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Computational approach confirming the therapeutic potential of selected mannose derivatives against fimH of Uropathogenic E. coli

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***Abstract*---**Urinary tract infection (UTI), mainly caused by uropathogenic *Escherichia coli* (UPEC), is a dreaded infectious disease globally. FimH is a key virulence factor in UTI pathogenesis and inhibition of FimH function can be an effective way to disarm the UPEC bacteria and can act as a vital target in the development of the non-antibiotic mediated approach to treat UTIs. The present study was undertaken to identify phytochemicals from the cranberry and bearberry plant parts and to evaluate their pharmacological properties. The pharmacological properties of those compounds were predicted using a computational approach. The compounds with similar pharmacophores with that of known fimH inhibitors were selected. After that, further studies were performed to determine their drug likeness, inhibitory potential, and IC_{50} values. The results were promising, and few compounds were found to have high drug likeness and a potent inhibitor of fimH with good IC_{50} value. Thus, the present study reports few novel fimH inhibitors from selected plant sources and is significant owing to their therapeutic implication as a non-antibiotic mediated therapy for UTI.

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Introduction

Urinary tract infection (UTI), mainly caused by uropathogenic *Escherichia coli* (UPEC), is a dreaded infectious disease globally [1]. UTI is most prevalent in almost 50% of women occurring in their lifetime [2-4]. Although antibiotics are effective against sensitive strains of UPEC but recurrent infections with a higher rate of 25-35% challenge the treatment regimen [5-9]. On the other hand, the latency of new antibiotics development calls for new therapeutic approaches to eliminate the infection [10-11].

One effective therapeutic approach can be to target the virulence factors [12-14] involved in the adhesion of UPEC to the host urothelial surface without killing the bacteria using antibiotics. This will help to not only disarm the bacteria but also to avoid the antibiotics resistance mediated by the selection pressure of viability in the host cell [11,15].

The adhesion is supported by the binding of FimH lection to the mannosylated glycoproteins present in the bladder epithelium coating [16-18]. The mannose-specific FimH lectin is located at the distal tip of type 1 pili that are highly expressed on the surface of UPEC and other Gram-negative uropathogen. These long hair-like structures are comprised of long repeating FimA based pilus rods and a FimF, FimG containing fibrillum, and one FimH capped adhesin. The FimH adhesin is again formed of one C-terminal pilin domain connecting the pilus rod and one N-terminal lectin domain that possesses a mannose-binding pocket.

This pocket naturally binds to a specific sequence of branched oligomannose present on the highly mannosylated uroplakin Ia (UPIa) glycoproteins present on the surface of epithelial umbrella cells of the host urinary tract [19]. It helps in bacterial invasion thereby facilitating colonization, proliferation, and formation of biofilm-like intracellular bacterial communities (IBCs) within the host bladder. Hence, it could be identified that FimH is a key virulence factor in UTI pathogenesis and inhibition of FimH function can be an effective way to disarm the UPEC bacteria and can act as a vital target in the development of the non-antibiotic mediated approach to treat UTIs. Therefore, there is a need for rationally designed, potent, and orally bioavailable, small-molecule FimH mannoside antagonists for future therapeutic use.

The hypothesis

During the literature survey it was found that cranberry and bearberry juice helps in curing UTI by unarming the bacteria from host urothelium. Hence in this study we took the bioactive compounds present in those fruits and their derivatives to find inhibitory activity and mode of action against UPEC fimH.

Need for New Drugs

Chronic and recurrent urinary tract infections pose a serious medical problem because there are few effective treatment options. Patients with chronic urinary

tract infections are commonly treated with long-term prophylactic antibiotics that promote the development of antibiotic resistant forms of uropathogenic Escherichia coli (UPEC), further complicating treatment [19-20].
UTIs are often a major problem throughout the life span of women, particularly when the infection becomes chronic, recurrent, or recalcitrant to treatment because of pathogenic mechanisms or antibiotic resistance. Multidrug-resistant uropathogen are becoming more prevalent and globally distributed, making UTI an increasingly pressing public health concern.

Selection of ligands
Mannosides that specifically inhibit the FimH type 1 pilus lectin of UPEC, which mediates bacterial colonization, invasion, and formation of recalcitrant intracellular bacterial communities in the bladder epithelium. Here, we optimized these compounds for oral bioavailability and demonstrated their fast-acting efficacy in treating chronic urinary tract infections [20-22]

Methodology

Prediction of toxicity and bioavailability
Prediction of toxicity and bioavailability is a must for any novel compound to pass drug-likeliness criteria. Physicochemical properties of the compounds including octanol/water partition coefficient (LogP) were determined using MolSoft server (http:// molsoft.com/mprop/). Absorption, distribution, metabolism, excretion, and toxicity (ADME/Tox) profiles of the compounds were analyzed using ADME/Tox server at Mobyle@RPBS (https://mobyle.rpbs.univ-parisdiderot.fr/)

Protein Quality Checking
The three-dimensional structure of UPEC FimH (PDB id: 5AAP) was obtained from RCSB Protein Databank (https://www.rcsb.org/structure/5AAP). Stereochemical quality of the structure was assessed using PROCHECK module available at PDBSum server (https://www.ebi.ac.uk/thornton-srv/software/PROCHECK/), by generating Ramachandran plot. The plot revealed that 93.2% and 6.8% of the amino acid residues fall in the most favoured and additional allowed regions, respectively, with no residues falling in the disallowed regions (Attached in Results), indicating a good quality protein for molecular docking analysis.

