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SPECTRAL CHARACTERIZATION AND DOCKING STUDY OF NEWLY SYNTHESIZED BENZOTRIAZOLE DERIVATIVES

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ABSTRACT

Some novel benzotriazole derivativeswere synthesized and their interaction with proteins was studied through docking. The reaction of o-phenylenediamine with sodium nitrite, yield benzotriazole. The title compounds were synthesized by treating benzotriazole with CH_3COCl, C_6H_5COCl, C_6H_5Cl,$C_6H_5NH_2Cl$ and $C_6H_5NH_2Cl$. Their structures were confirmed by IR ^{1}H-NMR and ^{13}C-NMR. Docking study was done and the compounds Aand C were found to fit well with the target protein COX-1.

KEY WORDS: Benzotriazole, O-phenylenediamine, Docking COX-1

1. INTRODUCTION

This aromatic compound is colorless and polar and can be used in various fields. Benzotriazole is used in chemical photography as a restrainer and fog-suppressant. Benzotriazole features two fused rings. Its five-membered ring can exist in tautomer's A and B, and the derivatives of both tautomer's, structures C and D can also s be produced. The chemistry and pharmacology of benzotriazole have been of great interest to medicinal chemistry [1], because its derivatives possessed various biological activities [2] such as antiviral [3], antifungal [4], antihypertensive [5],antitubercular [6],anti-cancer [7], anti-HIV [8], antimicrobial [9] etc. Moreover, benzotriazole is an important intermediate in organic reaction [10].In the present study it is planned to synthesize benzotriazole compounds and characterize these compounds by IR, ^{1}H NMR and ^{13}C NMR spectral analysis. It is further planned to carry out the docking study to find the binding capacity of the synthesized compounds with the protein molecule COX-1 [11].A synthesis of the BTA involves the reaction of *o*-

phenylenediamine, sodium nitrite and acetic acid. The conversion proceeds via diazotization of one of the amine groups.The synthesis can be improved when the reaction is carried out at low temperatures (5-10 ˚C) and briefly irradiated in an ultrasonic bath.

1.1 Drug precursor

Benzotriazole derivatives have chemical and biological properties that are versatile in the pharmaceutical industry. Benzotriazole derivatives act as agonists for many proteins. For instance, vorozole and Aliza pride have the inhibitory properties against different proteins and benzotriazole esters have been reported to work as mechanism-based in activators for severe acute respiratory syndrome (SARS) 3CL protease. The methodology is not only limited to heterocyclization but was also successful for polynuclear hydrocarbons of small carbocyclic systems. In the field of molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex [12]. The associations between biologically relevant molecules such as proteins, nucleic acids, carbohydrates, and lipids play a central role in signal transduction. Furthermore, the relative orientation of the two interacting partners may affect the type of signal produced (e.g., agonism vs. antagonism). Therefore docking is useful for predicting both the strength and type of signal produced.Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs [13].

2.2 Cyclooxygenase (COX)

Cyclooxygenase (COX) is an enzyme (EC 1.14.99.1) that is responsible for formation of important biological mediators called prostanoids, including prostaglandins, prostacyclin and thromboxane. Pharmacological inhibition of *COX* can provide relief from the symptoms of inflammation and pain. Non-steroidal anti-inflammatory drugs, such as aspirin and ibuprofen, exert their effects through inhibition of COX. In terms of their molecular biology, *COX-1* and *COX-2* are of similar molecular weight, approximately 70 and 72 kDa, respectively, and having 65% amino acid sequence homology and near-identical catalytic sites.

