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DOCKING STUDIES OF NOVEL 2-[5-SUBSTITUTED-1-*H* BENZO (d)IMIDAZOLE-2-YL SULFINYL] METHYL 3-SUBSTITUTED QUINAZOLINE-4(3*H*)ONE

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Abstract: In present investigation; we tried to authenticate quinazoline derivatives, which are extensively reported in the literature as antiulcer agent. In doing so we attempted virtual ligand screening; which found more promising as compare to high throughput screening in overcoming the limitations of unprecedented number of novel leads. We selected fifty one novel 2-[5-substituted -1-*H* benzo(d)imidazole-2-yl sulfinyl]methyl-3-substituted quinazoline-4(3H)one and subsequently docked them into newly generated homology model of H^+/K^+ ATPase at BioMed CAChe workstation V6.1.1.The analogues **42** and **43** were found promising. Omeprazole is selected as a standard. Structure based drug designing of these analogues may found a novel antiulcer drug at future study.
Keywords: Docking, Homology, Quinazoline, H^+/K^+ATPase, Antiulcer

Introduction

The drug discovery scientists are placing their all efforts to overcome the lead discovery bottleneck in drug development. The approach of 1980 and early 1990s; experimental high throughput screening(HTS) and combinatorial chemistry produces number of leads but few hits could be validated and further optimized into actual leads and preclinical candidates. In such lieu, a new technique, 'virtual screening' (VS) was coined and adopted by the community. In contrast to HTS, which is largely phenomenological and technology driven, in VS compounds are selected by predicting their binding to a macromolecular target using computer programme.We approached the VS technology in finding a lead molecule in the campaign of antiulcer chemotherapy.

The enzyme H^+/K^+ATPase, is responsible for gastric acid production[1,2] was selected for the study. The gastric H^+/K^+ATPase is the member of the P2 type ATpase family and undergoes cycle of phosphorylation and dephosphorylation coupled to the outward and inward transport of hydrogen and potassium ions,respectively, in the secretory canaliculus of the parietal cell. Conformations of the enzyme that bind ions for outward transport are defined as E1, where as that bind luminal ions for inward transport are termed E2. There is a functional similarity of H^+/K^+ATPase to the Na^+/K^+ATPase at pH 8.0. The primary structure of the gastric H^+/K^+ATPase (HK R 1)shows significant homology to the Na^+,K^+-ATPase (62%) and the sr Ca-ATPase 1 (29%). The ion binding sites of the Na^+/K^+ATPase are homologous to these, and other ,P2-type ATPases in that they have only carboxylate side chains as the counter charge species [3,4].This enzyme is located in the secretary membranes of the parietal cell. Gastric acid secretion is regulated by interaction of basolateral parietal cell receptors with their physiological stimulants gastrin, acetychloline, and histamine [5]. The irreversible inhibition of the H^+/K^+ATPase, a means of controlling gastric pH has attracted considerable attention in recent years.

Various therapeutics strategies have been utilized for the acid induced ulcer, such as acid neutralizing agents, acid inhibitory agents, antigastrin agents, ulcer insulators and promoters of ulcer healing agents. Various irreversible acting pyridinyl methyl sulfinyl benzimidazole derivatives have been synthesized but few of them are potent and are in current use such as Omeprazole, Pantoprazole, Rabeprazole, Esomeprazole, Lansoprazole.These accumulate in the acidic phase of the secretory canaliculus and then are acid- activated to form cationic, thiophilic intermediates.These react with one or more cysteine sulfhydryls at the luminal surface of the proton pump to form disulfide bonds. It is known that all proton pump inhibitors (PPIs) bind to cysteine 813(3), resulting in covalent inhibition of the enzyme via formation of this disulfide which stabilizes the enzyme in the E2 conformation [6,7].

