INTERNATIONAL JOURNAL
OF
PHYTOPHARMACY RESEARCH
www.phytopharmacyresearch.com

LIGAND DOCKING OF SOLUTE CARRIER FAMLY 12 MEMBER 1 WITH LIGANDS OF ISOLATED COMPOUNDS FROM PLANT EXTRACTS AND DRUG FUROSEMIDE

A.Malar Retna*, A. Anita, J. Lydia

Assistant Professor, Department of Chemistry and Research Centre, Scott Christian College (Autonomous),
Nagercoil- 629 003, Tamilnadu, India.

ABSTRACT

Solute carrier is a membrane protein that transport solutes such as ions, metabolites and peptides across biological membranes. The present study evaluates the ligand docking of solute carrier family 12 member 1(NKCC2) with 2-[1-(3-chloro-1H-pyrazol-4-yl)-ethylidene]-indan-1,3-dione isolated from methanol extract of *Dichrostachys cinerea* leaves, 2-bromoethyl 2- (chloromethyl) but-3-enoate, 6-furan-2-yl-2-oxo-1,2-dihydro-pyridine-3-carboxylic acid 2-bromo-ethylester isolated from methanol aerial and root extracts of *Aerva lanata* and ligands of standard drug furosemide. The docking analysis of solute carrier family 12 member 1 (NKCC2) was based on the measurement of binding energy against diseases like congestive heart failure and hypertension. The binding energies obtained for 2-[1-(3-chloro-1H - pyrazol-4-yl)-ethylidene]-indan -1, 3- dione, 2- bromo ethyl - 2- (chloro methyl) but- 3- enoate, 6-furan-2-yl - 2-oxo-1, 2-dihydro – pyridine - 3- carboxylic acid and standard drug furosemide were found to be - 7.30 KCal/mol, 6.73 KCal / mol, 7.04 KCal / mol, – 8.20 KCal/ mol which is exothermic and is possible. From the binding energies obtained for 2-[1-(3-chloro-1H - pyrazol-4-yl)-ethylidene]- indan -1, 3- dione, 2- bromo ethyl - 2- (chloro methyl) but- 3- enoate, 6-furan-2-yl - 2-oxo-1, 2-dihydro – pyridine - 3-carboxylic acid and standard drug furosemide, the best binding energy was obtained for standard drug furosemide and is found to be -8.20ΔG which is exothermic. Thus, from the above information, the ligand docking of solute carrier family 12 member 1(NKCC2) with standard drug furosemide is found to be very effective in the transport of solutes across the biological membranes which help to prevent congestive heart failure and hypertension.

Keywords: Hypertension, Furosemide, Binding, Docking, Solute carrier, Heart failure.

INTRODUCTION

The docking process involves the prediction of ligand conformation with in a targeted binding site [1]. Binding conformations generated by docking programs are thus defined by both position of the ligand on the receptor surface and a particular ligand conformer. The second part of docking program consists of a scoring function, which will distinguish among the generated binding mode the best solution that closely matches the actual mode of binding. The most – favourable predicted protein- ligand complex is considered to be biologically relevant one [2].

In the present investigation an attempt has been made to analyse the docking score of isolated compounds and standard drug furosemide with the target protein solute carrier family 12 member 1. It is a successful target type. They are studied on diseases like congestive heart failure and hypertension.

The HGNC approved gene symbol of solute carrier family 12 member 1 was SLC12A1. The gene type found in solute carrier family 12 member 1 is also known as BSC1, NKCC2. This gene encodes a kidney- specific sodium- potassium- chloride co-transporter that is expressed on the luminal membrane of renal epithelial cells of the thick ascending limb of Henle's loop and the macula densa. It plays a key role in concentrating urine and accounts for the most of the NaCl resorption. It is sensitive to such diuretics as furosemide and butmetamide. Some Bartter – like syndromes result from defects in this gene [3]. By mediating the coupled movement of Na^+, K^+, Cl^- ions across the membrane of most animal cells, the bumetanide - sensitive Na-K- Cl co-transporter (NKCC) plays a vital role in the regulation of ionic balance and cell volume. The transporter is a central element in the process of vectorial salt transport in secretory and absorptive epithelia [4-5].

MATERIALS AND METHODS

Corresponding Author: **A.Malar Retna Email:- iammalar80@gmail.com**

Template Search

Template search with Blast and HHBlits has been performed against the SWISS-MODEL template library. The target sequence was searched with BLAST [5] against the primary amino acid sequence contained in the SMTL. An initial HHblits profile has been built using the procedure outlined[6] followed by 1 iteration of HHblits against NR20. The obtained profile has then been searched against all profiles of the SMTL. A total of 22 templates were found [6].