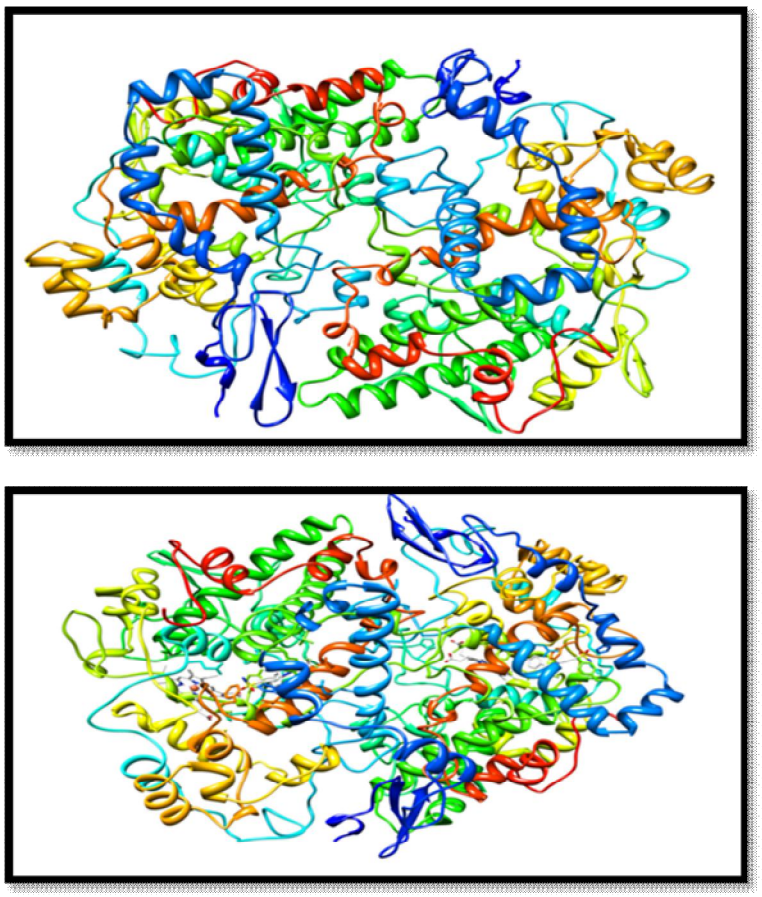


Figure.1COX 1Figure.2 COX 2

The classical *COX* inhibitors are not selective and inhibit all types of *COX*. The resulting inhibitionof prostaglandin and thromboxane synthesis has the effect of reduced inflammation, as well as antipyretic, antithrombotic and analgesic effects. The most frequent adverse effect of NSAIDs is irritation of the gastric mucosa as prostaglandins normally have a protective role in the gastrointestinal tract. Some NSAIDs are also acidic which may cause additional damage to the gastrointestinal tract [14].

2.3 Classical NSAIDs

The main *COX* inhibitors are the non-steroidal anti-inflammatory drugs (NSAIDs). The classical *COX* inhibitors are not selective and inhibit all types of *COX*. The resulting inhibition of prostaglandin and thromboxane synthesis has the effect of reduced inflammation, as well as antipyretic, antithrombotic and analgesic effects. The most frequent adverse effect of NSAIDs is irritation of the gastric mucosa as prostaglandins normally have a protective role in the gastrointestinal tract. Some NSAIDs are also acidic which may cause additional damage to the gastrointestinal tract [15].

2.4 Newer NSAIDs

Selectivity for *COX-2* is the main feature of celecoxib, rofecoxib, and other members of this drug class. Because COX-2 is usually specific to inflamed tissue, there is much less gastric irritation associated with COX-2 inhibitors, with a decreased risk of peptic ulceration. The selectivity of COX-2 does not seem to negate other side-effects of NSAIDs, most notably an increased risk of renal failure, and there is evidence that indicates an increase in the risk of heart attack, thrombosis, and stroke through an increase ofthromboxane unbalanced by prostacyclin (which is reduced by COX-2 inhibition). Rofecoxib (brand name Vioxx) was withdrawn in 2004 because of such concerns. Some other COX-2 selective NSAIDs, such as celecoxib, and etoricoxib, are still on the market [16].