Molecular docking analysis
The molecular docking was performed using BioSolveIT (LeadIT) FlexX 2.1.3 software to predict the binding pattern and binding energy of the novel compound against fimH. The structure of fimH was uploaded into LeadIT software followed by correction of amino acid residues having abnormal rotation and water molecules were removed from the workspace. As D-mannose was already bound to the enzyme as co-crystallized ligand, its binding site was selected as the docking site. For the validation of the docking results of selected compounds, few known fimH inhibitors were also included in the docking analysis. The three-dimensional structures of these compounds were retrieved from ChEMBL database (https://www.ebi.ac.uk/chembl/). The best docking poses of 1000 iterations for each compound were used for the analysis of binding pattern similarities.

Quantitative structure-activity relationship (QSAR) analysis
QSAR is one of the most vital tools in drug discovery research and is used to determine the IC_{50} value for any novel compound comparing its physicochemical properties with that of compounds having experimental IC_{50} values. For predicting IC_{50} values of the selected compounds, all selected fimH inhibitors were used in the QSAR analysis (Table 1). Multiple linear regression analysis was performed using EasyQSAR, a freeware. The QSAR equation was generated and the validity of the QSAR model was determined by plotting the predicted IC_{50} against the experimental IC_{50} values of the known inhibitors (Graph 1).

Molecular dynamic simulation
Initial simulation was run using Gromacs 5.0 to check the stability and residual bonding status for the best docked ligands. This was carried out to check the docking quality. Basic protein-ligand simulation procedure was followed during the study.

Result & Discussion

1000 mannose derivatives were prepared using side-chain modification by Ilib Diverse 2.0 for the docking study. Out of these, 124 ligands successfully cleared the ADMET filter with good oral bioavailability. No ligand was found with abnormal ADMET properties hence selected for further screening. The list of 124 selected ligands is given with their selected ADMET properties in Table1.

Table1: ADMET Properties of selected mannose derivatives showed high oral bioavailability

ID	SMILES	MW	logP	tPSA	RB	FB	HBD	HBA	SOL (mg/l)	Oral Bio-availability
M1	OC1OC(COC2CCC3C(CCC4C5CCCC5CCC34)C2)C(O)C(O)C1O	410.54	2.96	99.38	3	26	4	6	7137.12	Good
M2	OC1OC(COC2CCC3C2CCC2C3CCc3ccccc23)C(O)C(O)C1O	404.50	1.72	99.38	3	26	4	6	14825.93	Good
M3	OCc1ccccc1OCC1OC(O)C(O)C(O)C1O	286.28	-1.22	119.61	4	12	5	7	142280.17	Good
M4	OC1OC(CONc2nc3[nH]cnc3c(=O)[nH]2)C(O)C(O)C1O	329.27	-3.31	185.84	4	17	7	12	441180.13	Good
M5	CCC(O)CCOCC1OC(O)C(O)C(O)C1O	266.29	-1.97	119.61	6	6	5	7	308182.58	Good
M6	CC(=O)CC(=O)COCC1OC(O)C(O)C(O)C1O	278.26	-3.00	133.52	6	8	4	8	572123.47	Good
M7	CC(=O)C(=O)COCC1OC(O)C(O)C(O)C1	264.23	-3.21	133.52	5	8	4	8	633269.3	Good

	O									
M8	Nc1ncnc2n(OCC3OC(O)C(O)C(O)C3O)cnc12	313.27	-2.57	169.00	3	16	6	11	270941.08	Good
M9	CC(C)COCC1OC(O)C(O)C(O)C1O	236.26	-1.92	99.38	4	6	4	6	279699.71	Good
M10	OC1OC(CON2CCC(=O)NC2=O)C(O)C(O)C1O	292.24	-3.59	148.79	3	14	5	10	655488.03	Good
M11	OC1OC(COc2cc3ccccc3oc2=O)C(O)C(O)C1O	324.28	-0.59	129.59	3	18	4	8	74516.4	Good
M12	OC1OC(CON2CNc3ccccc3S2(=O)=O)C(O)C(O)C1O	362.36	-1.71	157.17	3	19	5	10	144836.71	Good
M13	OOCC1OC(O)C(O)C(O)C1O	196.16	-3.74	119.61	2	6	5	7	821345.5	Good
M14	OC1OC(COc2ccc3OCc4ccccc4Cc3c2)C(O)C(O)C1O	374.38	0.68	108.61	3	23	4	7	28573.37	Good
M16	OC1OC(CONc2ncnc3[nH]cnc23)C(O)C(O)C1O	313.27	-2.21	165.87	4	16	6	11	230696.12	Good
M18	OC1OC(CON2C3CCCCC3NC2=O)C(O)C(O)C1O	318.32	-1.97	131.72	3	17	5	9	218888.85	Good
M19	OC1OC(COc2ccc3oc(=O)ccc3c2)C(O)C(O)C1O	324.28	-0.80	129.59	3	18	4	8	85056.8	Good
M20	OC1OC(COC2=CC(=O)C=CC2=O)C(O)C(O)C1O	286.23	-2.47	133.52	3	14	4	8	329065.49	Good
M21	OC1OC(CON2c3ccccc3CCc3ccccc23)C(O)C(O)C1O	373.40	1.26	102.62	3	23	4	7	19968.8	Good
M22	OC1OC(COC2SC3CC(=O)N3C=C2)C(O)C(O)C1O	319.33	-2.45	144.99	3	16	4	8	295265.91	Good
M25	CC(Cc1ccccc1)NOCC1OC(O)C(O)C(O)C1O	313.35	-0.45	111.41	6	12	5	7	94197.67	Good
M26	OC1OC(COC2Oc3ccccc3Cc3ccccc23)C(O)C(O)C1O	374.38	0.69	108.61	3	23	4	7	28393.92	Good
M27	C\C=C\COCC1OC(O)C(O)C(O)C1O	234.25	-2.38	99.38	4	7	4	6	375195.05	Good
M28	OC1OC(CONc2ccnc(=O)[nH]2)C(O)C(O)C1O	289.24	-3.15	157.16	4	13	6	10	471352.47	Good