Template Selection

For each identified template, the template's quality has been predicted from features of the target-template alignment. The templates with the highest quality have been selected for model building.

Model Building

Models are built based on the target-template alignment using ProMod3. Coordinates which are conserved between the target and the template are copied from the template to the model. Insertions and deletions are remodelled using a fragment library. The side chains are then rebuilt. Finally, the geometry of the resulting model is regularized by using a force field. In case loop modelling with ProMod3 fails, an alternative model is built with PROMOD-II [7].

Primary amino acid sequence for which templates were searched and models were built

MPTNFTVVPVEAHADGGGDETAERTEAPGTPEGPEP ERPSPGDGNPRENSPFLNNVEVEQESFFEGKNMALF EEEMDSNPMVSSLLNKLANYTNLSQGVV EHEEDEESRRREAKAPRMGTFIGVYLPCLQNILGVIL FLRLTWIVGVAGVLESFLIVAMCCTCTMLTAISMSAI ATNGVVPAGGSYYMISRSLGPEFGGA VGLCFYLGTTFAGAMYILGTIEIFLTYISPGAAIFQAE AAGGEAAAMLHNMRVYGTCTLVLMALVVFVGVK YVNKLALVFLACVVLSILAIYAGVIKSAF DPPDIPVCLLGNRTLSRRSFDACVKAYGIHNNSATS ALWGLFCNGSQPSAACDEYFIQNNVTEIQGIPGAAS GVFLENLWSTYAHAGAFVEKKGVPSVPV AEESRASALPYVLTDIAASFTLLVGIYFPSVTGIMAG SNRSGDLKDAQKSIPTGTILAIVTTSFIYLSCIVLFGA CIEGVVLRDKFGEALQGNLVIGMLA WPSPWVIVIGSFFSTCGAGLQSLTGAPRLLQAIARDG IVPFLQVFGHGKANGEPTWALLLTVLICETGILIASL DSVAPILSMFFLMCYLFVNLACAVQT LLRTPNWRPRFKFYHWTLSFLGMSLCLALMFICSW YYALSAMLIAGCIYKYIEYRGAEKEWGDGIRGLSLN AARYALLRVEHGPPHTKNWRPQVLVMLNL DAEQAVKHPRLLSFTSQLKAGKGLTIVGSVLEGTYL DKHMEAQRAEENIRSLMSTEKTKGFCQLVVSSSLRD GMSHLIQSAGLGGLKHNTVLMAWPASWK QEDNPFSWKNFVDTVRDTTAAHQALLVAKNVDSFP QNQERFGGGHIDVWWIVHDGGMLMLLPFLLRQHK VWRKCRMRIFTVAQVDDNSIQMKKDLQMFLY HLRISAEVEVVEMVENDISAFTYERTLMMEQRSQM LKQMQLSKNEQEREAQLIHDRNTASHTAAAARTQA PPTPDKVQMTWTREKLIAEKYRSRDTSLSG

FKDLFSMKPDQSNVRRMHTAVKLNGVVLNKSQDA QLVLLNMPGPPKNRQGDENYMEFLEVLTEGLNRVL LVRGGGREVITIYS.

Model Quality Estimation

The global and per-residue model quality has been assessed using the Qualitative Model Energy Analysis (QMEAN) which is a composite scoring function describing the major geometrical aspects of protein structure [8]. For improved performance, weights of the individual QMEAN terms have been trained specifically for SWISS-MODEL.

Target protein model

The target protein model dimensions in Å were found to be X: 42.311, Y: 45.946, Z: 59.495. The protein model of Solute carrier 12 member 1 is presented in Fig.**1**.

RESULTS AND DISCUSSION

Docking analysis between 2-[1-(3-chloro-1H-pyrazol-4-yl)- ethylidene]- indan- 1,3-dione and solute carrier family 12 member 1

The docking analysis was carried with 2-[1-(3-chloro-1H-pyrazol-4-yl)-ethylidene]-indan-1,3-dione isolated from pure methanol extract of *Dichrostachys cinerea* leaves with solute carrier family 12 member 1.

Table.**1** and Fig.**2** reveals the interaction of solute carrier family 12 member 1 with ligand of 2-[1-(3-chloro-1H-pyrazol-4-yl)-ethylidene]- indan-1,3- dione which gives fullfitness value and binding energies corresponding to the element and cluster**.** Thus, the cluster 1, element 1 gives a full fitness value of – 1325.30 KCal / mol and ΔG as - 7.30 KCal / mol which is exothermic and is possible.