2.5 Natural COX inhibition

Culinary mushrooms, like maitake, may be able to partially inhibit COX-1 and COX-2. A variety of flavonoids have been found to inhibit COX-2. Fish oils contain a natural inhibitor of COX. Hyperforin has been shown to inhibit COX-1 around 3-18 times as much as aspirin. Calcitriol (vitamin D) significantly inhibits the expression of the COX-2 gene. Caution should be exercised in combining low dose aspirin with COX-2 inhibitors due to potential increased damage to the gastric mucosa. COX-2 is up regulated when COX-1 is suppressed with aspirin, which is thought to be important in enhancing mucosal defense mechanisms and lessening the erosion by aspirin [17].

2.6 Cardiovascular side-effects of COX inhibitors

COX-2 inhibitors have been found to increase the risk of atherothrombosis even with short-term use. A 2006 analysis of 138 randomized trials and almost 150 000 participants showed that selective *COX-2* inhibitors are associated with a moderately increased risk of vascular events, mainly due to a twofold increased risk of myocardial infarction, and also that high-dose regimens of some traditional NSAIDs such as diclofenac and ibuprofen are associated with a similar increase in risk of vascular events.Fish oils (e.g. cod liver oil) have been proposed as a reasonable alternative for the treatment of rheumatoid arthritis and other conditions as a consequence of the fact that they provide less cardiovascular risk than other treatments including NSAIDs [18].

3. Experimental Methods

All melting points were taken in open capillaries and are uncorrected.IR spectra were recorded in KBr on Shimadzu spectrometer. ^{1}H-NMR and ^{13}C-NMR in DMSO-d^{6} on Bruker

AC-400 spectrometer using MeOD as an internal standard. iGEMDOCK (3) is a Graphical-Automatic Drug Design System for Docking, Screening and Post-Analysis. This is maintained by Drug Design and Systems Biology Laboratory of National Chiao Tung University, Taiwan.

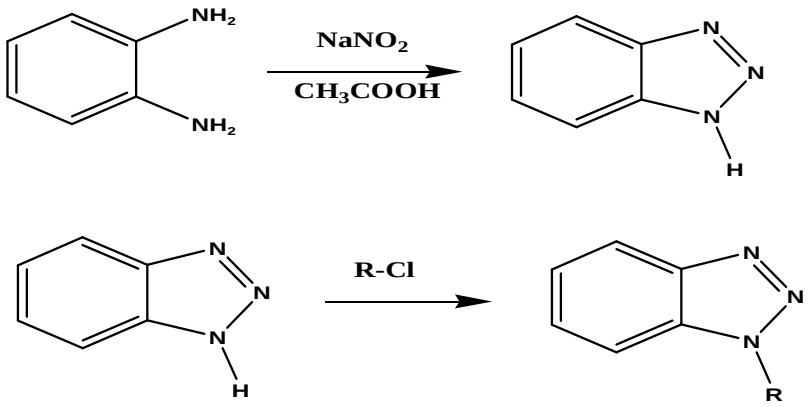
3.1 Thin Layer Chromatography

Thin layer chromatographic technique is a wonderful technique to find out the purity of the compound. The slurry was prepared by mixing of 50gram of silica powder, 9ml of $CHCl_3$and 1ml of methanol in the beaker. This slurry was coated uniformly in the activated TLC plate and dried, and the TLC plate was used for the experiment. A single spot on TLC silica gel glass plate was made and from that we confirmed the purity of the compound.

3.2 General Procedure for the Synthesis of Benzotriazole Compounds (A-E)

In a 100 ml conical flask 10.8gm of o- phenylenediamine was warmedwith 15ml of glacial acetic acid and 30ml of water until a clear solution is obtained. The flask is placed in an ice bath and when the temperature reaches 5^{o}C added with stirring 15ml cold sodium nitrite solution (7.5gm in 15ml water). The mixture turned dark green and allowed the temperature to rise to 70 degree Celsius.The flask was removed from ice bath and allowed it to cool at room temperature for 45-60 minutes. The flask is placed again in an ice bath and neutralized it to pH7 by adding 2N Sodium hydroxide solution. The mixture was stirred with glass rod until the oily layer solidified.The precipitatedsolid was filtered ona Buchner funnel and washed with cold water. Recrystallized benzotriazolefrom benzene. M.p.100-101 degree Celsius (Yield 7.8g). A white crystallineobtained by sublimation. The compounds are prepared by the following scheme (scheme-1)

Scheme 1.



