IDENTIFICATION OF TRIS (4-AMINOPHENYL) AMINE AS A POSSIBLE ANTI-INFLUENZA DRUG

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ABSTRACT

Current study is designed to compute the structural properties of N^1,N^1-bis(4-aminophenyl)benzene-1,4-diamine and highlight its role as an inhibitory neuraminidase, which interrupt and cuts down the life cycle of zoonotic orthomyxoviruses which are considered as the main cause of influenza. Computer aided structure of the concerned molecule is drawn, computational methods were applied to investigate the concerned molecule neuraminidase's inhibitory activity. Molecular docking results revealed that titled molecule is a potential neuraminidase inhibitor that merges to the active site of neuraminidase. Other tests like oral toxicity prediction reveals that compound belongs to class 4 according to Globally Harmonised System, cytotoxicity and stress response is inactive, hERG channel non blocker, Enters in to Blood brain barrier also suitable and plays the role as a COX-2 inhibitor, which minimizes inflammation in humans and computational quantum mechanical modelling was applied to calculate the infrared (I.R) spectrogram, ionization energy, electronic property, chemical potential, electronegativity, electron affinity, molecular softness, molecular hardness and electrophilic index. Prediction based Carbon and Hydrogen NMR results are also compiled in a comprehensive manner.

Key-words: Virtual drug, Orthomyxoviruses. 4,4',4''-triaminotriphenylamine, antiviral.

INTRODUCTION

An influenza or flu pandemic occurs; when a new influenza virus emerges after mutation and spreads across the globe, and population does not have immunity against it. Seasonal influenza (Zaraket *et al*., 2018) is an acute respiratory infection caused by influenza viruses and people with cardiovascular diseases become its victim easily (Panhwar *et al*., 2019). Many studies show that inflammatory response is triggered by influenza virus (Tavares *et al.*, 2017). So we must select a drug which does not play a role in cardiac toxicity and suppresses the inflammatory effect too. Inflammation is characterized by redness, swelling, heat, pain, and stop tissue working, which results in inflammatory cell responses in infected tissue (Takeuchi and Akira, 2010). Influenza infection is not life threatening in healthy adults, synthetic drugs are used for the prevention of influenza (Laborda *et al.*, 2016). Viruses have an ability to develop resistances against previous drug and the effectiveness becomes questionable and useless. Therefore, the discovery of high effective drugs is an imperative need (Rao *et al.*, 2019).

Surface bounded mushroom shape Glycoprotein neuraminidase assumes a key part in the development and life pattern of infection (Wang *et al*., 2017). So the drug which acts as a neuraminidase inhibitor also known sialidase inhibitor (Glanz *et al.*, 2019) can be considered as a suitable candidate against influenza virus.

Molecular visualization provides the edge to examine the 3D virtual confirmation of any molecule. The field now a days as chemical computational prediction is a product of the digital age (Engkvist *et al*., 2018). Through digital molecular editing it is possible to see the actual issue in a comprehensible way beforehand (Daina *et al.*, 2017a). With the advancement in science the practice of chemicals on animals and harming the animal is no more required (Doke and Dhawale, 2015) and the sale of product tested on animals is also banned in developing countries. There are many alternatives to animal testing methods (Doke and Dhawale, 2015) for the evaluation of chemical toxicity. Computer-aided design can shorten the cycle of drug development (Lindert, 2016), molecular docking technologies have been widely used for aforementioned (Meng *et al.*, 2011) purpose. Assessed molecule can be docked against target proteins easily using computational docking mechanism. It makes the process easy to find inhibitors, as it does not require lot of laborious work.

Analysis of chemical reactivity descriptors using DFT (Density-functional theory) is very essential to understand chemical and biological activities of the compounds against the biological target. It helps in characterizing the electrophilic or nucleophilic nature, their ionization, potential and affinity towards the target protein.

