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Protein Docking studies of target proteins using Metformin hydrochloride as a ligand

Eswari Beeram[1], K. Manisha[2], N. Bhavitha[3], S. Himagirish Kumar[4], Dr. Shabana Palyam[5]

[1]Assistant Professor, DBCS, SOLAS, Mohan Babu University, A.P., India.
[2]Research Scholar, DBCS, SOLAS, Mohan Babu University, A.P., India.
[3]Research Scholar, DBCS, SOLAS, Mohan Babu University, A.P., India.
[4]Assistant Professor, Dept. Of Organic Chemistry, Sri Padmavathi Mahila Viswavidyalayam, A.P., India.
[5]Associate Professor, Department of Chemistry, Sanskrithi School of Engineering, Puttaparthi, A.P., India.
Corresponding author email: eshu.sonu@gmail.com

ABSTRACT

Metformin hydrochloride is used as ligand to study its binding interactions with target proteins GLUT-1, GLUT -3, IR and Hexokinase through SWISSDOCK. Protein docking is performed using defined parameters of SWISSDOCK like high calculated affinity in each interaction and the drug showed fruitful interactions with the glycolytic enzyme HK and all the proteins like GLUT-1, GLUT-3 and IR that facilitate glucose uptake. GLUT- 1 interacts with metformin hydrochloride at ASn 98, Met 94, Ileu 196, Phe 137 and Trp386 positions where as metformin hydrochloride interacts with GLUT-3 transporter and shows protein ligand interactions at Phe 81 position, met77 and Ileu 78th positions through hydrogen bonds between carboxylate and 'N' of metformin. GLUT-3 transporter in glucose bound state shows interactions with ligand at ASn315 forming hydrogen bond with N of metformin and O of ASn carboxylate. Other possible interactions include Phe 414, Thr 411, Asn 409 (with O of Asn and N of metformin), Trp 410 and Gln 281positions. Metformin interacts with IR at ASP 1150, ASn 1137 (Hydrogen bonding b/n 'O' of carboxylate of ASn and N of the drug) and C, H &N of Tyr 1158 interacts with N of the drug. Metformin hydrochloride interacts with Hexokinase and Hexokinase bind to ATP analogue at positions like Glu 249 showing ionic interactions between N of drug metformin with C of Glutamic acid at 249 positions. Leu795 'O' interacts with N of metformin by hydrogen bonding interactions. Ileu 245, Val 248 and Arg 243 by hydrogen bonding between N of drug and O of the amino acids Ileu 245, Val 248 and Arg 243 (Hexokinase + ATP analogue). Drug does not show Hydrophobic interactions and π- interactions with the proteins studied.

KEYWORDS: Insulin Receptor, Hexokinase, GLUT-1 Transporter, GLUT-3 Transporter, SWISSDOCK, Metformin hydrochloride.

INTRODUCTION

Metformin is a drug used to treat Type II diabetes without impairing insulin secretion and causing weight gain. Recent docking studies of metformin and its four analogues [1-Carbamimidoyl-1, 2-dimethylguanidine hydrochloride, Metformin hydrochloride, N1, N1-Dimethyl-N5-methylbiguanide hydrochloride, and N1, N1, N5, N5-Tetrakis (methyl-biguanide hydrochloride] on GSK-3 was carried out by Lakshmi Rajagopal et al., (2022) to confirm the anti-diabetic activity of the metformin.

Synthetic studies on aryl imeglimin compounds and their efficacy as antidiabetic agents and simultaneous molecular docking studies using Zebrafish as a model carried out by Aylin Khodakhah et al., (2024) and the docking studies showed good correlation with experimental assays. Studies of Shi et al., (2023) on metformin correlation with Alzheimer's and Type II diabetes proven to be regulation of signalling pathways and ion transport by metformin. Studies by Deepti et al., (2016) include metformin show non-specific interactions and bind with serum BSA via hydrophobic, hydrogen bonding and electrostatic interactions.