Docking analysis between 2- bromo ethyl - 2- (chloro methyl) but- 3- enoate and solute carrier family 12 member 1

The docking analysis was carried with 2- bromo ethyl -2- (chloro methyl) but- 3- enoate isolated from pure methanol aerial extract of *Aerva lanata* with solute carrier family 12 member 1.

Table.2 and Fig. 3 reveals the interaction of solute carrier family 12 member 1 with ligand of 2- bromo ethyl - 2- (chloro methyl) but- 3- enoate which gives fullfitness value and binding energies corresponding to the element and cluster**.** Thus, the cluster 0, element 0 gives a full fitness value of – 1354.01 KCal / mol and ΔG as - 6.73 KCal / mol which is exothermic and is possible.

Docking analysis between 6-furan-2- yl - 2-oxo- 1, 2-dihydro- pyridine- carboxylic acid and solute carrier family 12 member 1

The docking analysis was carried with 6-furan-2-yl - 2-oxo- 1, 2-dihydro- pyridine- carboxylic acid isolated from pure methanol root extract of *Aerva lanata* with solute carrier family 12 member 1.

Table.**3** and Fig. **4** reveals the interaction of solute carrier family 12 member 1 with ligand of 6-furan-2yl - 2-oxo-1, 2-dihydro- pyridine-3-carboxylic acid which gives

fullfitness value and binding energies corresponding to the element and cluster. Thus, the cluster 3, element 3 gives a full fitness value of – 1349.01 KCal / mol and ΔG as - 7.04 KCal / mol which is exothermic and is possible.

Docking analysis between standard drug furosemide and solute carrier family 12 member 1

The docking analysis was carried with standard drug furosemide with solute carrier family 12 member 1.

Table.**4** and Fig.**5** reveals the interaction of solute carrier family 12 member 1 with ligand of standard drug furosemide which gives fullfitness value and binding energies corresponding to the element and cluster. Thus,

the cluster 2, element 1 gives a full fitness value of – 1442.87 Kcal / mol and ΔG as – 8.20 KCal/ mol which is exothermic and is possible.

From the above information, the standard drug furosemide showed best binding energy or Gibb's Free Energy, ΔG of -8.20 KCal/ mol than the docking studies done on solute carrier family 12 member 1 with the ligand of 2-[1-(3- chloro-1H - pyrazol-4-yl)-ethylidene]-indan -1,3- dione, 2- bromo ethyl - 2- (chloro methyl) but-3- enoate, 6-furan-2-yl - 2-oxo-1, 2-dihydro – pyridine - 3-carboxylic acid.

Table 1. Docking scores of 2-[1-(3-chloro-1H- pyrazol-4-yl)-ethylidene]- indan-1, 3-dione with target protein

S.NO	Cluster	Element	Fullfitness (KCal / mol)	Estimated ΔG (KCal / mol)
1	1	0	-1325.30	-7.30
2	1	0	-1323.96	-7.10
3	1	1	-1323.95	-7.10
4	1	2	-1322.09	-6.59
5	1	3	-1321.29	- 6.82
6	1	4	-1320.72	- 6.78
7	1	5	-1320.46	-6.42
8	1	6	-1320.32	-6.39
9	1	7	-1315.04	-6.17
10	2	0	-1323.91	-6.88
11	2	1	-1323.91	-6.88
12	2	2	-1323.91	-6.87
13	2	3	-1323.91	-6.87
14	2	4	-1323.91	-6.87
15	2	5	-1323.91	-6.87
16	2	6	-1323.87	-6.87
17	2	7	-1323.87	-6.87
18	3	0	-1323.38	-6.88
19	3	1	-1315.69	-6.27

Table 2. Docking scores of 2- bromo ethyl- 2- (chloro methyl) but- 3- enoate with target protein

S .NO	Cluster	Element	Fullfitness (KCal / mol)	Estimated ΔG (KCal / mol)
1	0	0	-1354.01	-6.73
2	0	1	-1348.25	-6.56
3	0	2	-1347.88	-6.21
4	0	3	-1345.76	-6.60
5	0	4	-1345.18	-6.35
6	0	5	-1341.13	-6.06
7	0	6	-1336.92	-5.66
8	0	7	-1330.85	-6.17
9	1	0	-1352.64	-6.63
10	1	1	-1352.61	-6.64
11	1	2	-1352.60	-6.64
12	1	3	-1347.56	-6.57
13	1	4	-1344.26	-6.69
14	1	5	-1337.51	-5.89
15	1	6	-1336.45	-5.86
16	1	7	-1335.19	-6.10
17	2	0	-1352.60	-6.42
18	2	1	-1352.60	-6.42
19	3	0	- 1352.57	- 6.49

