arg880
3	37	2	74.81	Phe914, Arg880
4	1	9	74.68	Phe914, Arg880, Phe1009
5	3	3	74.58	Phe914, Arg880, Asn768
6	28	3	73.83	Phe914, Arg880, Glu1261
7	38	2	73.46	Phe914, Arg880, Thr1010
8	39	2	72.91	Phe914, Arg880
9	12	2	72.81	Phe914, Arg880, Thr1010, Glu802
10	27	1	72.6	Phe914, Arg880, Glu1261
11	25	2	72.5	Phe914, Arg880, Glu1261
12	24	2	72.32	Phe914, Arg880, Thr1010
13	26	2	70.64	Phe914, Arg880, Thr1010
14	21	10	70.53	Phe914, Arg880, Thr1010
15	19	3	70.51	Phe914
16	15	2	70.12	Phe914, Arg880
17	46	1	70.11	Phe914, Arg880
18	2	1	69.89	Phe914, Arg880
19	14	1	69.84	Phe914, Arg880
20	6	3	69.51	Phe914, Arg880, Glu1261
21	32	4	69.42	Asn768, Arg880
22	14	2	69.39	Phe914, Arg880
23	29	3	69.06	Phe914, Arg880
24	34	1	68.99	Phe914, Arg880, Thr1010, Asn768
25	18	1	68.86	Phe914, Glu1261, Ser876
26	8	2	68.76	Phe914, Arg880, Thr1010
27	23	1	68.63	Phe914, Arg880, Thr1010
28	9	5	67.62	Phe914, Arg880
29	5	4	67.52	Glu1261

Table 5: Docking score of high-ranked xanthine inhibitors (Table 2 and 3) and amino acid residues involved in D-R interactions

S. no.	Compound	Docked Conformation	Gold score	Amino acid residues involved in D-R interactions
1	8f	3	73.45	Phe914, Glu802, Ala1072, MTE1333
2	8ak	1	76.11	Phe914, Glu802, Ala1079, Glu1261, MTE1333
3	8al	1	76.01	Phe914, Glu802, Ala1079, MTE1333
4	8as	3	71.91	Phe914, Glu802, Ala1079, Phe911, MTE1333
5	23r	1	73.7	Phe914, Arg880
6	23s	10	74.01	Phe914, Arg880

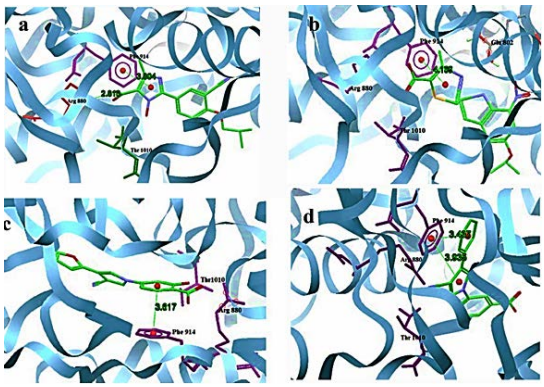


Fig. 3a. Docked conformation and binding interactions of compounds 8 (a), 12 (b), 23(c), and 24(d) with amino acid residues

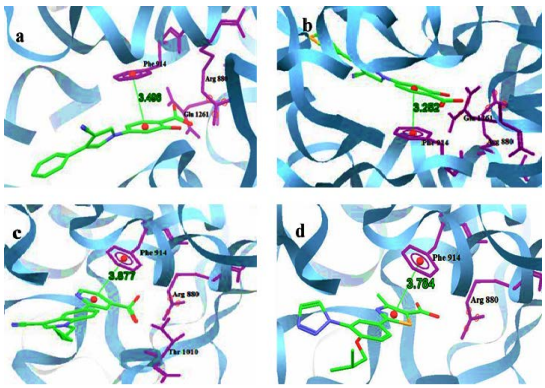


Fig. 3b. Docked conformation and binding interactions of compounds 27(a), 28(b), 34 (c) and 37(d) with amino acid residues

CONCLUSION

The present study was initiated to learn about docking studies and the interactions with amino acids required for xanthine oxidase inhibition for published and patented molecules from 2011-2020. The docking study of approximately 46 molecules with IC_{50} in the nanomolar range was performed. The docking studies showed interactions with amino acids such as Phe914, Phe1009, and Arg880. Molecules 8 and 23 can be potent molecules for xanthine oxidase inhibition based on their docking studies and interaction affinity with amino acids. Based on this, the derivatization of these two molecules was performed. It was observed that derivatized molecules i.e. 8f, 8ak, 8al, 8as, 23r and 23s, have shown around 10% increase in the gold score function compared to their parent molecules. The study provides a suitable docking model for researchers to design and develop potent and selective XO inhibitors.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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