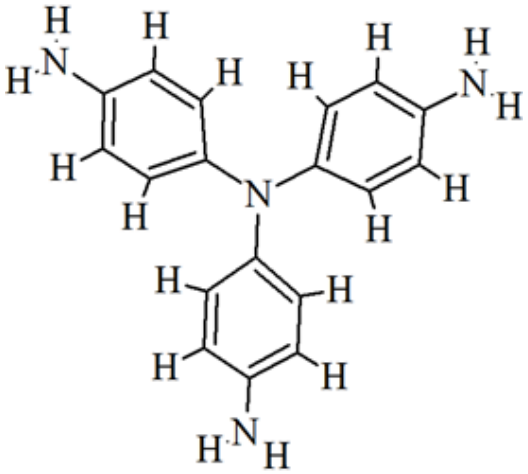

Fig.1. Graphical representation of Tris(4-aminophenyl)amine.

STEREOCHEMISTRY OF LIGAND

Generally, molecules comprising functional groups have certain properties that are similar to known drugs. Therefore, calculating the molecular property is significant parameters for oral drug, and it is shown to be an important feature in drug discovery and development. The Tris(4-aminophenyl)amine IUPAC name is N^1,N^1-bis(4-aminophenyl)benzene-1,4-diamine having molecular formula $C_{18}H_{18}N_4$. The molecule contains 40 atoms, there are three aromatic rings with the attachment of nitrogen atom with each benzene ring. Each aromatic rings contains six hydrogen atoms and six carbon atoms in this ways there are 18 hydrogen atoms and 18 carbon atoms. Each benzene ring contains central nitrogen atom and terminal nitrogen atoms. Nine double bonds, no triple bond are present there are three rotatable bonds, as shown (Fig. 1). The molecular weight is 200.4 g/mol, Smiles notation (Simplified molecular-input line-entry system) Nc1ccc(cc1)N(c1ccc(N)cc1)c1ccc(N)cc1

COMPUTATIONAL METHODS

To draw the structure of molecule Avogadro 1.2 (Hanwell *et al*., 2012) is used. Universal force field (UFF) is applied to optimize the geometry of molecule. For Oral toxicity, cytotoxicity and stress response calculation ProTox-II is used (Banerjee *et al.*, 2018). Web based application is used to predict lipophilicity (Daina *et al.*, 2017b). Protein structures i.e neuraminidase and Cox-2 are obtained from the protein data bank online database system, Computer aided molecular structure is conformed through I.R spectrogram which confirms the presence of functional groups. Carbon NMR and H-NMR is used for conformation of carbon and hydrogen atoms. Mestrelab research applications program is used for predicting NMR Carbon and Hydrogen (Cobas and Ceoane, 2015). Gamess is used for the calculation of the I.R spectroscopic analysis (Gordon and Schmid., 2005). Calculation is in basic set B3LYP (Becke3-Lee-Yang-Parr) 6-31G*. To view the results Gabedit 2.50 is used (Allouche, 2011). For Physicochemical parameters, Computational quantum mechanical modelling is applied for evaluation of electronegativity, electron affinity, electro-chemical potential, molecular softness and hardness, electrophilicity index and ionization energy. QSAR model is used to predict the potential hERG blockage (Braga *et al*., 2015). For blood brain barrier online system is use (Liu *et al*., 2014), Figure 2 & 3 draws with help of Chimera 1.13.1(Pettersen *et al.*, 2004).

COMPUTATIONAL RESULTS

1-Molecular docking

The virtual molecular docking of Tris (4-aminophenyl)amine interaction is advisement with Neuraminidase (uniprot p27907) (UniProt, 2008) and the name of the organism is influenza B virus. The predicting results score, which is calculating from mcule (Kiss *et al.*, 2012) shows that it's a good candidate for neuraminidases inhibitor.

