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IN SILICO DISCOVERY AND MOLECULAR DOCKING EVALUATION OF NOVEL 3-(TRI FLUORO METHYL) - 5,6,7,8 - TETRA HYDRO –[1,2,4]-TRIAZOLO (4,3-A)- PYRAZINE (SITAGLIPTIN INTERMIDIATE DERIVATES) INHIBITORS ON E-COLI DNA GYRASE-A

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Abstract:

The aim of present investigation is to identify the new potential inhibitors for E. coli DNA Gyrase-A by using in silico and molecular docking. A series of Sitagliptin title compounds were designed and were docked within the "Quinolone Resistance Determining Region" (QRDR) of E. coli DNA Gyrase-A (EcGyr-A) chain (QRDR-A). The obtained docking scores of Sitagliptin intermediate derivatives were compared with score of reference ligand ciprofloxacin and norflaxacin, under identical experimental sets. The Sitagliptin intermediate derivatives (7-(2-Nitrophenylsulfonyl)-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3,α] pyrazine), showed highest docking score i. e -8.5 kcal.mol^{-1}, and (7-(4-Chloro-3-nitrophenylsulfonyl)-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo [4,3,α] pyrazine), (7-(4- Flurophenylsulfonyl)-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4] triazolo [4,3,α] pyrazine), (7-(4-Nitrophenylsulfonyl)-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3,α] pyrazine) and (7-(4-Iodophenylsulfonyl)-3-(trifluoromethyl)-5,6,7,8-tetrahydro-[1,2,triazolo[4,3,α] pyrazine) shows moderate docking scores i.e -8.4, -8.0, -7.8 and -7.8 kcal.mol^{-1} respectively, though the presence of halides at different positions in the parent compound, the sifting position of nitro group from o-p (ortho to para) positions there was great observation of potent inhibitor activity. The results concludes among the tested synthesized analogues selected for docking studies, the compound 6a i.e (7-(2-Nitrophenylsulfonyl)-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3,α] pyrazine) worked as most potent inhibitor for E. coli DNA Gyrase-A.

Keywords: *Ciprofloxacin, Docking, DNA Gyrase-A, Norflaxacin, Protein Data Bank, Sitagliptin Intermediate Derivatives*

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INTRODUCTION:

Diabetes is a multifactorial disease that is classified as chronic hyperglycemia due to defects in insulin secretion, action or both, which results in abnormalities in carbohydrate, fat and protein metabolism. The World Health Organization (WHO) reported in 2000 a worldwide prevalence of 154.4 million subjects with diabetes and predicts that by the year 2025 there will be nearly 300 million diabetics. Management of type-II diabetes includes a prudent diet, regular exercise and medicine to reduce blood glucose levels. Pharmacological options available in the management of type 2 diabetes include thiazolidinediones, sulphonylureas, α-glucosidase inhibitors, metformin and insulin. These treatment options, although highly effective in reducing blood glucose levels, may be associated with an increased risk of hypoglycaemia, as seen with sulphonylureas and insulin; weight gain, as noted with insulin, sulphonylureas and thiazolidinediones; and gastrointestinal intolerance, as observed with metformin. These unwanted adverse effects may act as barriers to optimal glycaemic control [1].

Sitagliptin intermediate derivatives are chemically, (3R)–3 amino-1-[3-(trifluoromethyl)-5, 6 dihydro [1,2,4] triazolo [4,3-a] pyrazin-7 (8H)-yl]–4-(2,4,5, triflurophenyl) butan–1-one phosphate hydrate, is an oral dipeptidyl peptidase–4 (DPP-4) inhibitor for the treatment of type-II diabetes mellitus, and which improves glycaemic control by inhibiting dipeptidyl peptidase–4 (DPP-4) inactivation of the glucose–dependent insulin tropic polypeptide (GIP) and glucagon like peptide-1 (GLP-1). This inhibits releasing of glucagon from alpha cells of pancreas and slows the absorption of nutrients into the blood stream and further causes an enhances in the amount of insulin release from beta cells of pancreas [2-6].