Besides,studies that described simple chemical modification(insertion of pyrimidine ring instead of pyridine)of the benzimidazole sulfinyl methyl pyridine,this approach resulted in increase in antiulcer and antisecretory activity [8]. It has been further reported that 4-(arylamino)quinazolines,2,4 bis(aryl amino)quinazolines,and 2,4 bis(aryl amino)thieno pyrimidines showed good reversible proton pump inhibitory activity [9]. The rationale behind the use of quinazolinone is that the weakly basic nature of pyrimidine as compared with pyridine this makes the formation of sulfenamide intermediate difficult and compound acts as reversible proton pump inhibitors.

Furthermore,it had been reported that a proton pump inhibitors 8[(5-fluro-2-benzimidazolyl) sulfinyl-3-methyl 5,6,7,8 tetrahydroquinoline showed potent antisecretory and antiulcer activity. Biochemical studies suggest that this would be an appropriate lead compound for further modification.[10]

Virtual screening requires a prerequisite knowledge about spatial and energetic criterion responsible for binding of particular candidate ligand to the receptor under investigation. The quinazoline derivatives are docked in H^{+}/K^{+} ATPase (generated homology model) and corresponding dock scores are generated. Among the docked analogues, **42** and **43** showed dock score of -91.24 and -92.36 kcal/mol respectively, indicating that these analogues are promising.

Experimental

Homology modeling

In the present investigation we developed homology model of H^{+}/K^{+}ATPase by workspace platform of BioMedCache 6.1.1 with the template crystal structure of 3b8ec as shown in Figure 1 because which is not prepared in protein data bank. The modeled residue range is 42 to 1039. The template crystal structure is of 3.5 A° resolution. The sequence identity between the template and target is 65.63%. The corresponding E-value is 0.00, exhibiting high evolutionary resemblance. The alignment of sequences is done at BLOSSUM 5.0. Firstly the backbone alignment is completed. The CSP loop was built with anchor residues LEU 964 and ASN 967. Numbers of ligations found were 2, there were zero clashes. Side chains are optimized to build the loops. Final energy of the protein is -9578.411 KJ/mol. Model was evaluated by Ramchandran plot for its backbone confirmation [11,12] which is shown in Figure 2.

Docking

Molecular docking studies helped in understanding the interactions between the ligands and enzyme active site.Since it is highly probable that the investigated compounds **5a-o** undergo invivo H^{+}/K^{+}ATPase enzymatic inhibition.Molecular docking studies for these quinazoline-4(3*H*)-ones derivatives were performed.Structures of molecules were generated at workspace platform of BioMedCache 6.1.1, available at Nanded Pharmacy College (Nanded) India, using internal default settings for convergence criteria. Structures were beautified (cleaned) for valencey ring geometry. The beautified structures were energy minimized using MM3 force field (standard method).Such energy minimized structures were ready for docking. Structures were docked in to active site of homology generated H^{+}/K^{+}ATPase model using automated docking at workstation and scores are as shown in Table 1.While docking ligands were kept flexible and active site was kept rigid. The dock scores were generated as PMF scoring function.

Results and discussion

An in-silico method have been used inorder to generate the candidate model of gastric H^{+}/K^{+} - ATPase using MODELLER software. The model is confirmed for its backbone symmetry as 87.9% of amino acid residue lie with in the most acceptable region at Ramchandran plot Multiple sequences alignment was carried out using CLUSTLAW. Candidates were evaluated by using PROCHEK and PROFIT. Side chain directions were inspected visually using PYMOL to determine whether there were any violations of the experimentally determined data. PROCHEK produced a G-Factor which assessed the probabilities of the conformation having permitted stereochemistry by reference to Ramchandran plot and residues properties. Model producing high G-factors were selected. The models with low <5 A^{0} RMSD values were selected. The model ranked the highest under these evaluation criteria was used in subsequent ligand docking studies.The model is confirmed for its backbone symmetry as 87.9% of amino acid residue lie with in the most acceptable region at Ramchandran plot .Very few amino acids exhibits negative energy at GROMOS force field.

