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Original Research Article

Taurochotine Drug Design as Sodium Taurocholate Co-Transporting Polypeptide (NTCP) Inhibitor for HBV Treatment, in Silico Approach

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Abstract

***Background*:** Hepatitis B Virus (HBV) is a viral infection that attacks the liver and can cause both acute and chronic disease. The virus is most commonly transmitted from mother to child during birth and delivery. Also, by contact with patient blood or other fluids during sex with an infected partner. The virus enters the cell body by sodium taurocholate cotransporting polypeptide receptor (NTCP), then it replicate and invade other healthy cells. ***Aim*:** the aim of this study is to design a drug that can block sodium taurocholate cotransporting polypeptide receptor (NTCP), Using bioinformatics tools and servers. ***Material and method*:** Using CHemSketch the drug molecule was drawn and determined and using OpenBabel software the molecule was transferred and designed into MOL 2 format to be applied in the SwissDock server. The target protein from the protein database bank using the PDB ID of NTCP receptor protein was retrieved and then inserted it into SwissDock. The result of docking was then subjected to SwissADME to check the pharmacological effect. ***Results*:** A novel drug that can inhibit and block sodium taurocholate cotransporting polypeptide receptor (NTCP) hence can treat the HBV. ***Conclusion*:** With these predicted pharmacokinetics and chemical properties of NTCP inhibitor, a new emerging drug to treat hepatitis B virus disease, which affects the majority of people around the world, especially in poor countries, can be developed, and this can be achieved with more research and laboratory procedures.

Keywords: HBV, NTCP, ChemSketch, OpenBabel, SwissDock.

1. INTRODUCTION

Hepatitis B virus (HBV) is an enveloped virus with an internal icosahedral nucleocapsid (NC) enclosing a partially double-stranded, relaxed circular DNA (rcDNA) genome, which is replicated through reverse transcription of a RNA intermediate, pregenomic RNA (pgRNA) [1-3].

The Hepatitis B virus (HBV) was first discovered in an Australian aborigine by the detection of its antigen, currently known as surface antigen and originally called Australia antigen as reported by Blumberg and colleagues [4]. The presence of this antigen in hepatitis a patients was independently reported by Prince and by Okochi and Murakami in 1968 [5, 6]; the virus particles were visualized by an electron microscope in 1970 by Dane and colleagues [7]. At least three types of HBV particles are observed in the serum of infected patients: spherical structures of 42 nm in diameter, those with a diameter of 22 nm, and filament structures of variable length that are 22 nm in diameter (figure 1). The 42 nm particles, also called Dane particles, are infectious virions consisting of a lipid membrane with three viral surface antigens (HBs), large (L-HBs), middle (M-HBs), and small (S-HBs), that surround a nucleocapsid composed of hepatitis B core protein (HBc), viral polymerase (Pol), and viral genome DNA. The 22 nm particles, which are much more abundant in-patient serum, include sub viral particles (SVPs) that lack the nucleocapsid and are thus noninfectious. Moreover, other noninfectious particles are currently known to be produced by infection, including enveloped particles that lack a viral genome, those containing viral RNA, and envelope less particles (naked nucleocapsids) [8].

The virus specifically infects human hepatocytes by binding the cell surface receptor, sodium taurocholate cotransporting polypeptide (NTCP), which was only recently identified [9]. Upon cell entry, HBV is believed to deliver its internal NC to the nuclear pore complex where rcDNA is released into the nucleus. rcDNA is then converted, in an ill-understood process, to a covalently closed circular DNA (cccDNA), which serves as the transcriptional template for the production of all viral RNAs, subject to regulation by epigenetic mechanisms as well as ubiquitous and liver-enriched transcriptional factors. Upon translation, the viral reverse transcriptase assembles with pgRNA, with the help of host factors, into a ribonucleoprotein complex, which triggers the initiation of viral reverse transcription using a novel protein priming mechanism as well as the assembly of immature progeny NCs into the viral capsid protein [9].