DNA gyrase is a bacterial type II topoisomerase which couples the free energy of ATP hydrolysis to the introduction of negative supercoils into DNA. This plays essential roles in bacterial DNA replication. DNA gyrase is a heterotetrameric structure, consisting of two proteins Gyrase-A (GyrA) and Gyrase-B (GyrB), which form an A_2B_2 complex in the active enzyme. Broadly speaking, the A subunit is involved with interactions with DNA, it contains the active-site tyrosine responsible for DNA cleavage, and the B subunit contains the ATPase active site. There is currently no high-resolution structure for the gyrase holoenzyme (A_2B_2), but several X-ray crystal structures exist for individual domains from various bacterial species [7-8], a fusion of a GyrB with a GyrA domain, with and without DNA [9].

Norfloxacin is a bactericidal and is a class of quinolone / fluoroquinolone antibiotic drug. The mechanism of norfloxacin depends on blocking of bacterial DNA replication by binding of itself to DNA gyrase enzyme. Especially this drug has 100 times higher affinity for bacterial DNA Gyrase than mammalian. Ciprofloxacin is an antibiotic and is widely used for second generation of quinolones. Ciprofloxacin is a selective potent inhibitor of CYPIA2, CYP26 and CYP3A4. The applications of Ciprofloxacin is to inhibits DNA Gyrase, type -2 topoisomerase, type-4 isomerase and is necessary to separate bacterial DNA strands.

MATERIALS AND METHODS:

Protein preparation

The Crystal structure of signaling molecules which are involved in NF-KB activation pathway such as E coli DNA gyrase A is downloaded from PDB (Brook hooven, database). All Water molecules were removed and hydrogens were added and, energy minimization was carried out with force field grooms 96 using SWISS PDB viewer [10].

Crystal structure of Molecular targets constitutes with ligands which are separated using SWISS PDB viewer, the polymers and chains selected for each protein.

Ligand Preparation

The two-dimensional (2D) structures of Norfloxacin and Ciprofloxacin analogues were drawn using Chem Draw ultra 10.0 (Cambridge software) and was saved as MDL Mol files. The three-dimensional structures (3D) were generated using Glyco BioChem PRODRG2 online server (http://davapc1.bioch.dundee.ac.uk/prodrg/) [11]. To obtain 3D structure as PDB file format, the 2D structure (MDL Mol files) were used as input files for PRODRG. The finally obtained 3D structures were energy minimized using Hyperchem's MM^+ force field (http://www.hyper.com/) [12]. The minimization was executed until the root mean square (r.m.s) gradient value reached a value smaller than 0.001 $kcal.mol^{-1}$. Such energy minimized structures of ofloxacin analogues were considered for molecular docking studies.

Retrieval and preparation of 3D-structure of E coli DNA Gyr-A

The 3D X-ray crystal structure of target protein E coli DNA Gyr-A was retrieved from Brook Heaven Protein Data Bank (PDB database) (http://www.rcsb.org/pdb) (PDBID: 1AB4) at 1.60 Å RMSD resolution. Identification and analysis of protein template i.e. QRDR-A was considered as standard, reported by Yoshida et al, [1990]; Conrad et al, [1996]; Friedman et al, [2001] [13-15].

Molecular docking with Sitagliptin title compound derivatives and scoring

Molecular Docking is the process in which two molecules fit together in 3D space. It is a key tool