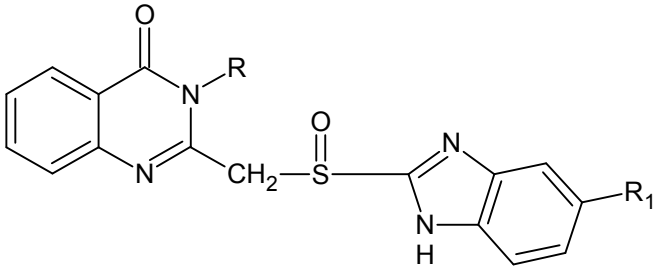
This study validates the model to be used for virtual screening in drug designing.Docking study also shows that model can be used for virtual screening of antiulcer activity.The flexible docking studies were carried out by using above said validated docking protocol.The analogs **42,43** showed higher dock score, this may be due to methoxy at fifth position of benzimidazole which stabilises the moiety as shown in figure 3..The analogs

18,26-28,33,35,37-41,46,51 showed dock score above -80 may be due to presence of methoxy and difluromethoxy at fifth position of benzimidazole.Almost all compounds showed good docking score.

The homology model of H^+/K^+ATPase is built at BioMedCAche (v 6.1.1). The detailed procedure has been discussed in experimental part. Energy minimization of H^+/K^+ATPase helped, relieve any steric clashes or improper geometries in the protein structure to produce a model with correct bond length and bond angles and where individual atoms are not too close together.The molecular mechanics calculations for energy minimization are used. The model is evaluated by ANOLEA, representing y axis of the plot, energy for each amino acid of the protein chain. Negative energy values represent favorable energy environment where as positive values unfavorable energy environment for a given amino acid. There are few amino acids lying in unfavorable energy environment which can be ignored in overall consideration of the model structure.

The distribution of main chain torsion angles phi and psi are examined in Ramchandran plot. The result clearly shows the vast majority of the amino acids are in phi-psi distribution constraint with right handed helices. The remaining residues that fall into the random or configuration geometries are very short segments and are primarily in the loop regions of the protein. The 87.9% of residues of the model lie within the most favored region indicating good quality model. The backbone root mean square deviation (RMSD) between the final model and the template crystal is 1.36 A^o validating the model for further structure based drug design. The sequence alignment of template (3b8eC) with target sequence (NP_115980) (H^+/K^+ATPase) is shown in Figure 4.

Table 1. Docking score of novel 2-((5-Substituted benzo[d] imidazol-2-yl sulfinyl) methyl)-3-substituted quinazolin-4(3H)-one



Compound	R	R_1	Free energy of binding (kcal / mol)
1	H	H	-64.252
2	CH_3	H	-68.364
3	$COCH_3$	H	-69.128
4	OCH_3	H	-58.689
5	OC_2H_5	H	-71.913
6	Br	H	-69.066
7	Cl	H	-67.989
8	H	OCH_3	-71.053
9	CH_3	OCH_3	-68.014
10	$COCH_3$	OCH_3	-64.144
11	OCH_3	OCH_3	-66.134
12	OC_2H_5	OCH_3	-64.573
13	Br	OCH_3	-63.486
14	Cl	CH_3	-57.694
15	H	$OCHF_2$	-77.114
16	CH_3	$OCHF_2$	-70.172
17	$COCH_3$	$OCHF_2$	-75.748
18	OCH_3	$OCHF_2$	-87.553
19	OC_2H_5	$OCHF_2$	-62.549
20	Br	$OCHF_2$	-60.884
21	Cl	$OCHF_2$	-75.662
22	CH_3	H	-72.397
23	C_2H_5	H	-74.007
24	isopropyl	H	-68.188
25	cyclohexyl	H	-58.761
26	pyridyl	H	-80.922