	R
A	CH_3CO
B	C_6H_5CO
C	C_6H_5
D	$C_6H_5NH_2$
E	$C_6H_5NH_2$

Table 1. Analytical data of benzotriazole compounds (A-E)

Compound	%yield	Molecular Formula	Molecular Weight
A	61	$C_8H_7N_3O$	161
B	72	$C_{13}H_9N_3O$	223
C	76	$C_{12}H_9N_3$	195
D	80	$C_{12}H_{10}N_4$	210
E	74	$C_{12}H_{10}N_4$	210

4. IR& NMR Spectral data of the synthesized compounds

4.1 Compound: A

1-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)ethanone

3059 (C-H_{str}), 1286(C-N_{str}), 3433(N-N_{str}), 1622(N=N_{str}), 1654(C=O_{str}), 1479(C-C_{str}), ^{1}H NMR:δ 7.8(aromatic proton), ^{13}C NMR: δ39.79**(**sp2 carbon in ppm**),**δ138.42 (Ar -C).

4.2 Compound: B

benzotriazole-1-yl1-phenyl-methanone

3067 (C-H_{str}), 1379 (C-N_{str}), 3421 (N-N_{str}), 1598 (N=N_{str}), 1710 (C=O_{str}), 1450(C-C_{str}), ^{1}H NMR:δ 8.2 (aromatic proton), ^{13}C NMR: δ39.97**(**sp2 carbon in ppm), δ138.6 (Ar -C).

4.3 Compound: C

1-Phenyl-1H-Benzotriazole

3080 (C-H_{str}), 1267 (C-N_{str}), 3361 (N-N_{str}), 1624 (N=N_{str}), 1707 (C=O_{str}), 1407(C-C_{str}), ^{1}H NMR:δ 7.9 (aromatic proton), ^{13}C NMR: δ39.69**(**sp2 carbon in ppm**),** δ138.3 (Ar -C)

4.4 Compound: D

2-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)benzenamine

3090 (C-H_{str}), 1267 (C-N_{str}), 3400 (N-N_{str}), 1591 (N=N_{str}), 1685 (C=O_{str}), 1423(C-C_{str}), 1654 (N-H_{str}) ^{1}H NMR:δ 7.9 (aromatic proton), ^{13}C NMR: δ39.95**(**sp2 carbon in ppm**),** δ138.98 (Ar-C)

4.5 Compound: E

4-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)benzenamine

3080 (C-H_{str}), 1267 (C-N_{str}), 3352 (N-N_{str}), 1597 (N=N_{str}), 1687 (C=O_{str}), 1462 (C-C_{str}), 1624 (N-H_{str}) ^{1}H NMR:δ 7.8 (aromatic proton), ^{13}C NMR: δ39.98**(**sp2 carbon in ppm**),** δ139.17 (Ar -C)

5. Dockingstudy

1. Target Protein Structure

The structure of the target protein was downloaded from PDB

Target PDB: THE CRYSTAL STRUCTURE OF WILD TYPE DIPHTHERIA TOXIN

PDB ID: 1FOL [19].

2. Structures of the compounds.The structures of the different compounds were drawn using Chemsketch software and the files were processed and saved as MOL files.

5.1 Methodology

1. The PDB structure with the ID 1FOL was loaded in to the iGEMDOCK software.
2. The binding site for the target was prepared with the radius of 4 A0.
3. The different ligands were drawn, prepared and uploaded into the software.
4. The following parameters were set.