M29	CC(C)(C)COCC1OC(O)C(O)C(O)C1O	250.29	-1.53	99.38	4	6	4	6	212453.88	Good
M31	OC1OC(CON2c3ccccc3Sc3ccccc23)C(O)C(O)C1O	377.41	1.11	127.92	3	22	4	7	21215.91	Good
M32	OC1OC(CON2CCC34CCCCC3C2Cc2ccccc42)C(O)C(O)C1O	405.48	0.83	102.62	3	26	4	7	25846.58	Good
M33	OC1OC(CON2c3ccccc3C=Cc3ccccc23)C(O)C(O)C1O	371.38	1.46	102.62	3	23	4	7	17599.25	Good
M34	OC1OC(CON2c3ccccc3Sc3cccnc23)C(O)C(O)C1O	378.40	0.38	140.81	3	22	4	8	33336.57	Good
M35	OC1OC(CON2CCN=Cc3ccccc23)C(O)C(O)C1O	324.33	-1.44	114.98	3	18	4	8	138776.19	Good
M38	CC1CN(OCC2OC(O)C(O)C(O)C2O)C(=O)NC1=O	306.27	-3.02	148.79	3	14	5	10	439745.15	Good
M39	Cn1c2ccccc2n(OCC2OC(O)C(O)C(O)C2O)c(=O)c2ccccc12	402.40	0.06	126.31	3	24	4	9	36786.37	Good
M40	OC1OC(COC23CCCC2C2CCc4ccccc4C2CC3)C(O)C(O)C1O	404.50	1.45	99.38	3	26	4	6	17575	Good
M41	CC(C)OCC1OC(O)C(O)C(O)C1O	222.24	-2.46	99.38	3	6	4	6	377540.3	Good
M42	CC(=O)OCC1OC(O)C(O)C(O)C1O	222.19	-3.22	116.45	3	7	4	7	609446.11	Good
M43	OCCCCCOCC1OC(O)C(O)C(O)C1O	266.29	-2.87	119.61	7	6	5	7	580385.41	Good
M44	OC1OC(CON2c3ccccc3C=NCC2=O)C(O)C(O)C1O	338.31	-2.01	132.05	3	19	4	9	189619.2	Good
M46	CCOCC1OC(O)C(O)C(O)C1O	208.21	-2.89	99.38	3	6	4	6	505903.8	Good
M47	NOCC1OC(O)C(O)C(O)C1O	195.17	-4.00	125.40	2	6	6	7	968565.79	Good
M49	OC1OC(COCC(=O)C=C)C(O)C(O)C1O	248.23	-2.26	116.45	5	8	4	7	361091.03	Good
M51	OC1OC(COC=C2c3ccccc3CCc3ccccc23)C(O)C(O)C1O	384.42	1.18	99.38	3	24	4	6	20331.15	Good
M52	CC(=O)C(OCC1OC(O)C(O)C(O)C1O)C(C)=O	278.26	-2.90	133.52	5	8	4	8	502881.63	Good
M53	OCCCCOCC1OC(O)C(O)C(O)C1O	252.26	-3.22	119.61	6	6	5	7	699975.07	Good