in structural biology and computer-aided drug design. The goal of ligand and protein docking is mainly to predict the predominant binding mode(s) of a ligand with a protein of known three-dimensional structure. In MVD the receptor and ligand coordinates were used in PDB format. Mol Dock docking engine of MVD automatically identifies potential binding sites, (hereafter referred to as cavity) using the cavity detection algorithm. During Docking at first the molecules were prepared and bonds, bond orders, explicit hydrogens, charges, flexible torsions, were assigned if they were missing, by the MVD program to both the protein and ligands. The molecular docking studies were performed for compounds against Ecoli Dna Gyrase by appropriate X, Y, and Z grid parameters by using AUTODOCK vina with PyRx [16]. The Autogrid is used to know the Best Binding pocket for each ligand molecule and docking Results were interpreted. Autodock uses Lamarckian genetic algorithm [17]. The interaction between protein and ligands was interpreted using PyMOL, bond length, key amino acid close to the ligand the interaction figures were analysed using PyMOL viewer (18). From the docking wizard, ligands were selected and the docking was performed in the QRDR-A including Ser83 and Asp87, taking bound fluoroquinolone molecule as standard ligand.36 An exhaustive systemic search of the conformational space was performed with the help of heuristic search algorithm to locate the possible position of ligand in the QRDR-A during docking simulation. The QRDR-A is defined as a spherical region, surface area: 305.92Å2, coordinates dimensions X (68.08 Å), Y (76.18 Å), Z (25.01 Å) axes, respectively. The potential binding site within QRDR-A; a cavity of volume 67.58 Å3 was observed close to amino acid residue Asp82, Ser83, Ala84, Tyr86, Asp87, Val90, Arg91, Gln94, Phe96 and Ser97 located within the constraints 17 Å (Fig. 3). The search algorithm was taken as Moldock SE and docking was performed using a grid resolution of 0.3 Å. For each of the 10 independent runs; a maximum number of 1500 iterations were executed on a single population of 50 individuals. Side chain flexibility of the amino acids present in the binding site of QRDR-A was incorporated during docking run was performed. For each benchmark complex, 10 independent runs were conducted and each of these runs returning one solution (pose). These 10 solutions were then re-ranked and the highest ranked (ranked by the lowest docking energy) solution was compared with the reference ligand, along with their docking score.

RESULTS AND DISCUSSION:

E coli DNA Gyrase A has shown best binding affinities for five organo synthetic compounds such as sitagliptin intermediate derivatives 6a (7-(2-Nitrophenylsulfonyl)-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3,α] pyrazine), 5a (7-(4-Chloro-3-nitrophenylsulfonyl)-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo [4,3,α] pyrazine), 1a (7-(4- Flurophenylsulfonyl)-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3,α] pyrazine), 4a (7-(4-Nitrophenylsulfonyl)-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3,α] pyrazine) and 2a (7-(4-Iodophenylsulfonyl)-3-(trifluoromethyl)-5,6,7,8-tetrahydro [1,2,triazolo[4,3,α] pyrazine) and which was shown in Table 1. E coli DNA Gyrase A was bound to compound 6a with binding energy -8.5kcal/mol and showed four hydrogen bonds with the Asp 338, Asp 338, Pro 274 and Pro 274, the bond angles were 2.4, 2.6, 2.7, and 2. 7 with Ser334, Gln275, Ile273, Ile27, Asp25, Thr34, Gly 35, His38,Arg 190, and Phe 41surrounding residues. Gyrase A showed best binding affinity -8.4kcal/mol for compound 5a and showed four hydrogen bond with Thr 336, Asp 338, Gln 275, and Cys 268 with 2.2, 2.5, 2.1, and 2.5 bond angle. Gyrase A interacts with 1a by showing binding affinity -8.0kcal/mol and exhibit two hydrogen bonds with Thr 336 and Asp 338 and the surrounding amino acid residues were Lys 337, Gln 275, Arg 276, Pro 274, Ile 273, Phe 41, His 38, Arg 190, Ile 186, and Lys 189. Gyrase A was bound to compound 4a molecule with binding affinity -7.8kcal/mol forming three hydrogen bonding interactions with Lys 89, Arg 192, and Glu 193 respectively. Gyrase A showed binding affinity -7.8kcal/mol with 2a and showed two hydrogen bonding interactions with Thr 336 and Asp 338, the bond angles were 2.3 and 2.6 respectively and which was shown in Table 1 and Fig.5-9. Though the presence of halides at different positions in the parent compound (data not shown), the sifting position of nitro group from *o-p* (ortho to para) positions there was great observation of potent inhibitor activity (Table 1).