METHODOLOGY

Protein – drug interactions using SWISSDOCK:

Protein drug interactions are studied using SWISSDOCK utilising advanced search option and AutoDock vina

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tool with defined parameters of:

Parameters: Box center:	25 - -10 - 15	**Sampling exhaustivity:**	64
Box size:		20 - 20 - 20	

We used metformin hydrochloride (CN(C)C(=[NH2+]) NC(N)=[NH2+]) as a ligand and target protein with already available proteins with mentioned protein ids 5c65; 4pyp; 4zw9; 1irk; 2nzt; 1qha and defined X-ray crystallographic structures. The binding interactions is studied and captured using high calculated affinities obtained through the Auto Dock vina tool.

RESULTS

SWISSDOCK studies on protein drug interactions using Metformin hydrochloride and GLUT-3 showed interactions like formation of hydrogen bonds at active residues ASn 98 and carbon – carbon interactions with met 94, Ileu 196, Phe 137 and Trp386 positions. Methionine interacts with Phe 137 at carboxylate position.
Protein docking studies was carried out using SWISSDOCK AutoDock vina tool to study the interactions of GLUT-1 transporter protein and metformin hydrochloride. Metformin hydrochloride interacts with the transporter at Phe 81position, met77 and Ileu 78th positions through hydrogen bonding between carboxylate and 'N' of metformin.

Similar interaction studies on GLUT-3 transporter bound to glucose using SWISSDOCK by using ligand metformin hydrochloride showed active interactions with the protein at amino acid positions ASn315 forming hydrogen bond between N of metformin and O of ASn aminoacid. The drug interacts with protein at other amino acid positions like Phe 414, Thr 411, Asn 409 (with O of Asn and N of metformin), Trp 410 and Gln 281.

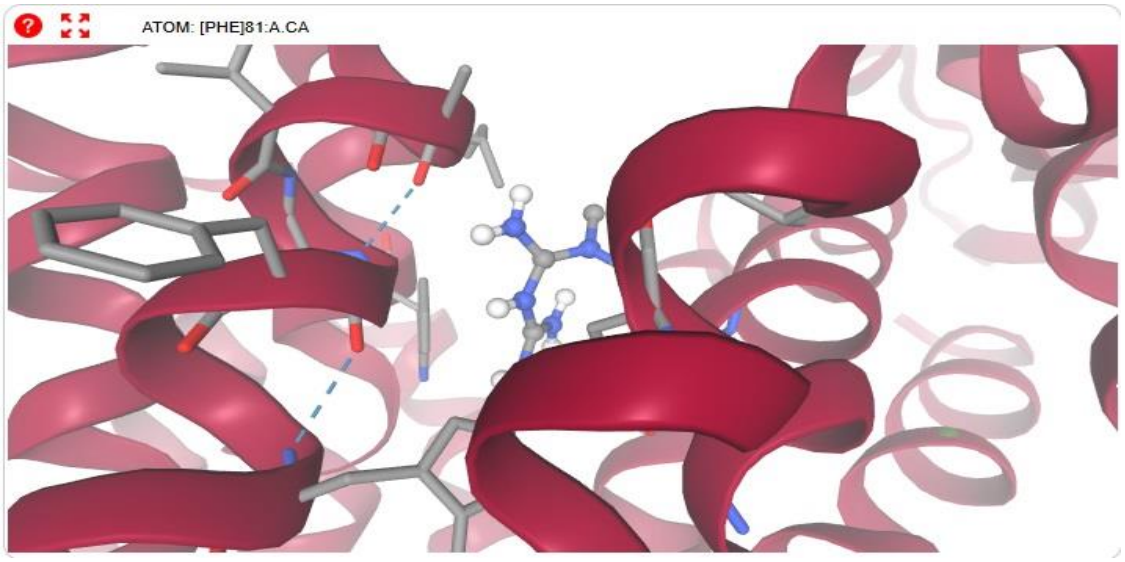

Figure: 1 Molecular Docking studies of GLUT-3 with metformin hydrochloride using SWISSDOCK; Protein id: 5c65 using highest calculated binding affinity: -4.671 (Bugnon M, Röhrig UF, Goullieux M, Perez MAS, Daina A, Michielin O, Zoete V. SwissDock2024: major enhancements for small-molecule docking with Attracting Cavities and Auto Dock Vina. *Nucleic Acids Res.* **2024**