M57	COCC1OC(O)C(O)C(O)C1O	194.18	-3.25	99.38	2	6	4	6	604479.03	Good
M58	CCCOCC1OC(O)C(O)C(O)C1O	222.24	-2.36	99.38	4	6	4	6	378674.62	Good
M59	OC1OC(COC2CCCC3CCC4C5CCCC5CCC4C23)C(O)C(O)C1O	410.54	3.15	99.38	3	26	4	6	6331.96	Good
M61	OC1OC(COc2ccc3ccc(=O)oc3c2)C(O)C(O)C1O	324.28	-0.72	129.59	3	18	4	8	80876.17	Good
M62	OC1OC(COC2Sc3ccccc3Cc3ccccc23)C(O)C(O)C1O	390.45	1.23	124.68	3	23	4	6	19075.13	Good
M64	OC1OC(COC2CCC3CCC4C5CCCC5CCC4C3C2)C(O)C(O)C1O	410.54	2.96	99.38	3	26	4	6	7137.12	Good
M67	CCCCCCOCC1OC(O)C(O)C(O)C1O	264.32	-0.92	99.38	7	6	4	6	170713.67	Good
M70	CCC(CCO)OCC1OC(O)C(O)C(O)C1O	266.29	-1.97	119.61	6	6	5	7	308182.58	Good
M71	CCCCOCC1OC(O)C(O)C(O)C1O	236.26	-2.00	99.38	5	6	4	6	314227.29	Good
M73	OC1OC(CON2C(=O)CC(=O)NC2=O)C(O)C(O)C1O	306.23	-3.62	165.86	3	15	5	11	641828.88	Good
M75	OC1OC(CON2CNS(=O)(=O)c3ccccc23)C(O)C(O)C1O	362.36	-1.75	157.17	3	19	5	10	148532.97	Good
M76	OC1OC(COC#N)C(O)C(O)C1O	205.17	-2.95	123.17	2	7	4	7	493879.26	Good
M77	OC1OC(COC(=O)c2ccccc2)C(O)C(O)C1O	284.26	-0.91	116.45	4	13	4	7	116914.02	Good
M80	CC(O)CCOCC1OC(O)C(O)C(O)C1O	252.26	-3.15	119.61	5	6	5	7	626998.86	Good
M83	OC1OC(CON2C3NCNC3C(=O)NC2=O)C(O)C(O)C1O	334.28	-4.29	172.85	3	18	7	12	897968.11	Good
M89	OC1OC(COCC=C)C(O)C(O)C1O	220.22	-2.61	99.38	4	7	4	6	444772.75	Good
M91	CCC(C)CCCOCC1OC(O)C(O)C(O)C1O	278.34	-0.02	99.38	7	6	4	6	93478.39	Good
M96	OC1OC(COC2C3SCCN3C2=O)C(O)C(O)C1O	307.32	-2.68	144.99	3	15	4	8	353861.3	Good
M98	CC(O)COCC1OC(O)C(O)C(O)C1O	238.24	-3.51	119.61	4	6	5	7	758619.66	Good

M99	CCC(C)OCC1OC(O)C(O)C(O)C1O	236.26	-1.93	99.38	4	6	4	6	281467.38	Good
M101	OC1OC(COC2=CN3C(CC3=O)C2)C(O)C(O)C1O	287.27	-3.03	119.69	3	15	4	8	466967.54	Good
M102	CCCC(CC)COCC1OC(O)C(O)C(O)C1O	278.34	-0.02	99.38	7	6	4	6	93478.39	Good
M103	NC1NC2NCNC2C(=O)N1OCC1OC(O)C(O)C(O)C1O	335.31	-5.01	181.80	3	17	9	12	1408698.41	Good
M104	OC1OC(COC2C=CN3C2CC3=O)C(O)C(O)C1O	287.27	-3.30	119.69	3	15	4	8	553554.24	Good
M108	Cn1c2ncn(OCC3OC(O)C(O)C(O)C3O)c2c(=O)n(C)c1=O	358.30	-2.35	161.20	3	18	4	12	209246.55	Good
M109	CC(CCCO)OCC1OC(O)C(O)C(O)C1O	266.29	-2.14	119.61	6	6	5	7	343021.25	Good
M111	CC(=O)CCOCC1OC(O)C(O)C(O)C1O	250.25	-3.60	116.45	5	7	4	7	836243.51	Good
M113	OC1OC(COCC(=O)Cc2ccccc2)C(O)C(O)C1O	312.32	-1.26	116.45	6	13	4	7	156294.92	Good
M120	OC1OC(COc2ccc3CCc4ccccc4C(=C)c3c2)C(O)C(O)C1O	384.42	1.53	99.38	3	24	4	6	16307.97	Good
M131	CC1CNC(=O)N(OCC2OC(O)C(O)C(O)C2O)C1=O	306.27	-3.02	152.36	3	14	5	10	439745.15	Good
M133	CCCC(C)OCC1OC(O)C(O)C(O)C1O	250.29	-1.57	99.38	5	6	4	6	232740.69	Good
M145	C\C=C(/C)OCC1OC(O)C(O)C(O)C1O	234.25	-1.90	99.38	3	7	4	6	259575.09	Good
M146	CCC(OCC1OC(O)C(O)C(O)C1O)C(C)=O	264.27	-1.92	116.45	5	7	4	7	280926.13	Good
M149	CC(CC(C)=O)OCC1OC(O)C(O)C(O)C1O	264.27	-2.52	116.45	5	7	4	7	409973.18	Good
M152	CC(O)CCCOCC1OC(O)C(O)C(O)C1O	266.29	-2.79	119.61	6	6	5	7	516612.14	Good
M154	OC1OC(COC2C3SCC=CN3C2=O)C(O)C(O)C1O	319.33	-2.45	144.99	3	16	4	8	295265.91	Good
M155	C\C=C\C(\OCC1OC(O)C(O)C(O)C1O)=C/C	260.28	-0.61	99.38	4	8	4	6	116316.33	Good
M158	CC(CO)OCC1OC(O)C(O)C(O)C1O	238.24	-3.51	119.61	4	6	5	7	758619.66	Good
M160	OC1OC(COc2ccc(cc2)C(=O)c2ccccc2)C(	360.36	1.01	116.45	5	19	4	7	27482.11	Good