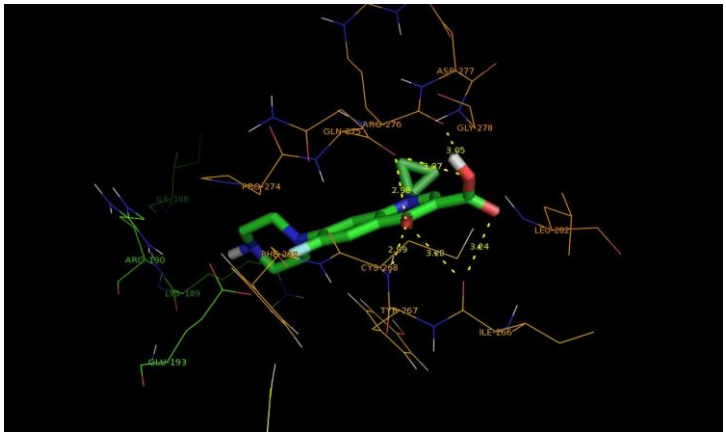

Fig.1: Interaction of reference ligand (Ciprofloxacin) with QRDR-A residue

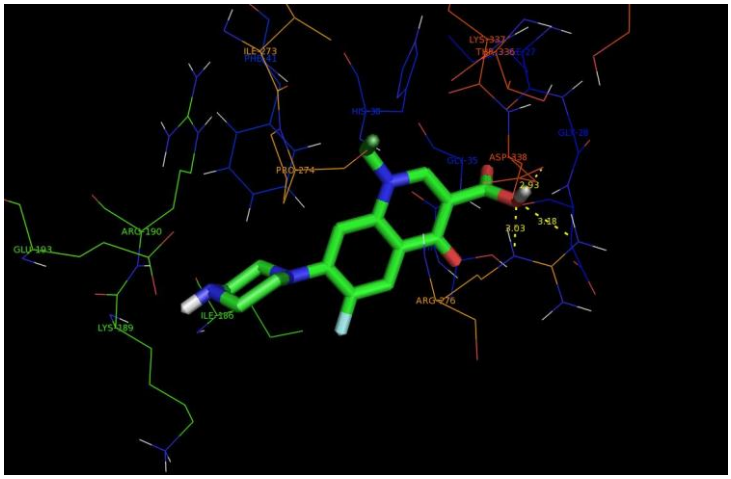

Fig. 2: Interaction of reference ligand (Norfloxacin) with QRDR-A residue