27	CH_3	OCH_3	-80.113
28	C_2H_5	OCH_3	-81.443
29	isopropyl	OCH_3	-76.433
30	cyclohexyl	OCH_3	-65.867
31	pyridyl	OCH_3	-77.449
32	CH_3	$OCHF_2$	-75.831
33	C_2H_5	$OCHF_2$	-84.667
34	isopropyl	$OCHF_2$	-69.608
35	cyclohexyl	$OCHF_2$	-82.797
36	pyridyl	$OCHF_2$	-73.789
37	3,4dimethoxy phenyl	H	-82.752
38	2-pyridyl	H	-55.896
39	3-pyridyl	H	-75.791
40	2-pyrazine	H	-83.466
41	5-tetrazole	H	-84.82
42	3,4dimethoxy phenyl	OCH_3	-91.24
43	2-pyridyl	OCH_3	-92.36
44	3-pyridyl	OCH_3	-68.21
45	2-pyrazine	OCH_3	-74.87
46	5-tetrazole	OCH_3	-81.10
47	3,4dimethoxy phenyl	$OCHF_2$	-49.74
48	2-pyridyl	$OCHF_2$	-68.83
49	3-pyridyl	$OCHF_2$	-65.09
50	2-pyrazine	$OCHF_2$	-79.60
51	5-tetrazole	$OCHF_2$	-84.32
Omeprazole			-71.382

Figure 1. Homology Model of H^+/K^+ATPase generated with mulsequence alignment using template (3b8eC).The model represents α subunit in the E_2-P conformation

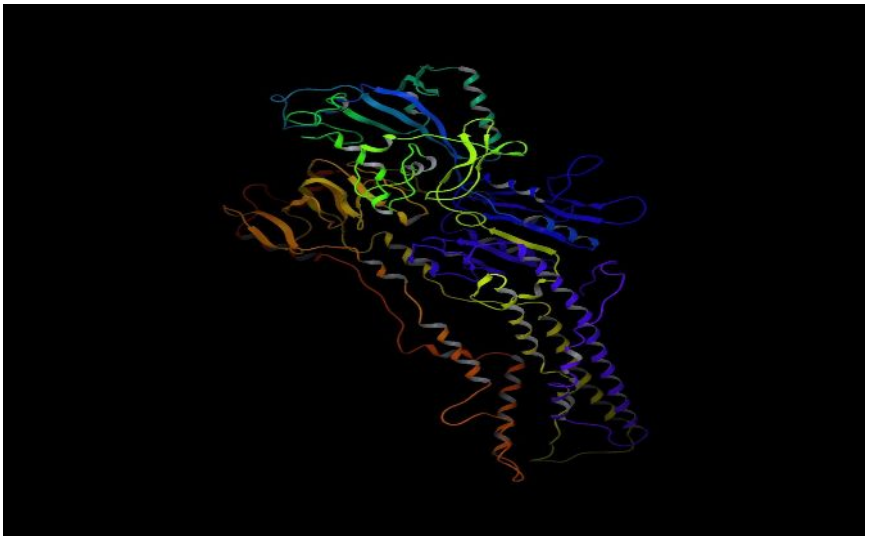
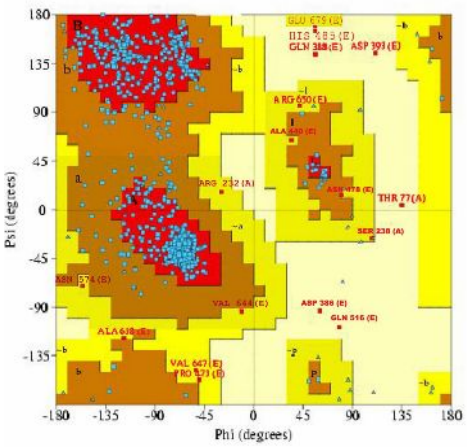


Figure 2. Ramachandran plot produced by PROCHECK of the homology model based on template 3b8eC with their statistics



	No. of residues	Percentage
Most favoured regions	595	85.2%
Additional allowed regions	87	12.5%
Generously allowed regions	9	1.3%
Disallowed regions	7	1.0%
Non Glycine and Non praline residues	698	100%
End residues	2	
Glycine residues	62	
Proline residues	35	

Figure 3. Equilibrated molecular dynamics snapshot of the docked compound 42,43 in the active site of H^+/K^+ATPase.