    Population size: 100

    Generations: 50

    Number of solutions: 2

5. The output path was set.
6. ‘Start docking’ option was clicked and when docking was complete post analysis of the docked ligands was done.
7. The predicted poses and the energy list of these poses will be outputted into the “best_Pose” and “fitness.txt” of the output location, respectively. The predicted poses and scores of ligands are saved in the user defined output path.
8. Fitness is the total energy of a predicted pose in the binding site. The empirical scoring function of iGEMDOCK is estimated as:

Fitness = vdW + Hbond + Elec

Here, the vdW term is van der Waal energy. Hbond and Elect terms are hydrogen bonding energy and electro statistic energy, respectively.

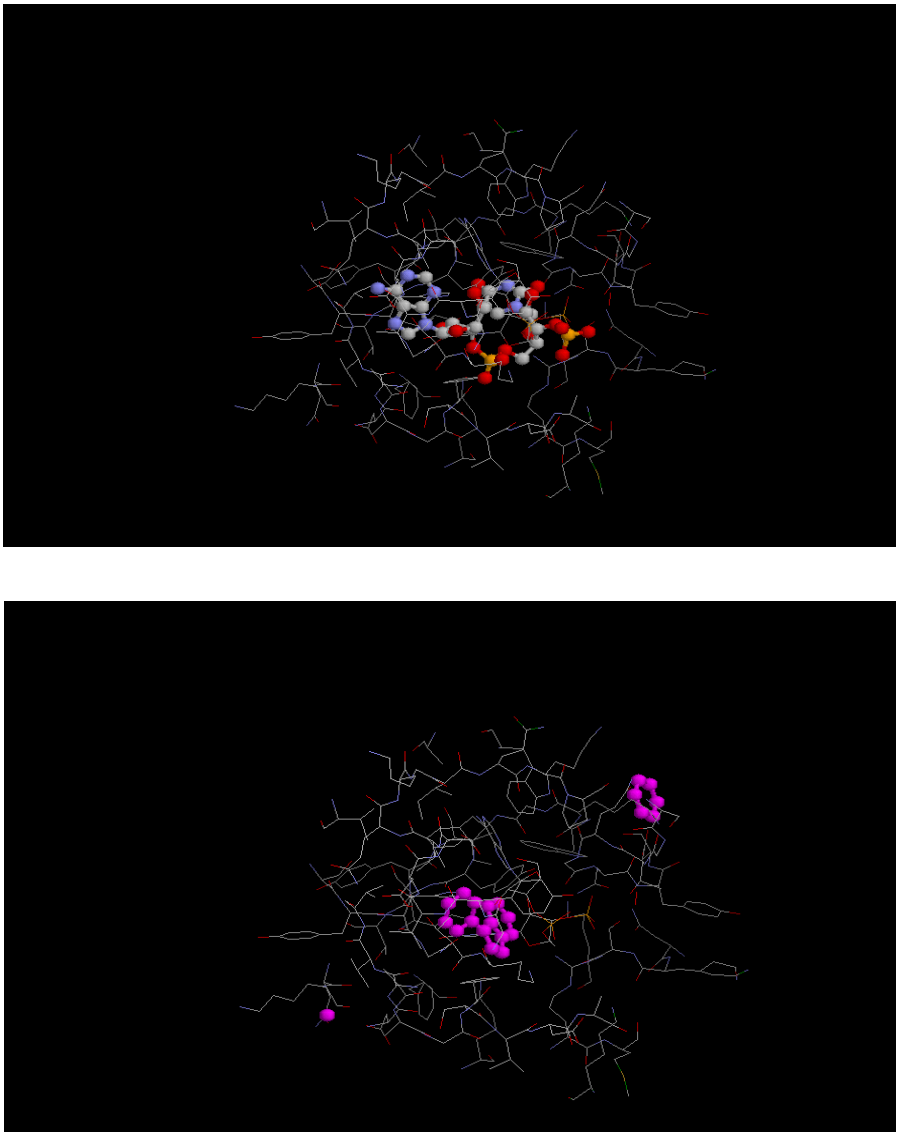
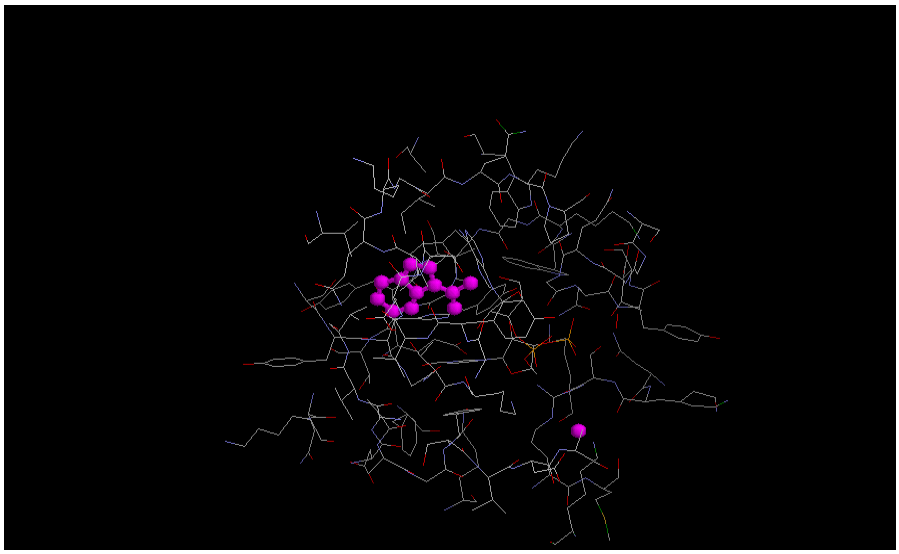