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Model	Calculated affinity (kcal/mol)
1	-4.671
2	-4.639
3	-4.573
4	-4.515
5	-4.501
6	-4.406
7	-4.406
8	-4.327
9	-4.325
10	-4.274
11	-4.250
12	-4.228
13	-4.201
14	-4.198
15	-4.173
16	-4.170
17	-4.147
18	-4.136
19	-4.128
20	-4.100

Table:1: Calculated affinity of GLUT-3 interactions with metformin hydrochloride through protein - drug docking studies using SWISSDOCK

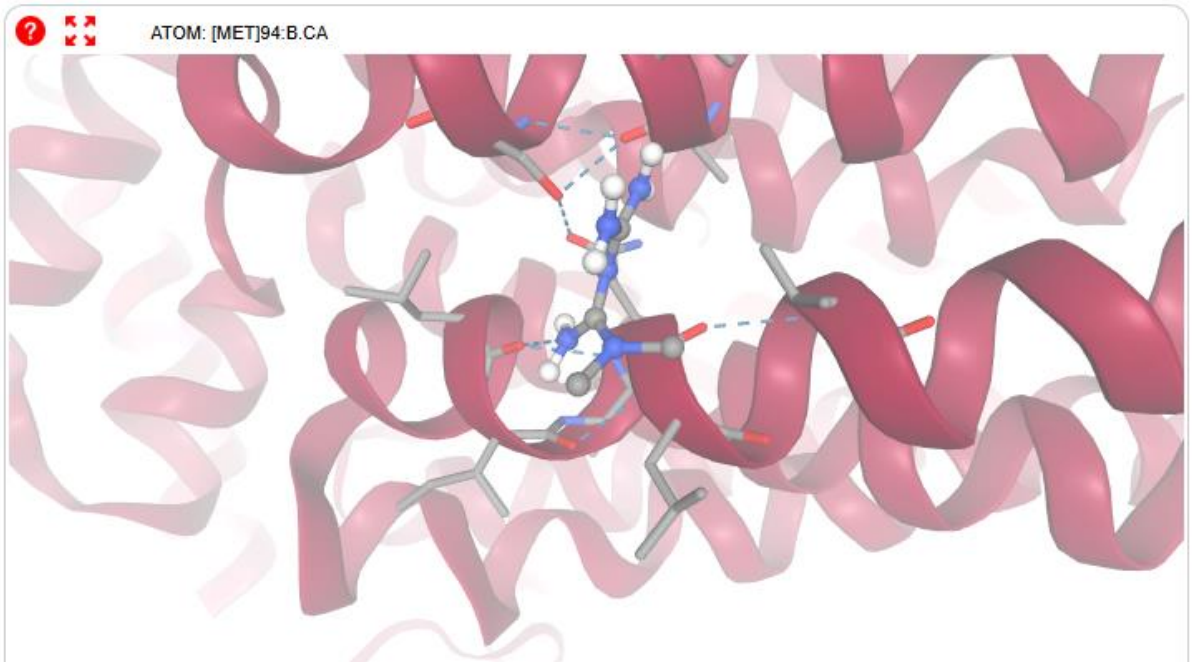

Figure:2 Molecular docking of GLUT- 1 with metformin hydrochloride using SWISSDOCK; protein id: 4pyp using high calculated affinity of -3.822 (Bugnon M, Röhrig UF, Goullieux M, Perez MAS, Daina A, Michielin O, Zoete V. SwissDock2024: major enhancements for small-molecule docking with Attracting Cavities andAutoDock Vina. *Nucleic Acids Res.* **2024**