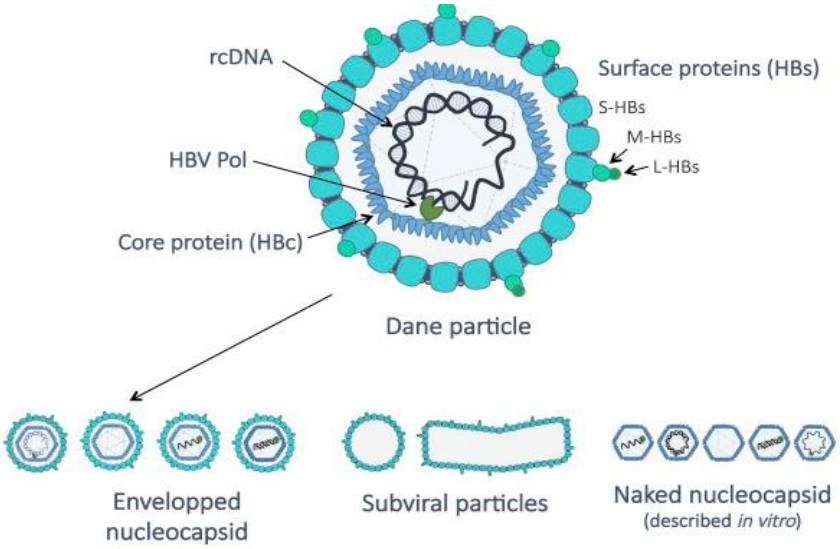

Figure 1: Showing the structure of HBV [1]

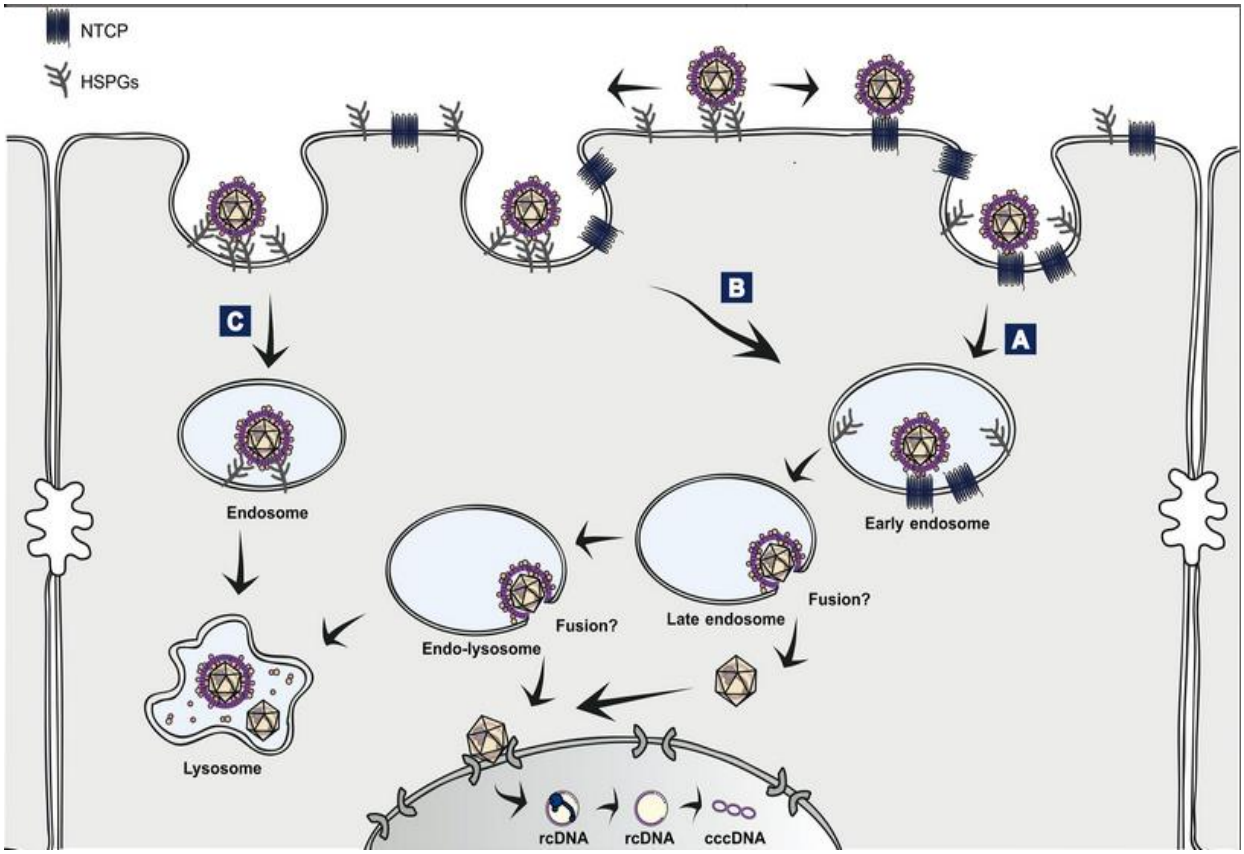

Figure 2: Showings the entry of HBV into hepatocytes and NTCP receptor [1]

The aim of this study was to design a drug that blocks sodium taurocholate cotransporting polypeptide receptor and prevents the virus from entering the cell and completing its cycle to invade other hepatocytes using bioinformatics tools.