Figure 3.Interaction of the ligand with the binding site of the target



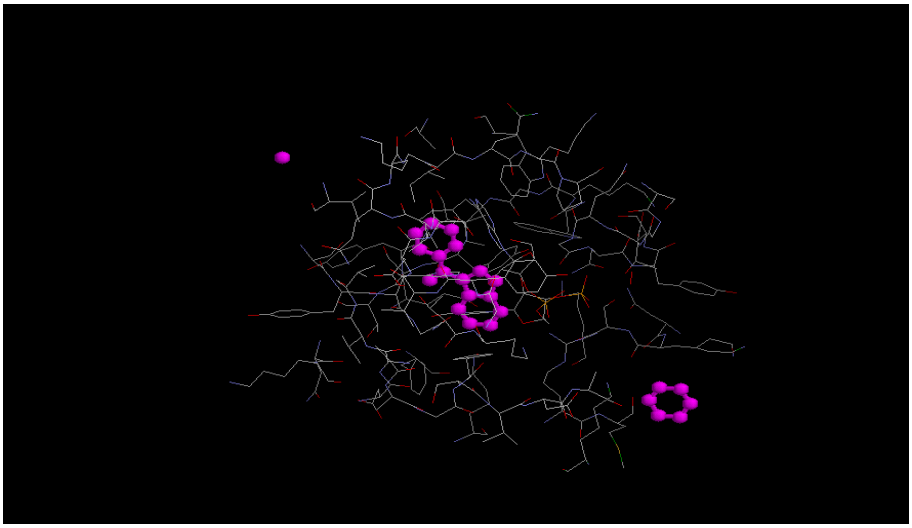
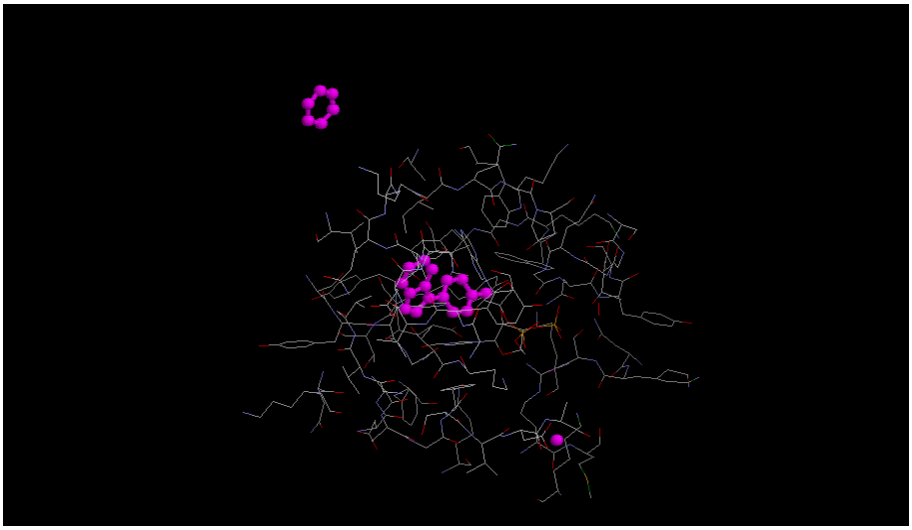