Table. 3 Docking scores of 6-furan-2- yl - 2-oxo-1, 2-dihydro - pyridine- 3 carboxylic acid with target protein

S .NO	Cluster	Element	Fullfitness (KCal /mol)	Estimated ΔG (KCal /mol)
1	0	0	-1360.18	-7.02
2	0	1	-1348.25	-6.56
3	0	2	-1356.44	-6.64
4	0	3	-1356.03	-6.54
5	0	4	-1350.21	- 6.75
6	1	0	-1347.63	- 6.36
7	1	1	-1360.07	-6.74
8	1	2	-1357.65	-6.70
9	2	0	-1356.17	-6.39
10	2	1	-1359.75	-6.96
11	2	2	-1359.28	-6.87
12	2	3	-1359.25	-6.94
13	2	4	-1356.62	-6.61
14	2	5	-1355.19	-6.52
15	2	6	-1336.45	-6.85
16	2	7	-1355.19	-6.10
17	3	0	-1359.37	-6.95
18	3	1	-1356.85	-6.86
19	3	3	-1349.01	-7.04

Table 4. Docking scores of standard drug furosemide with target protein

S .NO	Cluster	Element	Fullfitness (KCal / mol)	Estimated ΔG (KCal / mol)
1	0	0	-1442.94	-6.86
2	0	1	-1441.62	-7.4
3	0	2	-1439.85	-7.32
4	0	3	-1439.73	-7.31
5	0	4	-1439.46	- 6.75
6	1	5	-1438.06	- 6.42
7	1	6	-1438.00	-6.90
8	1	7	-1436.65	-6.95
9	2	8	-1435.18	-6.92
10	2	9	-1433.20	-.55
11	2	0	-1442.88	-8.19
12	2	1	-1442.87	-8.20
13	2	2	-1431.62	-7.69
14	2	3	-1431.57	-7.71
15	2	4	-1429.99	-8.05
16	2	5	-1423.04	-7.43
17	3	6	-1420.95	-7.50
18	3	7	-1420.74	-7.21
19	2	0	-1442.73	-6.65

Fig 1. The protein model of Solute carrier 12 member 1

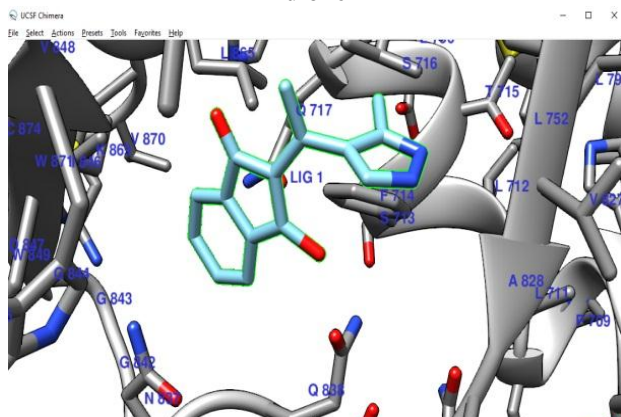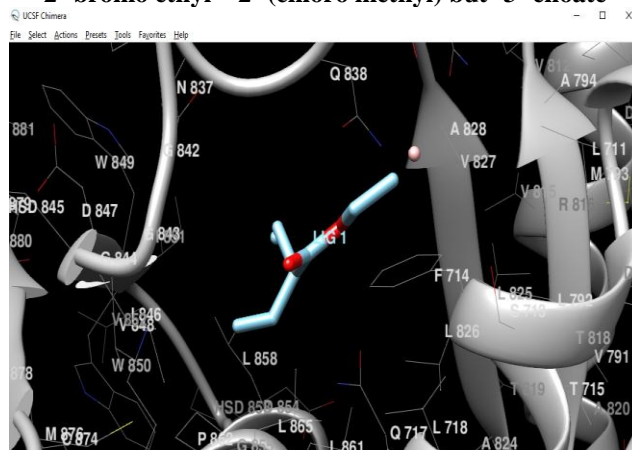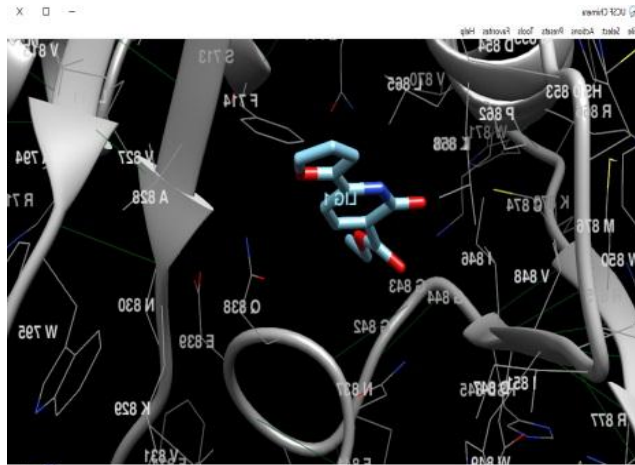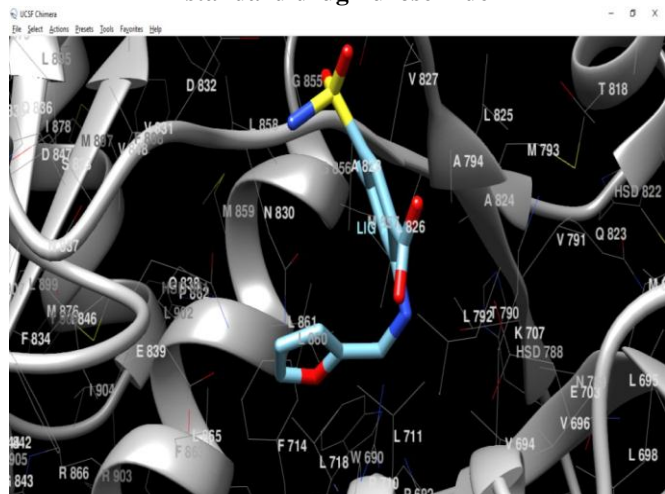|	
Fig 2. Binding of solute carrier family 12 member 1 with 2-[1-(3-chloro - 1H pyrazol-4-yl) - ethylidene]- indan-1,3-dione	**Fig 3. Binding of solute carrier family 12 member 1 with 2- bromo ethyl – 2- (chloro methyl) but- 3- enoate**
Fig.4 Binding of solute carrier family 12 member 1 with 6-furan-2- yl - 2-oxo-1, 2-dihydro- pyridine-3-carboxylic acid	**Fig 5. Binding of solute carrier family 12 member 1 with standard drug furosemide**