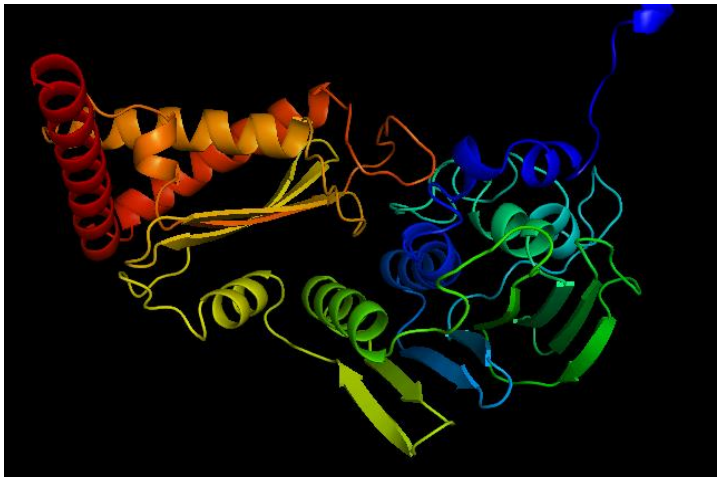

Fig. 3: Structure of E coli DNA Gyrase

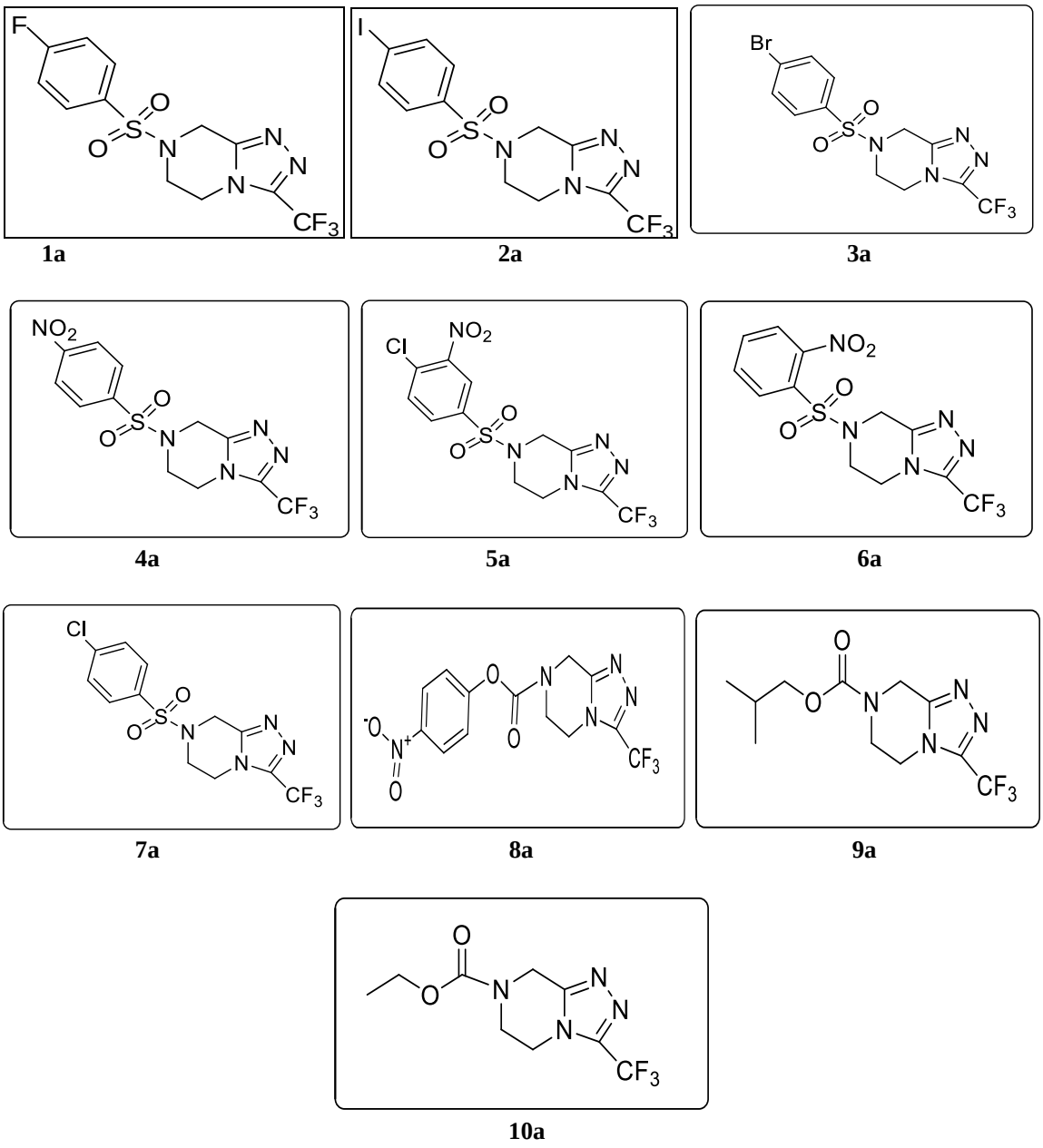

Fig. 4: Structures of 3-(Tri fluoro methyl)- 5,6,7,8- tetra hydro –[1,2,4]-triazolo (4,3-α)- pyrazine (Sitagliptin Intermediates) intermediates.