After obtaining best score result, the above mentioned ligand is studied with neuraminidase in Swiss docking online web system (Grosdidier *et al.*, 2011), Which shows the Interrelating amino acids of neuraminidase are Isoleucine, Tryphtophan and Alanine with terminal nitrogen atoms of Tris(4-aminophenyl)amine.

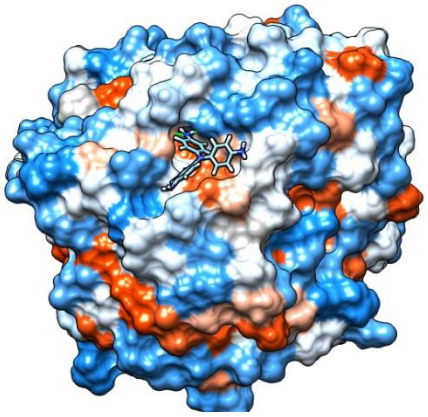

Fig. 2. Graphical representation of neuraminidase inhibitor. Tris(4-aminophenyl)amine shows an interaction with neuraminidase.

Current study is limited to Tris (4-aminophenyl) amine but the synergic effect of title compound on vital organs is beyond the scope of the current study. DFT studies and few parameters were studied for this compound without attachment of amino acids.

2-Oral toxicity

According to LD_{50}: 1120 mg/kg assessment, concern molecule belongs to toxicity class 4 as defined by "The Globally Harmonized System of Classification and Labelling of Chemicals" (GHS) and DL-AOT prediction, oral toxicity shows the same results (Madden, 2018).

3-Cytotoxicity

Inactive and Heat shock factor response also show inactive.

4-Lipophilicity

The ability to dissolve in oil. The role of lipophilicity in drug discovery and design is defined by many (Arnott and Planey, 2012). Consensus Log Po/w 5.81

5-Cyclooxygenase-2

Cyclooxygenase-2 (Cox-2) an enzyme which plays a major role in encourage inflammation. When Cyclooxygenase-2 activity is blocked by any mean, directly inflammation is reduced. Cox-2 become active only at the site of inflammation i.e peripheral tissues. COX-2 inhibitors as a safe promising treatment option, it can be used not only to treat inflammation, but also to treat cancer (Clària, 2003) .

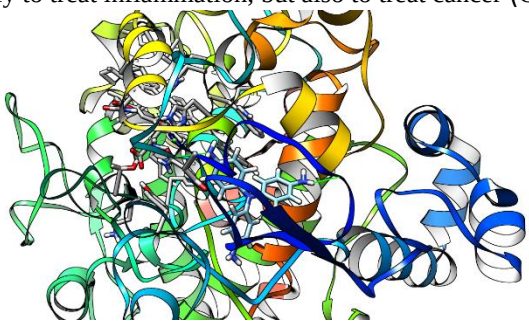

Fig.3. Graphical representation of Cox-2 inhibitor Tris(4-aminophenyl)amine shows an interaction with Cox-2.

Cox-2 amino acid Cysteine 159 interrelate with ligand nitrogen atom, Alanine 156 with terminal nitrogen and 41 Cysteine with Hydrogen atom of ligand.

5-Cardiac toxicity

hERG is the gene for potassium ion channels of cardiac myocytes. Any molecule that binds in the channel are known as hERG blockers (Di and Kerns, 2016) that can lead to cardiac arrhythmia (Kerns and Di, 2008). The drug which block off K^+ channel are strongly associated with dysrhythmic conditions collectively known as drug-induced

long-QT-syndrome (Kudaibergenova *et al.*, 2018). QSAR (quantitative structure-activity relationship) model reveal result that it a non-blocker. Binary Prediction non blocker 60% and Multiclass Prediction 80% non-blocker.

Blood brain barrier (BBB). The prediction reveal that the tile molecule is BBB+ with Molecular polar surface area is 81.31.

6-**DFT calculation**

Table 1. DFT results of Tris(4-aminophenyl)amine.