	O)C(O)C1O									
M164	OCCOCC1OC(O)C(O)C(O)C1O	224.21	-3.94	119.61	4	6	5	7	1021149.09	Good
M179	NC1NC2C(NCN2OCC2OC(O)C(O)C(O)C2O)C(=O)N1	335.31	-4.72	181.80	3	17	9	12	1173471.16	Good
M203	Nc1ccn(OCC2OC(O)C(O)C(O)C2O)c(=O)n1	289.24	-3.75	160.29	3	13	6	10	643940.39	Good
M215	CCCC(CC)OCC1OC(O)C(O)C(O)C1O	264.32	-0.39	99.38	6	6	4	6	114444.24	Good
M233	CCCC(CO)OCC1OC(O)C(O)C(O)C1O	266.29	-1.97	119.61	6	6	5	7	308182.58	Good
M242	CCC(C)CCOCC1OC(O)C(O)C(O)C1O	264.32	-0.37	99.38	6	6	4	6	113011.29	Good
M247	CC(=O)COCC1OC(O)C(O)C(O)C1O	236.22	-3.50	116.45	4	7	4	7	756877.39	Good
M250	CCCCC(C)COCC1OC(O)C(O)C(O)C1O	278.34	0.17	99.38	7	6	4	6	82932.77	Good
M252	C\C=C\C=C\COCC1OC(O)C(O)C(O)C1O	260.28	-1.74	99.38	5	8	4	6	253208.56	Good
M254	CCCCCOCC1OC(O)C(O)C(O)C1O	250.29	-1.46	99.38	6	6	4	6	231973.92	Good
M262	N\C=N\OCC1OC(O)C(O)C(O)C1O	222.20	-3.60	137.76	3	7	6	8	774288.79	Good
M263	OC1OC(COC2CC3CCC4C(CCc5ccccc45)C3C2)C(O)C(O)C1O	404.50	1.90	99.38	3	26	4	6	13236.49	Good
M284	CCC(CO)OCC1OC(O)C(O)C(O)C1O	252.26	-2.33	119.61	5	6	5	7	374033.26	Good
M291	CC(C)CC(C)COCC1OC(O)C(O)C(O)C1O	278.34	-0.77	99.38	6	6	4	6	140362.78	Good
M314	CC(C)CCCOCC1OC(O)C(O)C(O)C1O	264.32	-1.21	99.38	6	6	4	6	191844.99	Good
M315	CC(C)CCCCOCC1OC(O)C(O)C(O)C1O	278.34	-0.67	99.38	7	6	4	6	140784.5	Good
M319	CC(=O)CCCOCC1OC(O)C(O)C(O)C1O	264.27	-3.24	116.45	6	7	4	7	689310.45	Good
M332	OC1OC(COC2C3CC=CN3C2=O)C(O)C(O)C1O	287.27	-2.74	119.69	3	15	4	8	388992.38	Good
M333	CO\N=C\OCC1OC(O)C(O)C(O)C1O	237.21	-2.62	120.97	4	7	4	8	433915.31	Good
M336	CC(CCO)OCC1OC(O)C(O)C(O)C1O	252.26	-2.50	119.61	5	6	5	7	416316.06	Good
M337	OC1OC(COC=C)C(O)C(O)C1O	206.19	-2.51	99.38	3	7	4	6	399301.12	Good

M338	CC(C)CCOCC1OC(O)C(O)C(O)C1O	250.29	-1.57	99.38	5	6	4	6	232740.69	Good
M345	OC1OC(COc2cccc(c2)C(=O)c2ccccc2)C(O)C(O)C1O	360.36	0.55	116.45	5	19	4	7	36720.5	Good
M364	OCCCOCC1OC(O)C(O)C(O)C1O	238.24	-3.58	119.61	5	6	5	7	846915.17	Good
M369	OC1OC(COc2cccc3oc(=O)ccc23)C(O)C(O)C1O	324.28	-0.80	129.59	3	18	4	8	85056.8	Good
M385	CCC(C)(C)OCC1OC(O)C(O)C(O)C1O	250.29	-1.74	99.38	4	6	4	6	242505.63	Good
M403	OCc1cccc(OCC2OC(O)C(O)C(O)C2O)c1	286.28	-1.22	119.61	4	12	5	7	142280.17	Good
M408	OC1OC(COc2ccc3CCc4ccccc4Cc3c2)C(O)C(O)C1O	372.41	1.43	99.38	3	23	4	6	18065.97	Good
M419	CC(=O)C(OCC1OC(O)C(O)C(O)C1O)c1ccccc1	312.32	-1.16	116.45	5	13	4	7	137379.16	Good
M423	CCC(C)COCC1OC(O)C(O)C(O)C1O	250.29	-1.57	99.38	5	6	4	6	232740.69	Good
M424	CC(C)CCC(C)OCC1OC(O)C(O)C(O)C1O	278.34	-0.13	99.38	6	6	4	6	93787.38	Good
M427	OC1OC(COc2cccc3COc4ccccc4Cc23)C(O)C(O)C1O	374.38	0.68	108.61	3	23	4	7	28573.37	Good
M428	OC1OC(COC2CN3C(CC3=O)S2)C(O)C(O)C1O	307.32	-2.65	144.99	3	15	4	8	347236.12	Good
M431	CCC(OCC1OC(O)C(O)C(O)C1O)C(C)O	266.29	-1.89	119.61	5	6	5	7	274319.2	Good
M432	OC1OC(COc2cc(=O)oc3ccccc23)C(O)C(O)C1O	324.28	-1.08	129.59	3	18	4	8	101465.54	Good
M437	OC1OC(COc2ccc3Cc4ccccc4CCc3c2)C(O)C(O)C1O	372.41	1.43	99.38	3	23	4	6	18065.97	Good
M439	CC(C)C(OCC1OC(O)C(O)C(O)C1O)C(C)C	278.34	-0.53	99.38	5	6	4	6	112959.61	Good
M448	OC1OC(COC2Cc3ccccc3Cc3ccccc23)C(O)C(O)C1O	372.41	0.88	99.38	3	23	4	6	25547.26	Good
M453	C\C=C\OCC1OC(O)C(O)C(O)C1O	220.22	-2.28	99.38	3	7	4	6	338208.81	Good
M462	OC1OC(CON2C(=O)CCNC2=O)C(O)C(O)C1O	292.24	-3.59	152.36	3	14	5	10	655488.03	Good
M464	OCc1ccc(OCC2OC(	286.28	-	119.61	4	12	5	7	142280.17	Good

