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MOLECULAR DOCKING STUDIES OF TETRAZOLE DERIVATIVES ON COX-2 PROTEIN RESIDUE

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ABSTRACT

Tetrazoles are the hetrocyclic compounds having diverse biological activities such as analgesic, anti-inflammation, antimicrobial, anticancer, antidiabetic etc., and an impending source in biosciences. *Invivo* analysis was carried out on anti-inflammatory property of synthesized five tetrazole derivatives (a, b, c, e and g) which revealed that the compound(g) exhibited potential anti-inflammatory activity of 49% when compared to standard Indomethacine at10µg/ml in the previous report by the authors.In the present investigation, molecular docking studies were performed for the above tetrazole derivatives to evaluate the *in- silico* anti-inflammatory activity against Cycloxigenase-2 (COX-2) protein as model compound and the data was compared with Indomethacine.

KEYWORDS: Inflammation, Tetrazoles, paw oedema method, Indomethacine, COX-2protein.

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INTRODUCTION

Inflammation is a highly complicated biological reaction observed in tissues because of stimuli such as pathogens, allergic and damaged cells. Its symptoms are redness, swollen joints, joint pain, its stiffness and loss of joint function.A number of tetrazole derivatives exhibit biological activities and have found applications as carboxylic surrogates. The development of tetrazole chemistry during the past two decades can be ascribed to their diverse biological applications in medicine, biochemistry, agriculture, photography, information recording systems, explosives and others[1]. The ability of tetrazole compounds to mimic the carboxylic functionality has motivated the incorporation of tetrazole derivatives into biologically active molecules. This has led to applications in therapy resulting in compounds with antibacterial[2], antifungal[3], antiviral[4-6,] analgesic[7,8], anti-inflammatory[9-11], anti-ulcer[12] and anti-hypertension[13] activities. Tetrazoles have also been utilized in organo metallic chemistry as effective stabilizers of metallo peptide structures and as peptide chelating agents. Various modifications of the structure of tetrazoles have been carried out in order to obtain compounds with different physicochemical properties and subsequently with different pharmacodynamic and pharmacokinetic properties[14] The synthesis of tetrazole derivatives from quinazolin-4-one was reported earlier by the author[15]. The structures of all the five synthesized compounds were assigned on the basis of IR, ^{1}HNMR, ^{13}CNMR, Mass and elemental analysis[15].The synthesized compounds were tested for anti-inflammatory activity against carrageenan induced paw oedema method[15] which was also reported by the authors in earlier study. Indomethacine was used as the standard drug for comparison. Molecular docking can be defined as an optimization problem, which would describe the “best-fit” orientation of a ligand that binds to a particular protein of interest and is used to predict the structure the structure of the intermolecular complex formed between two or more molecules. The most interesting case is the protein ligand interaction, because of its applications in medicines. Docking was carried on COX2 protein residue using the tetrazole derivatives[16].Cyclooxygenases (COX) or prostaglandin endo peroxide synthases (PGHS) are the key enzymes in the synthesis of prostaglandins, the main mediators of inflammation, pain and increased body temperature (hyperpyrexia). The body produces two main isoforms COX proteins i.e., cyclooxygenases –1 (COX-1) and cyclooxygenases-2 (COX-2). The COX-1 is responsible for formation of important biological mediators such as prostanoids, including prostaglandins, prostacyclin and thromboxane and involved in pain causing, blood clotting and protecting the stomach[17]whereas COX-2 involved in the pain by inflammation and plays a major role in prostaglandin biosynthesis in inflammatory cells and central nervous system [18]. When COX-1 is inhibited, inflammation is reduced, but the protection of the lining of the stomach is also lost. This can cause stomach upset as well as ulceration and bleeding from the stomach and even the intestines. Whereas, COX-2 is usually specific to inflamed tissue, there is much less gastric irritation associated with COX-2 inhibition together with the decreased risk of peptic ulceration[19].The use of coxib drugs such as rofecoxib (Vioxx®) and valdecoxib (Bextra®) were withdrawn from the market in 2004 and 2005, respectively, because of increased risk of heart attacks and strokes with long term use[20].Molecular docking is a great promising field of computer based drug design, which screens small molecules by orienting and scoring them in the binding site of a protein as a result, novel ligands for receptors of known structure were designed and their interaction energies were calculated using the scoring functions. In view of the above, the present investigation merits in understanding the authoritative role of tetrazole derivatives exhibiting anti-inflammatory properties against COX-2 protein based on the fitness score, type of binding pattern, energy values etc.