Figure 4. Interaction of compound A, B, &C with the binding sit



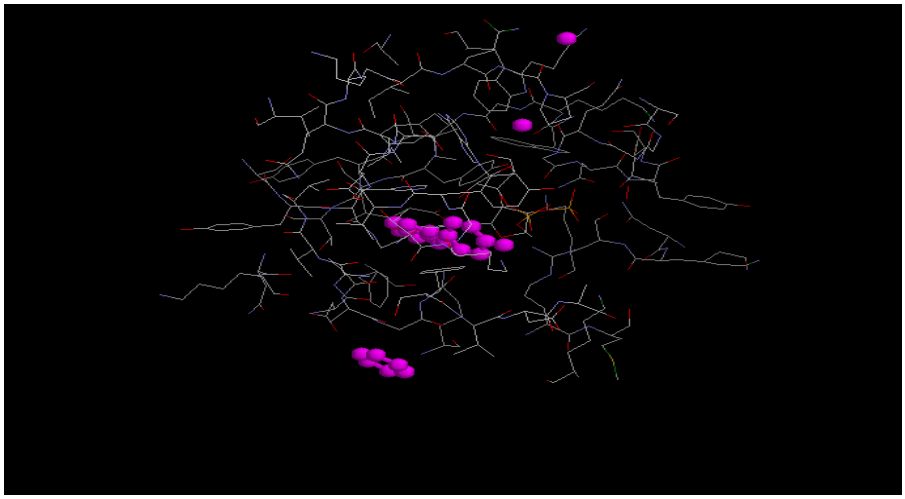


Figure 5. Interaction of compound D&E with the binding site

6. RESULTS AND DISCUSSION

Experimental results show that iGEMDOCK keeps the advantages of GEMDOCK and provided graphical-integrated environment for virtual screening and docking. iGEMDOCK, integrates the structure-based virtual screening and post-screening analysis, is a useful system for drug discovery[20]. iGEMDOCK (3) is a Graphical-Automatic Drug Design System for Docking, Screening and Post-Analysis. This is maintained by Drug Design and Systems Biology Laboratory of National Chiao Tung University, Taiwan.

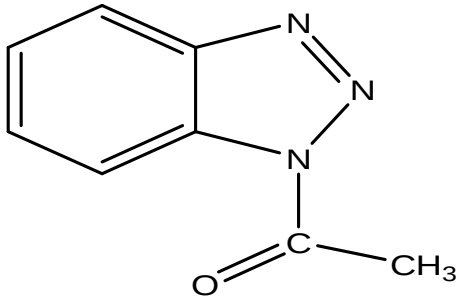
Table 4. The interaction residues and energy values of the compounds with the target.

S.No	Compound	Energy	VDW	HBond	Interaction
1	A	-73.88	-71.85	-2.03	His 21, Tyr54, Ser55, Tyr65
2	B	-72.54	-69.2	-3.34	His 21, Tyr54, Ser55, Tyr65
3	C	-65.13	-54.12	-11.01	His 21, Tyr54, Ser55, Tyr65
4	D	-56.22	-47.42	-8.8	His 21, Tyr54, Ser55, Tyr65
5	E	-55.24	-53.72	-1.53	Gly44, Tyr54, Ser55, Tyr65