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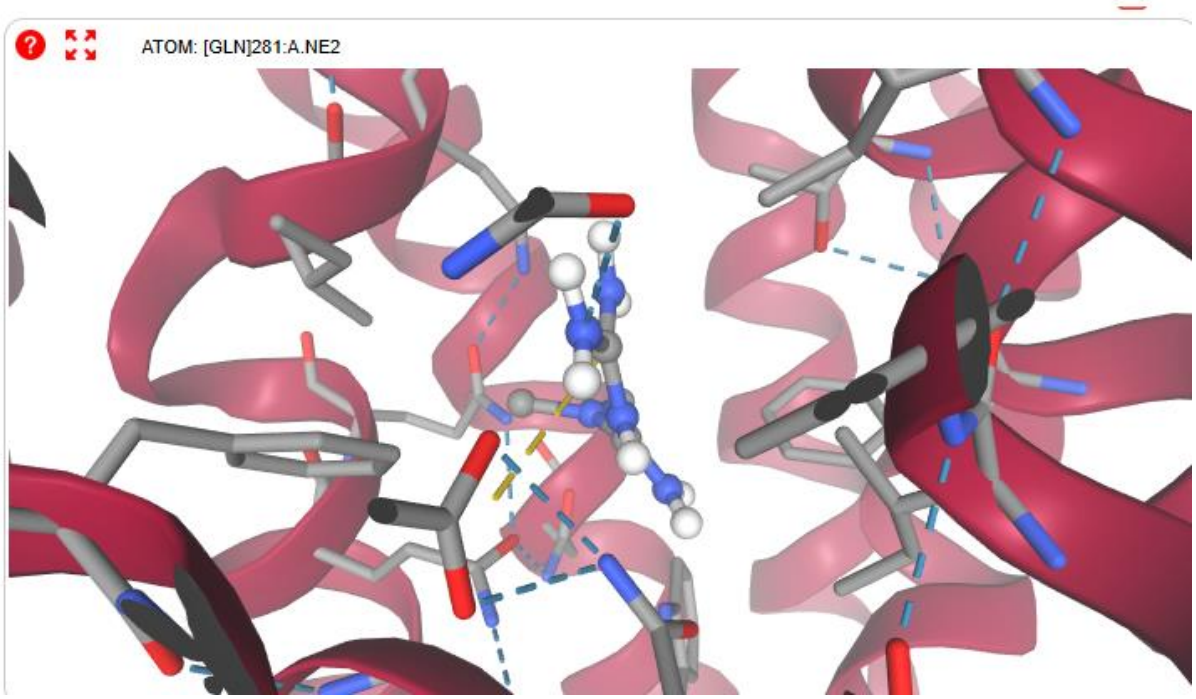

Figure:3 Docking studies of GLUT-3 bind to glucose with metformin hydrochloride using SWISSDOCK with protein id: 4zw9 using high calculated affinity of -4.875 (Bugnon M, Röhrig UF, Goullieux M, Perez MAS, Daina A, Michielin O, Zoete V. SwissDock2024: major enhancements for small-molecule docking with Attracting Cavities and Auto Dock Vina. *Nucleic Acids Res.* **2024**

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Model	Calculated affinity (kcal/mol)
1	-3.822
2	-3.748
3	-3.716
4	-3.707
5	-3.584
6	-3.582
7	-3.548
8	-3.514
9	-3.369
10	-3.357
11	-3.356
12	-3.331
13	-3.251
14	-3.241
15	-3.202
16	-3.145
17	-3.132
18	-3.108
19	-3.036
20	-2.972

Table :2 Calculated affinity of GLUT-3 and metformin hydrochloride interaction studies

Insulin receptor interaction studies with metformin using SWISSDOCK online tool proved to show interactions between drug and protein at positions ASP 1150, ASn 1137 (Hydrogen bonding b/n ‘O’ of carboxylate of ASn and N of the drug) and C, H &N of Tyr 1158 with N of the drug.