MATERIALS AND METHODS

The synthesis of tetrazoles from 3-[(4-(4-Amino-benzenesulfonyl)-phenyl]-2-phenyl-

quinazolin-4-(3*H)*-one were carried out and reported by the author earlier[15]. The quinazoline was treated with different acid chlorides to get the corresponding amides (2a-g). The amides were then treated with phosphorous penta chloride to get the imidoyl chloride derivatives. The imidoyl chloride on treatment with sodium azide and acetone yielded the new tetrazole derivatives (3a-g)[15]. Melting points were determined with digital melting point apparatus and were uncorrected. FTIR spectra were recorded on a Shimadzu FT-IR model spectrophotometer. ^{1}HNMR spectra were recorded in DMSO on Bruker AV III 500 MHz using TMS as internal standard. ^{13}C NMR spectra were recorded on Bruker AV III 500 MHz instrument using DMSO as the solvent. The mass spectra were recorded in JEOL GCmate instrument. The purity of compounds was checked by TLC. All these results were reported by the author in the earlier paper[15]. Five of the synthesized compounds (3a, 3b,3c, 3e and 3g) were tested for anti-inflammatory activity against carrageenan induced paw oedema method. Indomethacine was used as the standard drug for comparison[15].

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DOCKING STUDIES

(i) Protein preparation

Docking studies were performed for five derivatives of tetrazoles used Schrodinger L.L.C (Maestro 9.1) software into the 3D structure of the catalytic site of COX-2 enzyme (pdb code: 1cx2).Water molecules from the protein structure were removed and was prepared using protein preparation wizard of Maestro 2009. Hydrogen atoms were added and partial atomic charges were assigned using OPLS-2005 force field.The three-dimensional structure of the cyclooxygenase or COX-2 enzyme is given in the following Fig 1

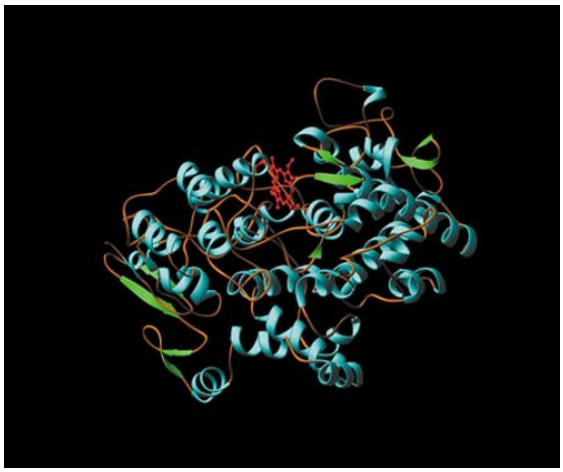


Figure 1
COX-2 enzyme

(ii) Ligand preparation

The five synthesized compounds (3a,3b,3c,3e and 3g)were taken as ligands and were drawn using chemsketch ACD LAB software. The ligands were further geometrically optimized and energy was minimized using steepest descent protocols of LigPrep module using Maestro9.1. A rigid receptor docking using the glide program was carried out against the receptor using the ligands. The scaling factor for protein van der waals radii was 1.0 in the receptor gird generation. The ligands in the active sites were used as a centroid to generate the grid file for docking. The default grid size was adopted from the glide program. No constraints were applied for all the docking studies. The Glide XP(extra precision) was used for all docking calculations the more negative the gilde score, the more favorable the binding. All the images were taken using PyMOL Program.