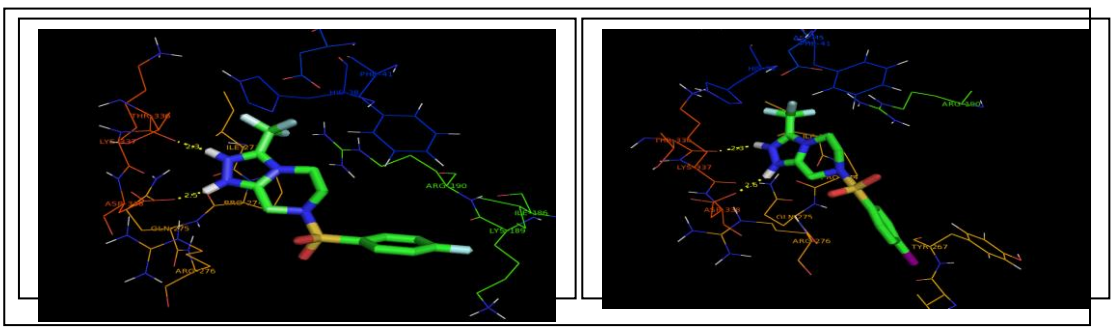

Fig. 5: Interaction of compounds 1a and 2a with QRDR-A residues.

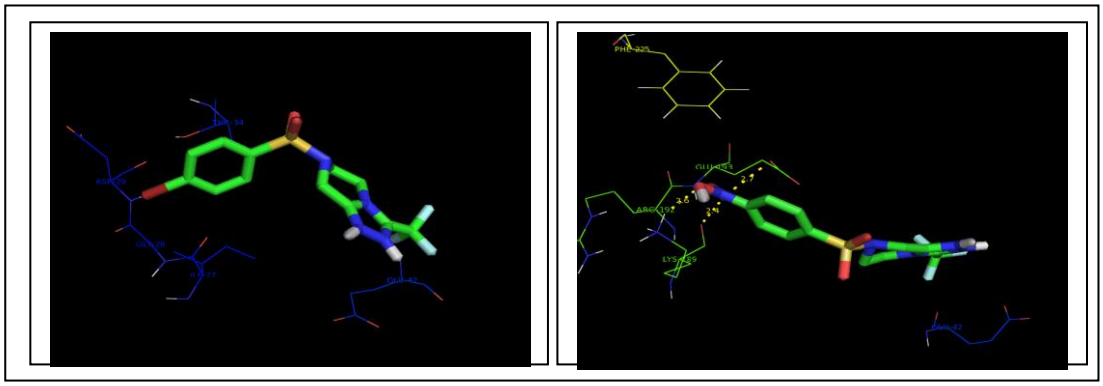

Fig. 6: Interaction of Sitagliptin Intermediate compounds 3a and 4a with QRDR-A residues

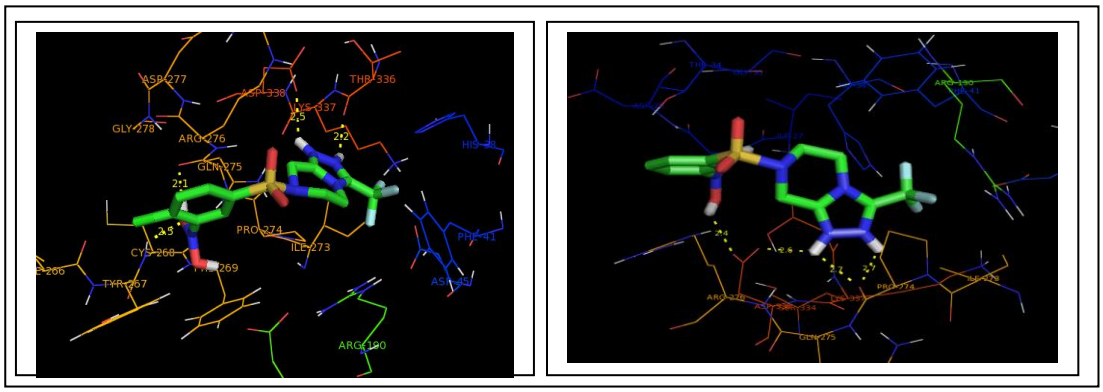

Fig. 7: Interaction of Sitagliptin Intermediate compounds 5a and 6a with QRDR-A residues