	O)C(O)C(O)C2O)cc1		1.22							
M487	OC1OC(COc2cccc3Cc4ccccc4COc23)C(O)C(O)C1O	374.38	0.68	108.61	3	23	4	7	28573.37	Good

The protein 5AAP was retrieved from Protein Databank. The structure was determined using X-ray diffraction at resolution of 1.30 Å, has 1 chain and 158 amino acid residues. The active site residues were found to be Asp, Tyr, Arg, etc. The protein was bound to mannoside in its wild type hence the ligand bounding site was selected as the active site for initial screening.

Docking with known drugs and mannosides had some similar amino acid residues in their bonding pattern.

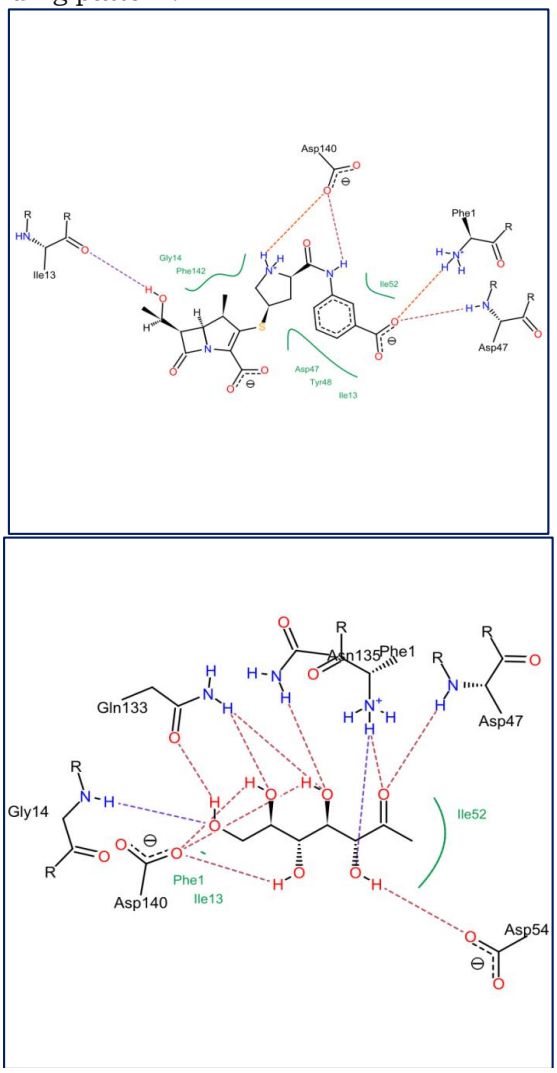

Fig1: Docking Poses of Mannoside M4 (left) and Ertapenem antibiotic (right) showing similar bonding patterns

The above docking pattern shows that the mannoside and known drugs have common bonding residues Phe1, Asp 37, and Asp140. The docking score of mannoside is much better than that of ertapenem. The numbers of H-bonds were also more in the case of mannoside M4 suggesting that M17 has better efficacy towards fimH. The docking scores of the selected ligands were given in Table2.

Table2: Top 10 docking scores showed by the selected ligands with bonding properties

Compounds	**Total Score (Kcal/mol)**	**Hydrogen Bond Properties**		
		Hydrogen Bonds	**Bond Energy (Kcal/mol)**	**Bond Length (A)**
M4	-30.0785	OASN23A - H34	-4.7	2.14
		OVAL35A - H30	-4.7	2.20
		OASP37A - H32	-4.7	2.13
		HASP37A - O4	-4.4	1.92
		HE22GLN41A - O12	-4.7	1.97
M25	-28.5698	OASN23A - H34	-4.3	1.97
		OLEU24A - H18	-3.9	2.08
		OVAL35A - H30	-4.7	2.04
		HASP37A - O4	-4.4	2.20
		OASP37A - H32	-4.2	1.99
		HE22GLN41A - O12	-4.6	1.88
M338	-28.5689	OASN23A - H34	-4.3	1.97
		OLEU24A - H18	-3.9	2.08
		OVAL35A - H30	-4.7	2.04
		HASP37A - O4	-4.4	2.20
		OASP37A - H32	-4.2	1.99
		HE22GLN41A - O12	-4.6	1.88
M73	-27.0363	OASN23A - H32	-4.7	2.08
		OVAL35A - H28	-4.7	1.81
		HASP37A - O4	-4.4	2.10
		OASP37A - H30	-4.7	2.19
		HE22GLN41A - O12	-4.7	2.18
M111	-26.4970	OASN23A - H30	-3.9	2.26
		OVAL35A - H26	-4.6	1.85
		HVAL35A - O17	-4.1	1.77
		OASP37A - H28	-4.6	2.20
		HASP37A - O4	-4.4	2.12
		HE22GLN41A - O12	-4.7	2.12
M358	-25.8892	OASN23A - H36	-4.7	2.09
		OVAL35A - H32	-4.7	2.08
		HASP37A - O4	-4.4	2.05
		OASP37A - H34	-4.7	2.14
		OASP37A - H38	-3.4	1.83
		HE22GLN41A - O12	-4.7	2.01
M345	-25.6424	OASN23A - H35	-4.7	2.17
		OVAL35A - H31	-4.5	1.94