RESULTS AND DISCUSSION

1. Spectral studies

The IR spectra of the compounds revealed absorption bands in the region 1590-1678 cm^{-1} (C=N),1399-1466 cm^{-1} (N=N),1156-1281 cm^{-1} (N-N=N) and 1110-1020 cm^{-1} (tetrazole ring) revealing the presence of a tetrazole ring in the synthesized compounds. The SO_2 (symmetric) and SO_2 (asymmetric) stretching vibrations are observed at 1353-1315 $cm^{-1}$and 1143-1156 cm^{-1} respectively[15]. In the ^1H NMR spectra, the aromatic protons appeared as a multiplet in the regions δ 7.15 to 8.04 and also confirmed the presence of CH_3, OCH_3, groups as singlet peaks at δ 2.3, δ 3.8 and in the tetrazole derivatives 3c & 3e respectively[15].In the ^{13}C NMR spectra, the appearance of signals in the region 121.0-153.7 may be attributed to the quinazolinone carbons. Signals at 121.5-135.6 may be assigned to the phenyl carbons of the compounds[15]. The mass spectrum for the tetrazole derivatives showed the molecular ion peaks at their respective molecular weights which are illustrated in the Table 1. The author had reported that the anti-inflammatory activity of the compound (3g**)** to be of 49%[15].

Table 1

List of Tetrazole derivatives with their Mol.formula, M.W, Physical properties

Compound No	Compound's Name	Molecular formula	M.wt	Colour	M.pt
3a	2-Phenyl-3-(4-(4-(5-phenyl-2*H*-tetrazol-2-yl)phenylsulfonyl)phenyl) quinazoline-4(3*H*)-one	$C_{33}H_{22}N_6O_3S$	582	Palegreen powder	291-294 °C
3b	3-(4-(4-(5-(4-Nitrophenyl)-2*H*-tetrazol-2-yl)phenylsulfonyl)phenyl)-2-phenylquinazolin-4(3*H*)-one	$C_{33}H_{21}N_7O_5S$	628	Pale yellowpowder.	281-283 °C
3c	2-pheny-3-{4-[4-(5-(4-methyl)phenylterazol-2-yl)-phenylsulfonyl]-phenyl}-quinazolin-4(3H)-one	$C_{34}H_{24}N_6O_3S$	597	Pale brownpowder	182-184 °C
3e	3-(4-(4-(5-(4-Methoxyphenyl)-2*H*-tetrazol-2-yl)phenylsulfonyl)phenyl)-2-phenylquinazolin-4(3*H*)-one	$C_{34}H_{24}N_6O_4S$	613	Pale yellowpowder	149–150 °C
3g	3-(4-(4-(5-(4-Chlorophenyl)-2*H*-tetrazol-2-yl)phenylsulfonyl)phenyl)-2-phenylquinazolin-4(3*H*)-one	$C_{33}H_{21}ClN_6O_3S$	618	Pale brownpowder	103–105 °C

2. Molecular Docking

The five tetrazole compounds (3a, 3b, 3c,3e and 3g) which were screened for the *invivo* anti-inflammatory activity were taken for the docking studies and molecular docking was performed against anti-inflammatory protein COX-2. All these ligands showed hydrogen bonds with anti-inflammatory protein (COX-2). The top score pose was selected for each compound and their results were shown in the Table 2. The glide score of the compounds were found to be -6.30(3a),-7.02(3b),-6.8(3c), 6.57(3e),-7.70(3g). It is worthy to note that the compound 3g has shown the highest glide score of -7.70. It is evident from the table that for each compound, the binding site and the hydrogen bonding interaction varied. It was interested to observe that even though the core structure of all the compounds were same, the degree of interaction and binding site were found to be different. From the (Fig 6) it is evident that the Tetrazole ring N6 (3g) is involved in hydrogen bond interaction with amino acid residue ARG120: NH of COX-2 protein with distance of 2.1Å. The (Fig 3) shows that the amino acid residue TYR122: NH and OH groups are involved in hydrogen bond with oxygen atom of the NO_2 and SO_2 groups with a distance of 2.5 and 2.6 Å respectively. The N atom of quinazoline ring of compound 3b is involved in hydrogen bond interaction with amino acid residue ASN43: NH with a distance of 2.2 Å. The amino acid residue (figure 4) TYR122: OH is involved in hydrogen bond interaction with carbonyl oxygen atom of quinazoline ring (3c) with the bond distance of 2.5Å. The compound 3e forms hydrogen bond with the amino acid residue such as LYS473: NH and ASN43: NH with the bond distance of 2.1 and 2.2Å respectively. Calculated affinities may be a good tool for interpretation of the difference of activity together with the

measured distance between specific functional groups in the compounds with the previously mentioned residues. The best observation here was that all the measured hydrogen bond distance are within the range of 2.1-2.6Å which is considered as a good sign that indicate the absence of classes between ligand and protein.