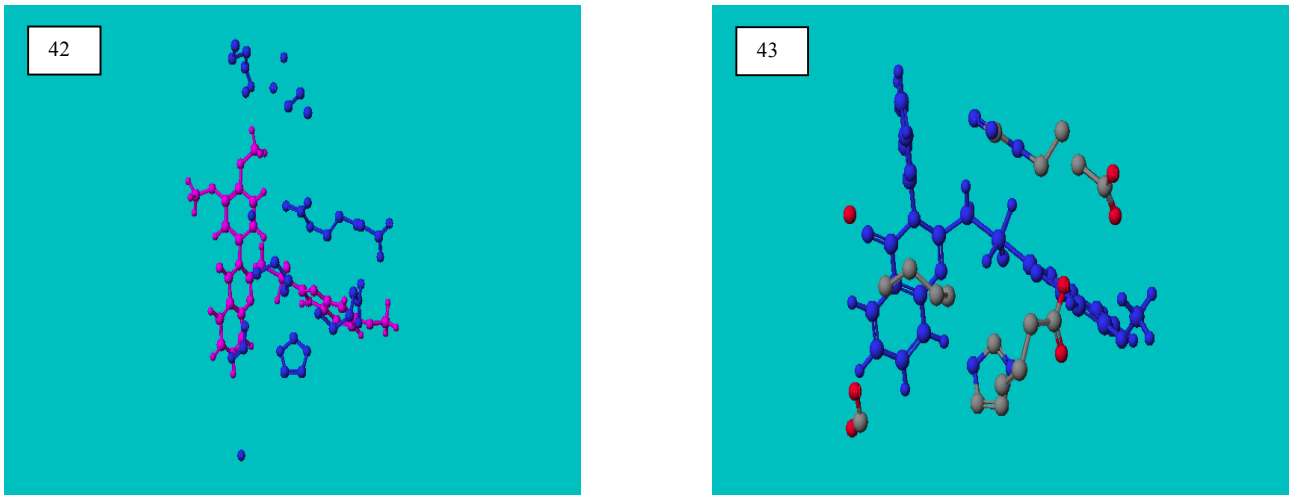


Figure 4. Multiple Sequence alignment of template (3b8eC, Na^+/K^+ATPase) with target sequence (NP_115980)

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TARGET   42      KKKNHKE EFQKELHLDD HKLSNRELEE KYGTDIIMGL SSTRAAELLA
3b8eC    19    A—KKERDMD ELKKEVSMDD HKLSLDELHR KYGTDLSRGL TPARAAEILA

TARGET   89    RDGPNSLTPP KQTPEIVKFL  KQMVGGFSIL LWVGAFLCWI AYGIQYSSDK
3b8eC    67    RDGPNALTPP PTTPEWVKFC RQLFGGFSML LWIGAILCFL  AYGIQAATEE

TARGET   139   SASLNNVYLG CVLGLVVILT GIFAYYQEAK STNIMSSFNK MIPQQALVIR
3b8eC    117   EPQNDNLYLG VVLSAVVIIT  GCFSYYQEAK SSKIMESFKN MVPQQALVIR

TARGET   189   DSEKKTIPSE  QLVVGDIVEV  KGGDQIPADI  RVLSSQGCRV DNSSLTGESE
3b8eC    167   NGEKMSINAE EVVVGDLVEV KGGDRIPADL RIISANGCKV  DNSSLTGESE

TARGET   239   PQPRSSEFTH ENPLETKNIC FYSTTCLEGT VTGMVINTGD RTIIGHIASL
3b8eC    217   PQTRSPDFTN ENPLETRNIA FFSTNCVEGT ARGIVVYTGD RTVMGRIATL

TARGET   289   ASGVGNEKTP IAIEIEHFVH  IVAGVAVSIG ILFFIIAVSL KYQVLDSIIF
3b8eC    267   ASGLEGGQTP  IAAEIEHFIH  IITGVAVFLG VSFFILSLIL EYTWLEAVIF