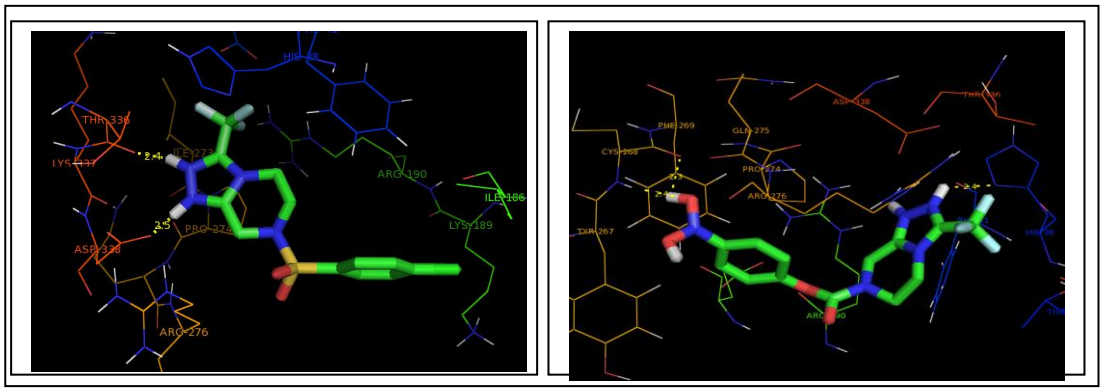

Fig. 8: Interaction of Sitagliptin Intermediate compounds 7a and 8a with QRDR-A residues

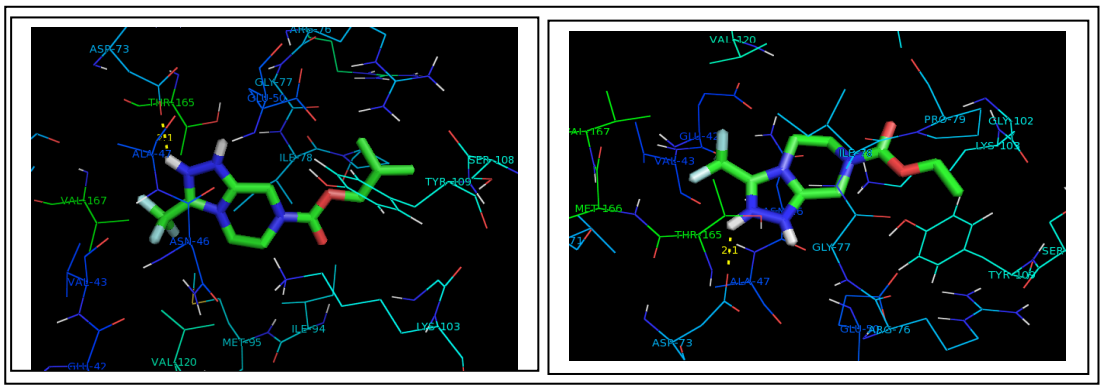

Fig. 9: Interaction of sitagliptin intermediate compounds 9a and 10a with QRDR-A residues

Table 1: Docking results of 3-(Tri fluoro methyl) - 5,6,7,8- tetra hydro –[1,2,4]-triazolo (4,3-α)- pyrazine (sitagliptin intermediate) with Quinolone Resistance Determining Region of E. coli DNA Gyrase-A.