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Model	Calculated affinity (kcal/mol)
1	-4.875
2	-4.770
3	-4.765
4	-4.727
5	-4.569
6	-4.502
7	-4.493
8	-4.471
9	-4.461
10	-4.391
11	-4.332
12	-4.307
13	-3.861
14	-3.829
15	-3.764
16	-3.761
17	-3.694
18	-3.547

Table:3 Calculated affinity of interactions between metformin hydrochloride and GLUT-3 transporter bound to glucose

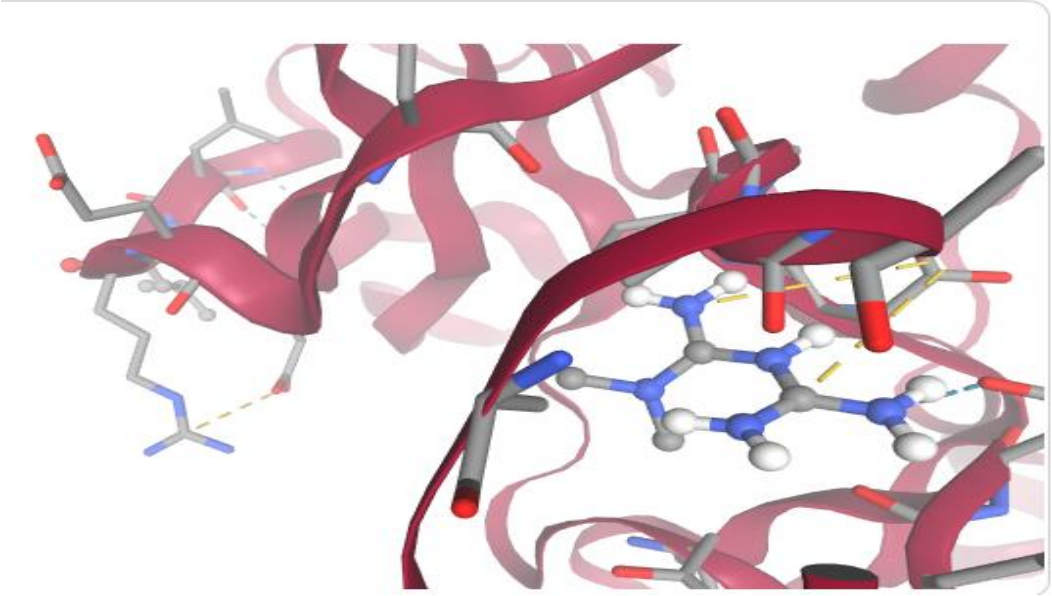

Figure:4 Docking studies of Insulin receptor with metformin hydrochloride using SWISSDOCK; Protein id:1irk using high calculated affinity of -5.207. (Bugnon M, Röhrig UF, Goullieux M, Perez MAS, Daina A, Michielin O, Zoete V. SwissDock2024: major enhancements for small-molecule docking with Attracting Cavities and Auto Dock Vina. *Nucleic Acids Res.* **2024**

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Model	Calculated affinity (kcal/mol)
1	-5.207
2	-5.187
3	-5.130
4	-5.005
5	-4.980
6	-4.891
7	-4.887
8	-4.865
9	-4.561
10	-4.535
11	-4.490
12	-4.448
13	-4.434
14	-4.379
15	-4.360
16	-4.294
17	-4.272
18	-4.221
19	-4.218
20	-4.093

Table: 4 Calculated affinities of binding studies of Insulin receptor using metformin hydrochloride as ligand

Metformin is an antidiabetic drug used to treat type ii diabetes and recent works on formulation of metformin nanoparticles proved to reduce blood sugar effectively compared to conventional metformin. Protein docking studies proved the interaction of drug with proteins like Insulin receptor and GLUT transporter present on the surface of the cells and glycolytic pathway enzymes like Hexokinase there by facilitating the glucose uptake.

Model	Calculated affinity (kcal/mol)
1	-3.360
2	-3.331
3	-3.238
4	-3.197
5	-3.196
6	-3.185
7	-3.173
8	-3.134
9	-3.123
10	-3.085
11	-3.080
12	-3.051
13	-3.006
14	-2.985
15	-2.971
16	-2.964
17	-2.956
18	-2.929
19	-2.924
20	-2.871

Table:5 Calculated affinities of metformin hydrochloride and Glycolytic enzyme Hexokinase studied using SWISSDOCK