TARGET   339   LIGIIVANVP EGLLATVTVT LSLTAKRMAK KNCLVKNLEA VETLGSTSII
3b8eC    317   LIGIIVANVP EGLLATVTVC LTLTAKRMAR KNCLVKNLEA VETLGSTSTI

TARGET   389   CSDKTGTLTQ NRMTVAHLWF DNQIFVADTS EDHSNQVFDQ SSRTWASLSK
3b8eC    367   CSDKTGTLTQ NRMTVAHMWS DNQIHEADTT ENQSGVSFDK TSATWLALSR

TARGET   439   IITLCNRAEF KPGQENVPIM KKAVIGDASE TALLKFSEVI LGDVMEIRKR
3b8eC    417   IAGLCNRAVF QNAQENLPIL KRAVAGDASE SALLKCIELC CGSVKEMRER

TARGET   489   NRKVAEIPFN STNKFQLSIH EMDDPHGKRF LMVMKGAPER ILEKCSTIMI
3b8Ec    467   YTKIVEIPFN STNKYQLSIH KNPNTAEPRH LLVMKGAPER ILDRCSSILI

TARGET   539   NGEEHPLDKS TAKTFHTAYM ELGGLGERVL GFCHLYLPAD EFPETYSFDI
3b8eC    517   HGKEQPLDEE LKDAFQNAYL ELGGLGERVL GFCHLFLPDE QFPEGFQFDT

TARGET   589   DAMNFPTSNL CFVGLLSMID PPRSTVPDAV TKCRSAGIKV IMVTGDHPIT
3b8eC    567    DDVNFPLDNL CFVGLISMID PPRAAVPDAV GKCRSAGIKV IMVTGDHPIT

TARGET   639   AKAIAKSVGI ISANSETVED IAHRLNIAVE QVNKRDAKAA VVTGMELKDM
3b8eC    617    AKAIAKGVGI ISEGNETVED IAARLNIPVS QVNPRADAKAC VVHGSDLKDM

TARGET   689   SSEQLDEILA NYQEIVFART SPQQKLIIVE GCQRQDAVVA VTGDGVNDSP
3b8eC    667   TSEQLDDILK YHTEIVFART SPQQKLIIVE GCQRQGAIVA VTGDGVNDSP

TARGET   739   ALKKADIGIA MGIAGSDAAK NAADMVLLDD NFASIVTGVE EGRLIFDNLK
3b8eC    717    ASKKADIGVA MGIAGSDVSK QAADMILLDD NFASIVTGVE EGRLIFDNLK


TARGET   789   KTIAYSLTKN IAELCPFLIY  IIVGLPLPIG TITILFIDLG  TDIIPSIALA
3b8eC    767   KSIAYTLTSN  IPEITPFLIF   IIANIPLPLG  TVTILCIDLG TDMVPAISLA


TARGET   839   YEKAESDIMN RKPRHKNKDR LVNQPLAVYS YLHIGLMQAL GAFLVYFTVY
3b8eC    817    YEQAESDIMK RQPRNPKTDK LVNEQLISMA  YGQIGMIQAL  GGFFTYFVIL

TARGET   889   AQEGFLPRTL INLRVEWEKD YVNDLKDSYG  QEWTRYQREY LEWTGYTAFF
3b8eC    867    AENGFLPIHL LGLRVNWDDR WINDVEDSYG QQWTYEQRKI  VEFTCHTPFF

TARGET  939   VGILVQQIAD   LIIRKTRRNS   IFQQGLFRNK   VIWVGITSQI IIGLILSYGL
3b8eC    917   VTIVVVQWAD LVICKTRRNS VFQQGM-KNK ILIFGLFEET   ALAAFLSYCP

TARGET   989   GSVTALSFTM   LRAQYWFVAV PHAILIWVYD EVRKLFIRLY PGSWWDKNMY
3b8eC    966   GMGVALRMYP LKPTWWFCAF  PYSLLIFVYD  EVRKLIIRRR PGGWVEKETY

TARGET  1039   Y
3b8eC    1016  Y-
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