Table 2

Glide score , glide energy and hydrogen-bond distance parameter for the compounds 3a,3b,3c,3e and 3g with protein COX-2 (PDB id: 1cx2)

Compound	Glide Score	Glide energy	Donor	Acceptor	Distance Å
3a	-6.30905	-50.2759	YS473:NH	Tetrazole ringN	2.3
			ASN43:NH	Quinazoline ring O	2.2
3b	-7.02242	-56.764	TYR122: NH	O of NO_2	2.5
			YR122:OH	O of SO_2	2.6
			ASN43: NH	Quinazoline ring N	2.2
3c	-6.82313	-56.5501	TYR122: OH	C=O of Quinazoline ring	2.5
3e	-6.57034	-54.715	LYS473: NH	N6 of tetrazole ring	2.1
			ASN43: NH	N2 of Quinazoline ring	2.2
3g	-7.7077	-56.2662	ARG120: NH	N6 of Tetrazole ring	2.2

Figure 2

Structure of Phenyl-3-(4-(4-(5-phenyl-2H-tetrazol-2-yl)phenylsulfonyl)phenyl) quinazoline-4(3H)-one (3a) and its docking pose with the amino acid residues of COX-2

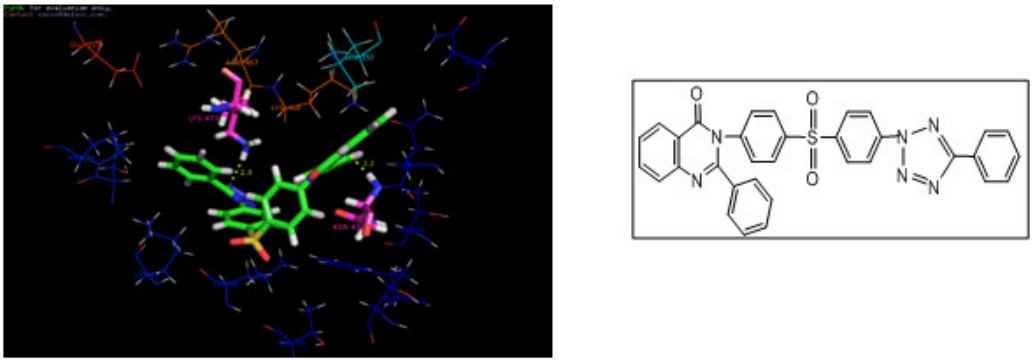


Figure 3

Structure of 3-(4-(4-(5-(4-Nitrophenyl)-2H-tetrazol-2-yl)phenylsulfonyl)phenyl)-2-phenylquinazolin-4(3H)-one (3b) and its docking pose with COX-2

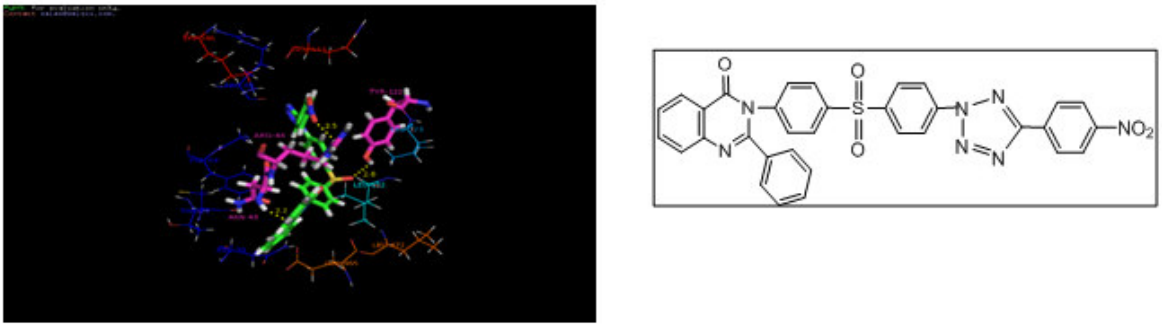
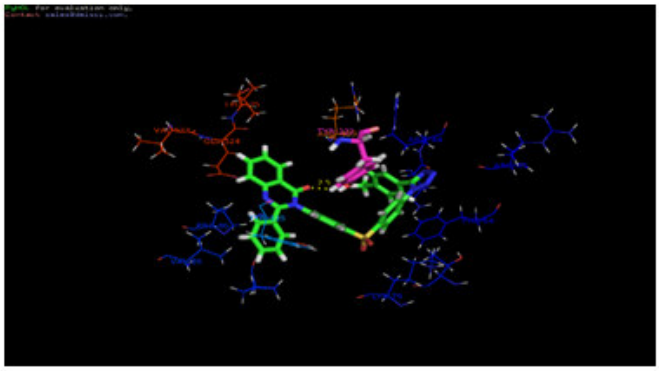