Parameter name	Symbolic Representation	Result
Energy of HOMO	$E_{HOMO\ \ Energy\ (eV)}$	-107.21
Energy of LUMO	E_{LUMO}	1.77
I (ionization energy)	$-E_{HOMO}$	107.21
E_a (electron affinity)	$-E_{LUMO}$	-1.77
η hardness	(I-A)/2	54.49
μ (Chemical potential)	-(I+A)/2	-52.72
S (softness)	1/η	0.01835
Ω (electrophilicity index)	$\mu^2/2\eta$	25.50
χ_m (Electronegativity)	½(I+E_a) Kcal/mol	52.72

Structural conformation

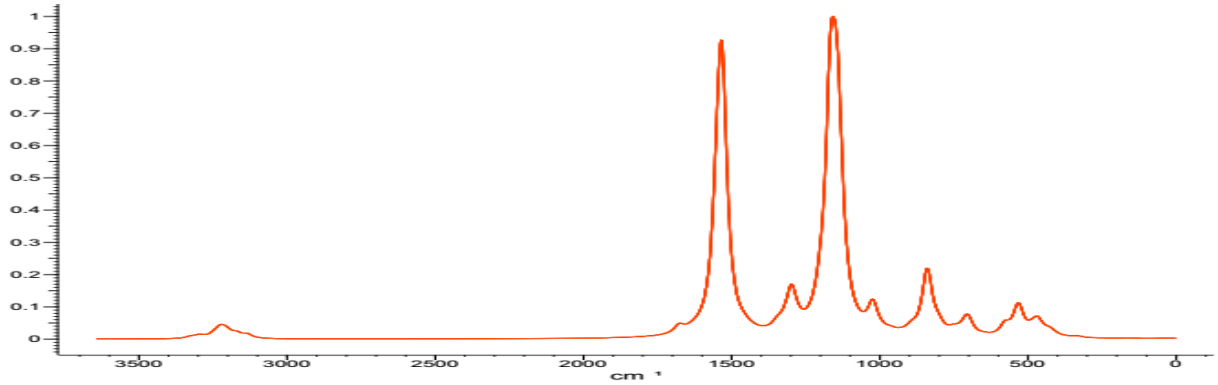

Fig.4. Spectrogram of Tris(4-aminophenyl)amine.

7-Infared spectroscopy

The Infrared spectrogram for Tris(4-aminophenyl)amine lies in 0-3500 cm^{-1}. It is clearly interpreted from (Fig. 4).

- Aromatic ring: Each aromatic ring contains di-substitute attachments, CH 1286 cm^{-1} ,1025 cm^{-1} peak due to in plain H bending, CH weak binding. CH deforming at 836 cm^{-1}, Ring 1582 cm^{-1} stretching. 3168 CH stretching.
- Amine: (C) 3N 1360 cm^{-1} C-N medium stretching. NH 3375cm/1 anti-symmetric stretching, NH symmetric stretching , NH_2 1664 Cm^{-1} Strong deforming, 1549 cm/1,NH deforming,1249 C-N stretching.

8-NMR C^{13} Predication

The C-13 NMR is used to predict the carbon atoms in the molecule. The current molecule contains only 18 carbon atoms, each benzene ring contain six carbon atoms, carbon atoms no 1,12,17 nmr shift visible at 145 ppm, double bonds carbon atoms no 6,7,13 show nmr shift at 140 ppm. Carbon atoms 4,5,8,9,14,15 nmr shift at 122, carbon atoms 2,3,10,11,16 & 19 show nmr shift at 115 ppm , There are four paramount peaks of carbon 13 NMR which is visible in 115-145ppm as show in (Fig.4). Carbon nmr predation in n-chloroform.