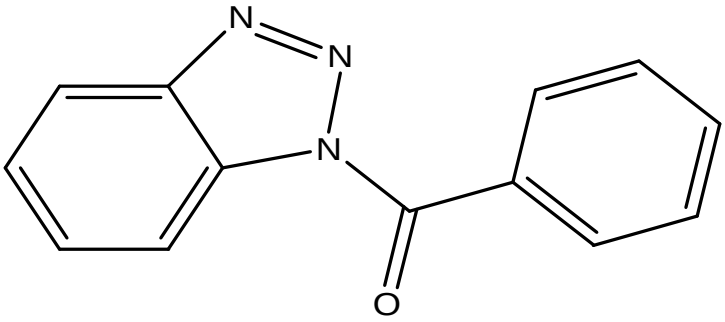
6.1 DISCUSSION

The reaction of o-phenylenediamine with $NaNO_2$/CH3COOH, yielded benzotriazole. The purity of all the synthesized compounds were confirmed by their sharp melting points (uncorrected) and column chromatography and thin layer chromatographic techniques. The chemical structures were confirmed by IR, ^{1}H-NMR and ^{13}C-NMR techniques. The aromatic (Ar-H) stretching frequencies for all the derivatives were found to be at the range of 2900-3100 cm-1.The presence of NH stretching was confirmed by the peaks at 3100-3550 cm-1. N-H (1646 cm^{-1}), C=O (1664 cm^{-1}), C-C (1489 cm^{-1}), N-N, (3389 cm^{-1})*N*=N, (1599 cm^{-1}).Also 1H-NMR spectra were useful for identifying protons. The peaks at the frequency

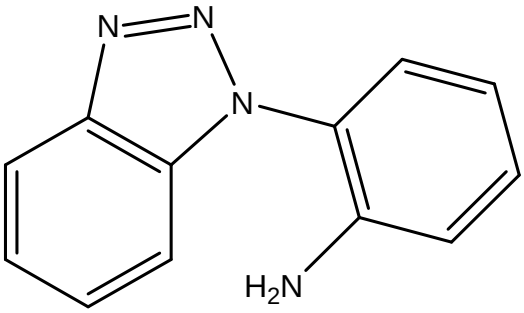
range 7.8 – 8.0 confirm the aromatic protons and 2.0-3.9 confirms the NH2 protons. From the results of docking, the compounds A and B were found to fit well with the binding sites of the target protein. The compounds A and B were found to have minimum energy of -73.88 and -72.54 respectively. They also interacted with the residues of His 21, Tyr54, Ser55, and Tyr65 of binding pocket. The compound efficiently inhibits the Diphtheria toxin. Hence the compound can be used as a cure for diphtheria but further research is needed to formulate it as a drug [21-23].Further toxicity studies have to be done to ensure the safety and efficacy of the compound to act as drug in treating inflammation.



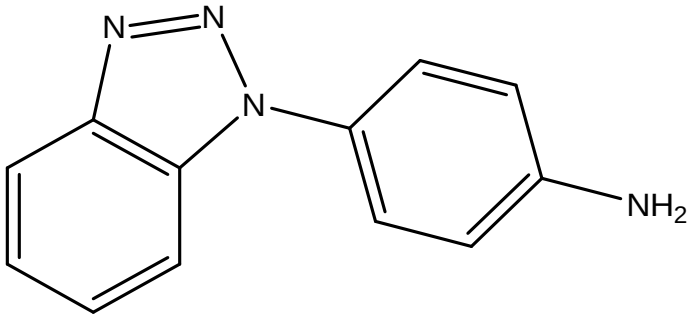
1-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)ethanone



benzotriazole-1-yl1-phenyl-methanone



2-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)benzenamine



4-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)benzenamine

Figure. 6 Compounds A, B, C, D&E

7. CONCLUSION

1. Compounds A-E are prepared using scheme 1.
2. IR, ^{1}H NMR, ^{13}C NMR spectra are taken. The results are in good agreement with the reported results.
3. Docking study is done and the compounds A and B are found to fit well with the target protein.

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