CONCLUSION

The docking analysis of solute carrier family 12 member 1 with the ligands of isolated compounds and standard drug furosemide was based on the measurement of binding energies. From the binding energies obtained for 2-[1-(3-chloro-1H - pyrazol-4-yl)-ethylidene]- indan -1, 3-dione, 2- bromo ethyl - 2- (chloro methyl) but- 3- enoate, 6-furan-2-yl - 2-oxo-1, 2-dihydro – pyridine - 3-carboxylic acid and standard drug furosemide, the best binding energy ΔG obtained was found to be -8.20 KCal/ mol. Hence, from the above information, the ligand docking of solute carrier family 12 member 1 with standard drug furosemide is found to be very effective in the transport of solutes across the biological membranes which help to prevent congestive heart failure and hypertension.

ACKNOWLEDGEMENTS

REFERENCES

1. Kitchen BD, Decornez H, Furr RJ, *et al.* Docking and scoring in virtual screening for drug discovery: methods and applications. *Nature reviews*, 3, 2004, 935-949.
2. Kuntz ID, Blaney JM, Oately SJ, *et al.* A geometric approach to macromolecular- ligand interaction. *Journal of Molecular Biology,* 161, 1982, 269-288.
3. SLC12A1 Solute carrier family 12 member 1[Homo sapiens (human)] –Gene- NCBI.htm, 2017.
4. Xu CJ, Lytle C, Zhu CL, *et al.* Molecular cloning and functional expression of the bumetanide- sensitive Na- K-Cl Co-transporter. *National academy of Sciences*, 9, 1994, 2201-2205.
5. Altschul SF, Madden TL, Schaffer AA, *et al.* Gapped BLAST and PSI- BLAST: a new generation of protein data base search programs. *Nucleic Acids Research*, 25, 1997, 3389-3402.
6. Benkert P, Biasini M, Schwede T. Toward the estimation of the absolute quality of individual protein structure models. *Bioinformatics,* 27, 2011, 343-350.

7.   Guez N, Peitsch MC. SWISS- MODEL and the swiss-pdb viewer: an environment for comparative protein modeling. *Electrophoresis,* 18, 1997, 2714-2723.

8.   Benkert P, Tosatto SC, Schomburg D. QMEAN: A comprehensive scoring function for model quality assessment. *Protein,* 7, 2008, 261-277.