S No	Compound	Rank	Binding energy (K cal mol^{-})	Binding interaction	Bond length (A^{o})	Bond type	Surrounding amino acids
1	Ciproflo xacin	R	-7.0	Pro276---O—H-N Pro 276 ---O---O---C Gln 275 ---O---O---C Ile 266 ---O---O---C Ile 266 ---O---O---C Cys 268 ---N---O---C	3.05 3.07 2.98 3.28 3.24 2.99	H-don H-acc H-acc H-acc H-acc H-acc	Asp 277, Gly 278, Arg 276, Gln 275,Pro 274, Ile 186, Arg 190, Lys 189, Glu 193, Phe 263, Cys 268, Tyr 267, Ile 260.
2	Norfloxa cin	R	-7.3	Arg 276 ---N---O---C Arg 276 ---N---O---C Asp 338 ---O---H---O	3.0 3.1 2.9	H-acc H-acc H-don	Glu 193, Arg 190, Lys 189, Ile 186, Arg 276, Gly 35, Gly 28, Thr 336, Lys 337, Ile 27, Ile273, Phe 41, Pro 274.
3	1a	3	-8.0	Thr 336---O---H---N Asp 338---O---H---N	2.3 2.5	H-don H-don	Lys 337, Gln 275, Arg 276, Pro 274, Ile 273, Phe 41, His 38, Arg 190, Ile 186, Lys 189
4	2a	5	-7.8	Thr 336---O---H---N Asp 338---O---H---N	2.3 2.6	H-don H-don	Lys 337,His 38, Asp 15, Arg 190, Tyr 267, Pro 274, Ile 273, Gln 275, Arg 276
5	3a	7	-7.8	---	--	--	Asp 29, Gly 28, Ile 27, Glu 42, Thr 34
6	4a	4	-7.8	Lys 89---O---H Arg 192---H---O Glu 193---O---H---O	2.4 2.6 2.7	H-acc H-acc H-acc	Phe 224, Glu 42
7	5a	2	-8.4	Thr 336---O---H---N Asp 338---O---H---N Gln 275---O---H---O Cys 268---H---O---N	2.2 2.5 2.1 2.5	H-don H-don H-acc H-acc	Asp277, Gly 278, Arg 276,Lys337,Thr267, Thr269,Pro274,Ile273,Arg190,Phe41, Asp45, His38
8	6a	1	-8.5	Asp 338---O---H---O Asp 338---O---H---N Pro 274---O---H---N Pro 274---O---H---N	2.4 2.6 2.7 2.7	H-acc H-don H-don H-don	Ser334,Gln275, Ile273, Ile27, Asp25, Thr34, Gly35, His38,Arg190,Phe 41
9	7a	6	-7.8	Thr 336---O---H---N Asp 338---O---H---N	2.4 2.5	H-don H-don	Lys337, Arg276, Pro274, Ile273, His38, Phe41,Arg190,Lys189,Ile186
10	8a	8	-7.6	Cys268---O---H---O Cys268---H---O---N His 38---N---H---N	2.3 2.4 2.4	H-acc H-don H-don	Thr267, Phe269, Gll275, Pro274, Arg276, Asp338, Thr336, His38.
11	9a	9	-7.5	Asp73---O---H---N	2.1	H-don	Val197, Val43, Glu42, Vil 126, Asn46, Ala 47, Thr165.Met95, Ile94, Lys103, Tyr 109, Ser108, Arg 76, Gly77, Glu50.
12	10a	10	-7.1	Asp73---O---H---N	2.1	H-don	Val120, Met166, Thr165, Ala47, Gly77, Glu50, Arg 76, Tyr109, Lys163, Gly 102, Pro79, Ile78

CONCLUSION:

Finally it may be concluded, that a series of Sitagliptin intermediate derivatives of 3-(Tri fluoro methyl)- 5,6,7,8- tetra hydro –[1,2,4]-triazolo (4,3-α)- pyrazine (sitagliptin intermediate derivative) have been docked successfully and analyzed to investigate the role of these derivatives, which indicates the importance of trizole, pyrazine, and oximes moieties along with the nitro-group. The docking scores showed significance in prediction of inhibition of E coli DNA Gyr A. Thus it is summarized that derivatization of Sitagliptin intermediate derivatives 6a (7-(2-Nitrophenylsulfonyl)-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3,α] pyrazine) is an optimum and a determinant for generation of bio-activity with regard to structure-activity relationships. The findings of this work should be helpful to medicinal chemists involved in further drug development of novel antimicrobials against *E.coli*.

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CONFLICT OF INTEREST

No conflict of interest

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