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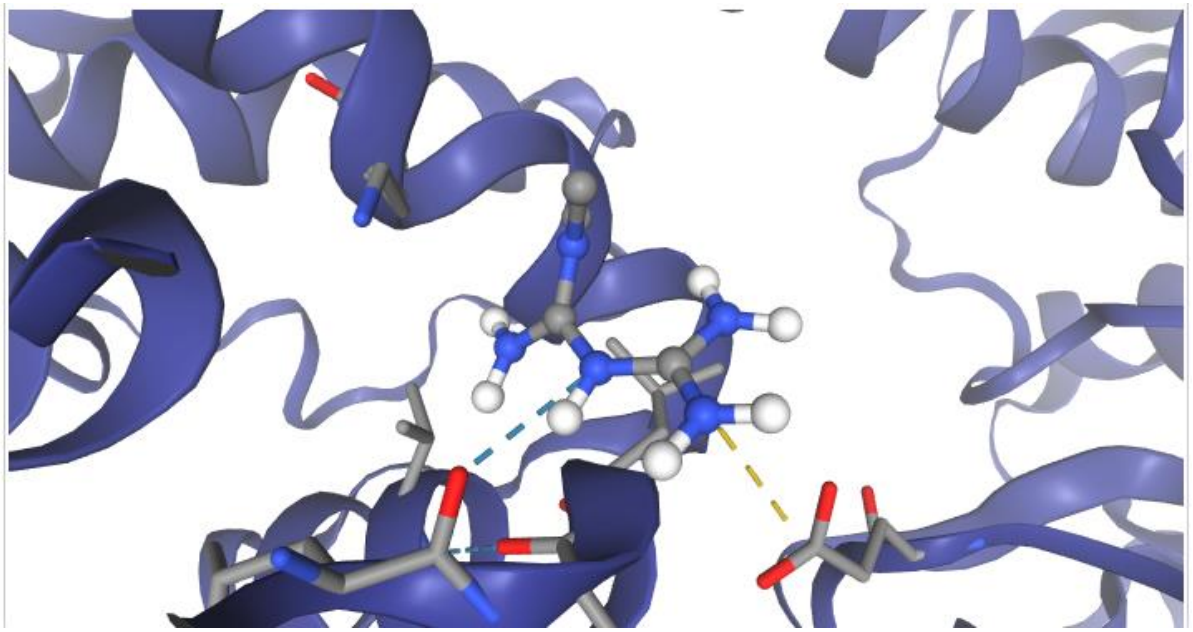

Figure :5 Docking studies of hexokinase with metformin hydrochloride using SWISSDOCK with protein id:2nzt and high calculated affinity: -3.360 (Bugnon M, Röhrig UF, Goullieux M, Perez MAS, Daina A, Michielin O, Zoete V. SwissDock2024: major enhancements for small-molecule docking with Attracting Cavities and Auto Dock Vina. *Nucleic Acids Res.* **2024;**

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Metformin hydrochloride interacts with Hexokinase at positions like Glu 249 showing ionic interactions between N of drug metformin hydrochloride with C of Glutamic acid at 249 positions. Leu795 'O' interacts with N of metformin hydrochloride by hydrogen bonding.

Hexokinase bound with ATP analogue interacts with the drug metformin hydrochloride at amino acid positions Ileu 245, Val 248 and Arg 243 by hydrogen bonding between N of drug and O of the amino acids Ileu 245, Val 248 and Arg 243.

Model	Calculated affinity (kcal/mol)
1	-5.073
2	-5.062
3	-5.009
4	-4.990
5	-4.967
6	-4.950
7	-4.920
8	-4.901
9	-4.895
10	-4.880
11	-4.866
12	-4.769
13	-4.653
14	-4.621
15	-4.605
16	-4.569
17	-4.562
18	-4.410
19	-4.364
20	-4.315

Table: 6 Calculated affinities of binding studies of Metformin hydrochloride and Hexokinase bound to ATP analogue