		HASP37A - O4	-4.4	2.16
		OASP37A - H33	-4.7	2.18
		HE22GLN41A - O12	-4.7	1.99
M314	-25.4987	OASN23A - H33	-4.7	2.18
		OVAL35A - H29	-4.6	2.20
		HVAL35A - O24	-3.4	2.27
		OASP37A - H31	-4.3	2.02
		HASP37A - O4	-3.3	2.30
		HE22GLN41A - O12	-4.7	1.90
M309	-25.1242	OASN23A - H36	-3.2	2.32
		OVAL35A - H32	-4.3	2.05
		OASP37A - H38	-4.4	1.73
		OASP37A - H34	-4.7	2.19
		HASP37A - O4	-3.9	1.97
		HE22GLN41A - O12	-4.7	1.88
M385	-25.1083	OASN23A - H35	-4.7	2.07
		OVAL35A - H31	-4.4	1.92
		OASP37A - H37	-3.6	1.92
		OASP37A - H33	-4.7	2.14
		HE22GLN41A - O12	-4.7	1.99

The simulation result suggested that after 1ns of run, the protein-ligand complex of M4-FimH became stable and there was not much fluctuation in the radius of gyration and radius of fluctuation studies. The minimization state was attained at 128 steps with energy -2.4x10-8KJ/mol. This indicates that after binding to M4, the system remained stable, indicating the stable binding of M4.

The numbers of H-bonds were found less in number (only 2) after simulation indicating that few of the bonds were weak and hence got eliminated during the 10ns run. However, the remaining bonds were high energy bonds which need more energy to break and hence, the bonding can be treated as strong.

QSAR study was performed to predict the probable bioactivity of selected ligands. 21 known inhibitors were taken from the bindingdb database and used to generate the QSAR model based on the descriptors viz. molecular weight (MW), Molar Refractivity, Molar Volume, Parachor, Index of Refraction, Surface Tension, Density, LogP and Polarizability (Pol) against their bioactivities ($Log(IC50)^{-1}$). From the analysis, the following QSAR equation has been generated. The analysis shows the descriptor Surface Tension contributes 49% to the activity with descriptor activity correlation 0.71.

Activity = -13.26+0.16*(Surface Tension)

In the multiple linear regression analysis the R^2 was found to be 49.87% and adjusted R^2 was found to be 47.23%. The F Statistics was 18.90 with a critical F value of 4.35, indicating the high significance of the QSAR equation. From the above QSAR equation, the bioactivities of the 21 known inhibitors were predicted and compared with the experimental bioactivities and plotted in a scattered plot (Fig.2). It was clearly seen in the scattered plot that most of the points fall on or

close to the trend line indicating a good QSAR equation. From the equation, the bioactivity [Log(IC50)$^{-1}$)] of the selected compound M4 with Surface Tension 113.4 dyne/cm was found to be 4.82 which is equal to IC50 = 1.514x10^{-5}nM. Thus, the inhibitory concentration of M4 was predicted to be lower than all 21 known fimH inhibitors.