Figure 4
Structure of 2-pheny-3-{4-[4-(5-(4-methyl)phenylterazol-2-yl)-benzenesulfonyl]-phenyl}-quinazolin-4(3H)-one (3c) and its docking pose with COX-2



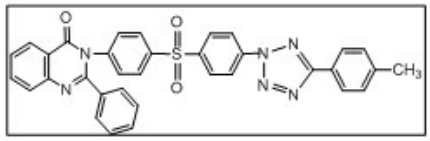
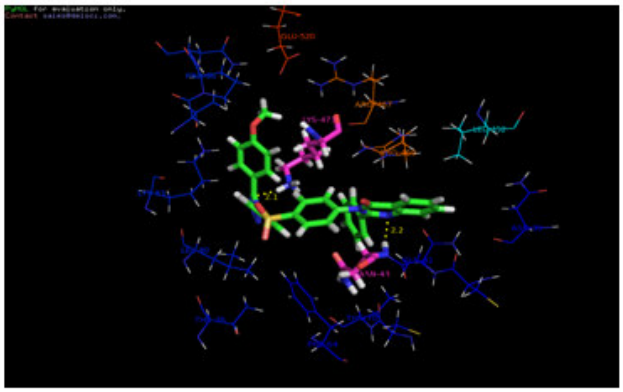


Figure 5
Structure of 3-(4-(4-(5-(4-Methoxyphenyl)-2H-tetrazol-2- yl)phenylsulfonyl)phenyl)-2-phenylquinazolin-4(3H)-one (3e) and its docking pose with COX-2



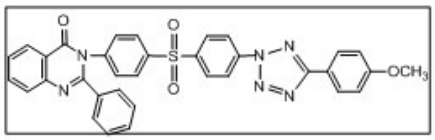
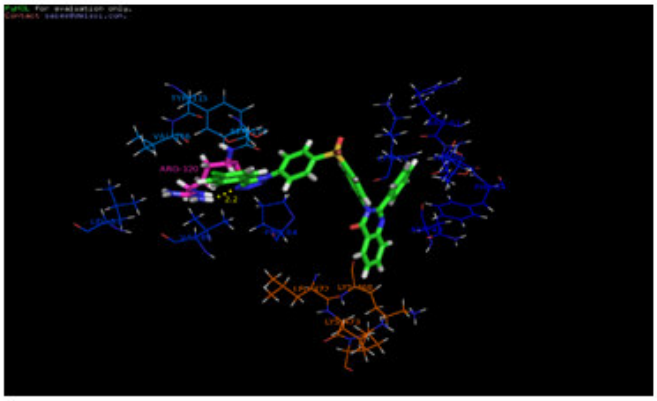
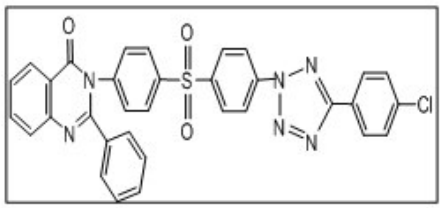


Figure 6
Structure of 3-(4-(4-(5-(4-Chlorophenyl)-2H-tetrazol-2-yl)phenylsulfonyl)phenyl)-2-phenylquinazolin-4(3H)-one (3g) and its docking pose with COX-2





CONCLUSION

The Protein-Ligand interaction plays a significant role in structural based drug designing. In the present work we have docked the ligands, tetrazole derivatives with COX2 protein. It was evident from the docking study that for each compound, the binding site and the hydrogen bond interactions varied. Therefore the study would be a fruitful matrix for the development of interesting lead molecule for further synthetic and biological evaluation. It is convincing that this class compounds certainly holds great promise towards the pursuit to discover novel classes of anti-inflammatory agents.Further biological studies are in progress to know the potency of the compounds.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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