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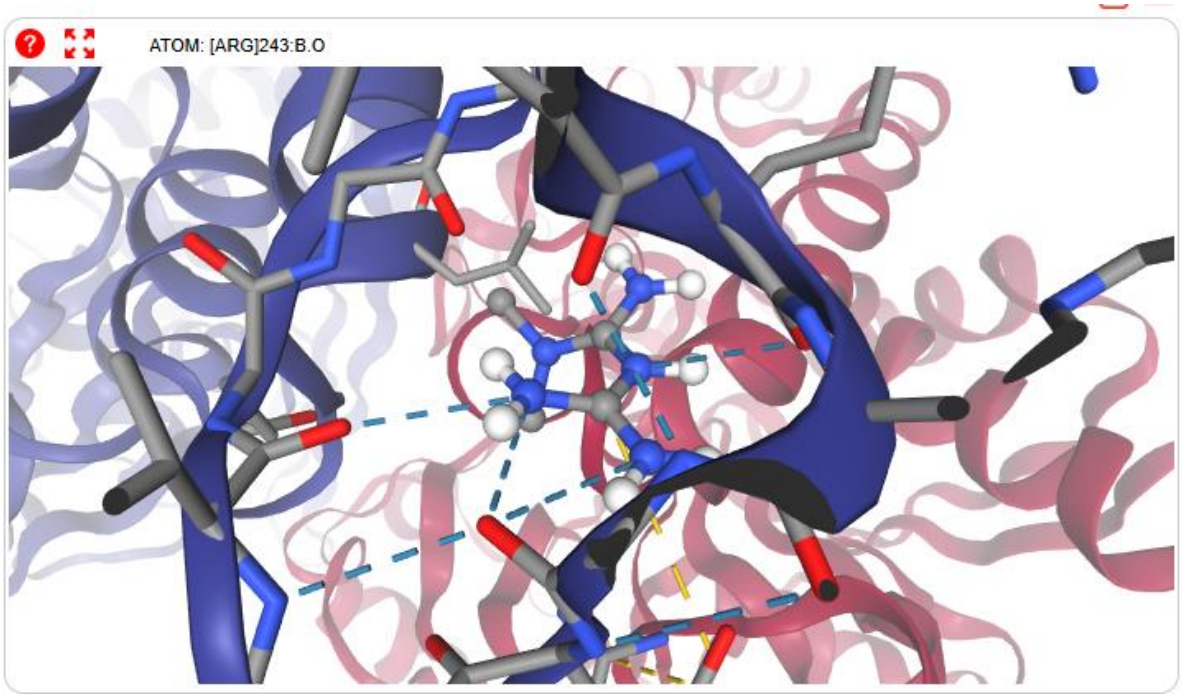

Figure: 6 Protein – drug docking studies of hexokinase bound to ATP analogue with metformin hydrochloride using SWISSDOCK with protein id:1qha showing high calculated affinity: -5.073 (Bugnon M, Röhrig UF, Goullieux M, Perez MAS, Daina A, Michielin O, Zoete V. SwissDock2024: major enhancements for small-molecule docking with Attracting Cavities and AutoDock Vina. *Nucleic Acids Res.* **2024**

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DISCUSSION AND CONCLUSION

Metformin is one of the oral antidiabetic drugs used to treat type II diabetes and some of the studies has found that the drug acts as common endocrine – disrupting chemical even though is doesn't possess the structure similar to endocrine disrupting chemical (Baptista M et al., (2022)). Metformin is known for its immunomodulatory effects and shows anti-viral and inflammatory responses. Recent molecular docking studies on metformin confirmed the modulated mitochondria mediated antiviral innate immunity (Ihsan Nalkiran (2025)).

Novel three nickel complexes of metformin were synthesised by Rajeshwari et al., and complex I of them shows the DNA binding capacity in groove binding mode (K. Rajeshwari et al., (2022)). Metformin is also used to treat certain diseases like cancers (e.g., breast cancer, endometrial cancer, bone cancer, colorectal cancer, and melanoma), obesity, liver diseases, cardiovascular disease, and renal diseases (Ziquan Lv et al., (2020)). Molecular mechanisms underlying are remains to be elucidated. Mostly the drug acts through regulating signalling mechanisms.

Clinical trials with metformin indeed proven that MILES (Metformin In Longevity Study) and TAME (Targeting Aging with Metformin) as an antiaging effect. MILES study has proven that metformin induces antiaging transcriptional changes but however the molecular mechanisms remain unelucidated (Soukas et al., (2019)).

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