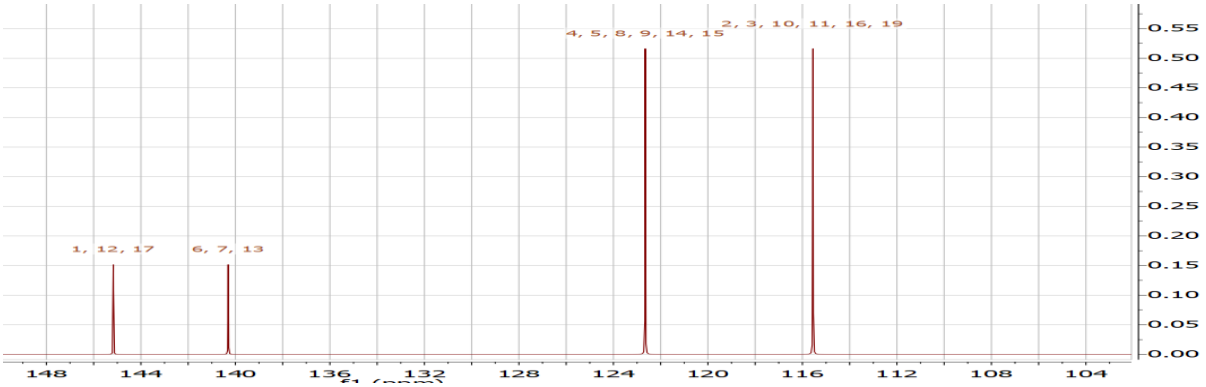

Fig.5 .NMR C-13 predication of Tris(4-aminophenyl)amine.

9- NMR H[1] Predication.

Fig. 6. NMR H1 Spectrogram of Tris(4-aminophenyl)amine.

The molecule under consideration contains a total of 18 hydrogen atoms. The investigation of H^1 NMR is more important than C^{13}. NMR result use to predict the structure. H-NMR shift for concern molecule lie 6.67-6.82 ppm. There are only two peaks. Hydrogen atoms which is attach with each benzene ring i.e (4x 12 hydrogen atoms is show in H-NMR. Hydrogen atom attached with each nitrogen atoms i.e (3x3) nine are minuscule from spectrogram as shown in (Fig. 6). Hydrogen atoms no 23, 24, 29, 34, 30&33 show shift at 6.67 ppm. Hydrogen atoms no 25, 27,28,31,32 and 26 show shift at 6.81ppm.

DISCUSSION

Identifying of ligand binding site on desired protein is an important step. Protein plays any important role in replication of viruses. If the ligand binding ability is proved it means that the ligand is capable of cutting down the life cycle of virus. Through molecular docking application we are able to discover binding sites in protein (neuraminidase).

To know a molecules side effect during the early phase, reduce the risk of drug attrition due to cardio toxicity during pre-clinical stages.

Hydrophilic or + ve charged NH_2 atoms, which interact with the negatively charged atoms and does not make interaction with positive charge atoms. The functional group NH_2 attached with molecule; is electron donating group and hence is very activating towards the electrophilic substitution reaction and make interaction with electron accepter atoms of neuraminidase and Cox-2. (Fig. 1) show a prototypical three aniline is attached, the weak basicity of aniline is attributed to a combination of inductive effect from the more electronegative sp2 carbon and resonance effects, as the lone pair on the nitrogen is partially delocalized into the pi system of the benzene ring.

N.M.R confirmation are essential, in the early stages of drug discovery, especially for fragment-based clue generation (Peng *et al.*, 2016).

Computer based model is used to predict the molecular side effect like oral toxicity, cytotoxicity, cardiac toxicity, lipophilicity, inflammation, other miscellanies effect play a vital role toward the drug effeteness.

CONCLUSION

The obtaining results revel that; current computer synthetic compound is founding stone to predict even a branch chain amine precursors. This results show that drug will attach at the active site of glycoprotein of

neuraminidase and stop the replication of virus. There is need to test Neuraminidase inhibition assay against the concern molecule which do not play a role toward cardiac toxicity and helpful in inflammation.

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