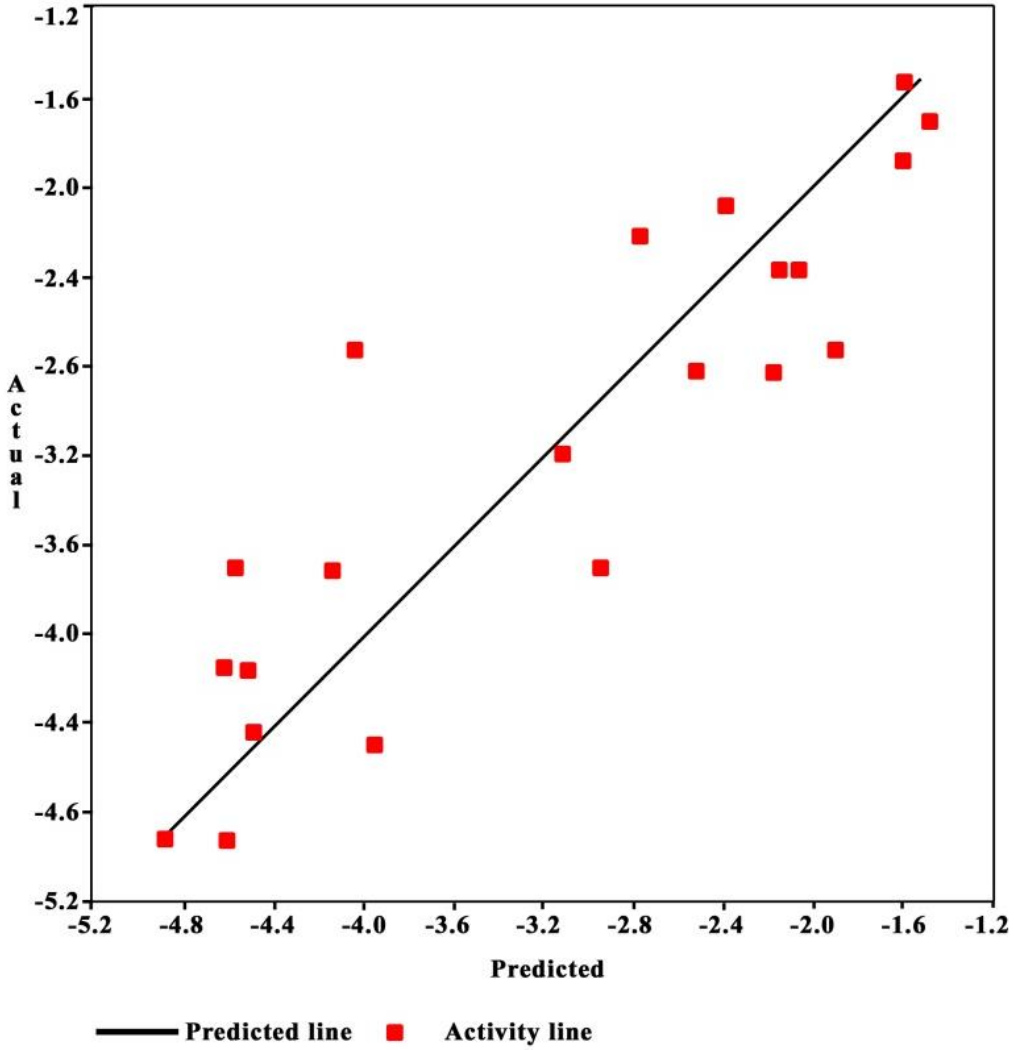

Fig2: QSAR multiple regression plot showing good correlation

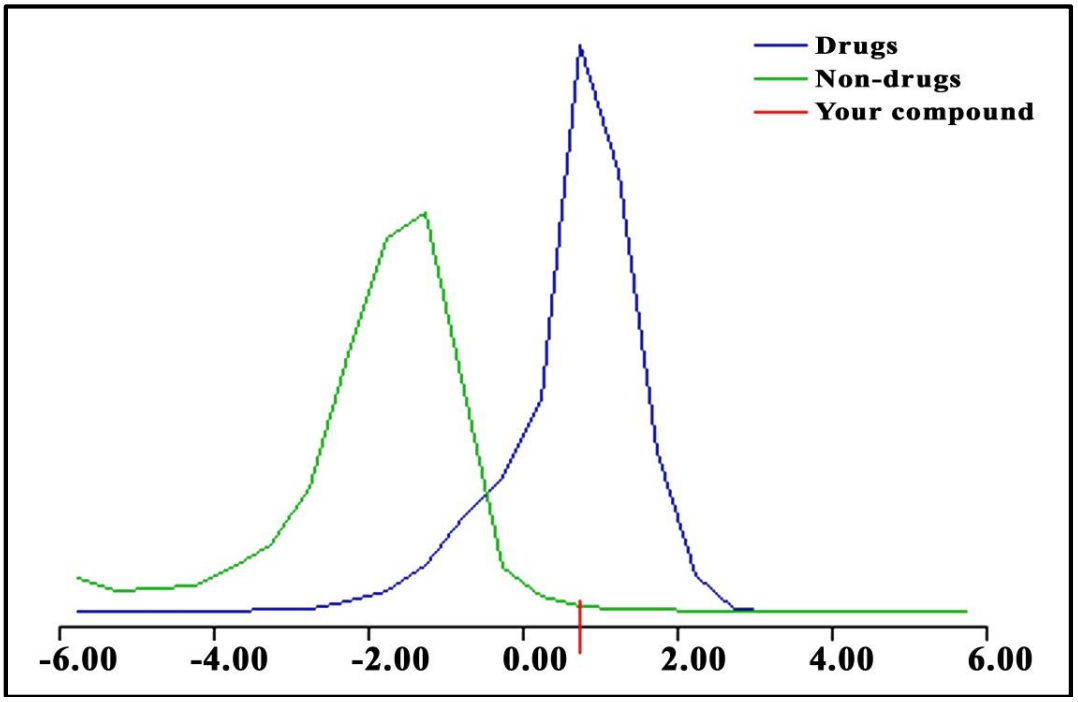

Fig3: High drug-likeness was shown by the best docked ligand M4 (Molsoft Score: 0.76)

Conclusion

From the above work, it was found that the selected mannosides could bind more effectively to the adhesin fimH than other molecules. Hence, the utilization of such ligands as non-antibiotic based inhibitors can be of immense use for treatment against UTIs. The increased binding score, good oral bioavailability, and lower IC_{50} confirms the credibility of the ligand M4 i.e 2-(((3,4,5,6-tetrahydroxytetrahydro-2H-pyran-2-yl)methoxy)amino)-1H-purin-6(9H)-one as the probable drug candidate to treat UTI.

Authors' contribution

All the authors contributed equally to the hypothesis preparation and design of the experiments. AC and MAL did all the experiments and compilation of data. MDC did final checking and overall proof checking before it was submitted for publication.

Conflict of interest: The authors report no conflict of interest arising out of this article. The work does not involve human or animals for its experiments hence ethical constrain does not arise.

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