2. MATERIAL AND METHODS

Using the following bioinformatics tools (Figure 3) to design the NTCP receptor inhibitor and HBV drug:

2. 1. Chem Sketch/ACD lab

It's an online server for drawing chemical structures and elements used in chemistry and pharmaceuticals. The website provides a variety of software tools that can be used in a variety of chemical applications, ranging from drawing to applying the software in various forms. It's available at (https://www.acdlabs.com/resources/freeware/index.php) [10].

2. 2. Open Babel GUI

It's a chemical toolkit that can communicate in a variety of chemical data languages. It's a free, open-source project that lets anyone search, convert, analyze, and store data from molecular modeling, chemistry, biochemistry, and other related fields. Its available at (https://sourceforge.net/projects/openbabel/) [11].

2. 3. RCSB PDB

Is website server is used to find the protein ID that is used in bioinformatics tools analysis, and it explains the 3D structure of the protein, nucleic acid, and complex assembles that aid students and researchers in understanding all aspects of biomedicine and agriculture, from protein synthesis to health disease. It's available at (https://www.rcsb.org/#Category-welcome) [12].

2. 4. Swiss Dock

Is a web service that predicts potential molecular interactions between a target protein and a micro molecule, most commonly a medication or chemical compound. Its available at (http://www.swissdock.ch/) [13].

2. 5. SwissADME

To aid drug discovery, this website allows you to compute physicochemical descriptors and estimate ADME parameters, pharmacokinetic properties, drug-like nature, and medicinal chemistry friendliness of one or more small compounds. (http://www.swissadme.ch/) [14].

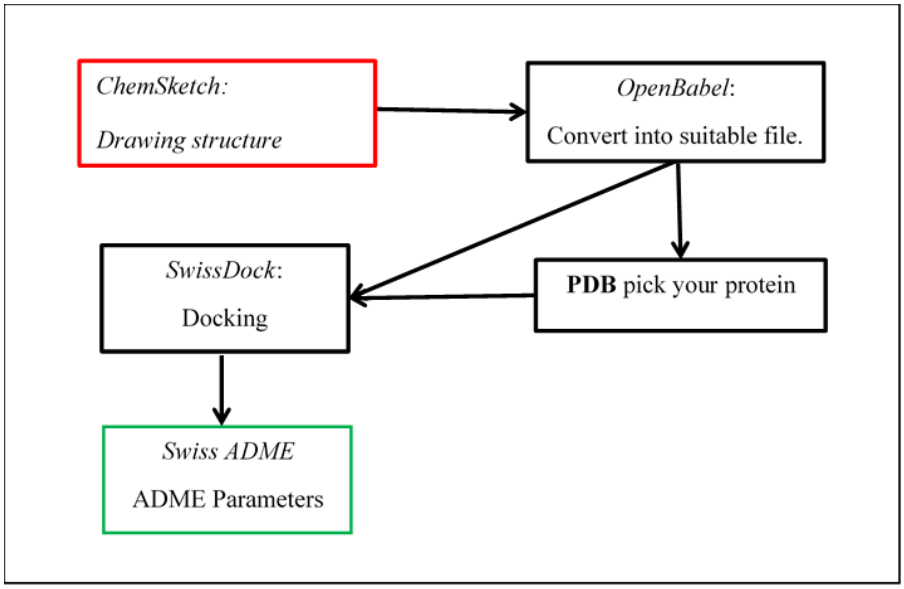

Figure 3: Show the workflow of the *in silco* analysis

3. RESULTS AND DISCUSSION

Using the Chem Sketch to draw a suitable chemical structure, this can block the sodium taurocholate cotransporting polypeptide (NTCP) cycle and prevent the virus from entering the host cell. The candidate base drug is already used to block the NTCP cycle [15] which is myrcludex B The drug structure had been modified to taurochotine The suitable predicted chemical drug is $C_{26}H_{24}NOPS$ (taurochotine) (Figure 4). After developing the drug structure, it was subjected OpenBabel software which convert the chemical appearance to a mol2 reading file as a ligand. The protein ID of NTCP cycle receptor was retrieved from RCSB. The ligand and the receptor were then read and applied by the SwissDock server, which docks the ligand and target. The target is sodium taurocholate cotransporting polypeptide cycle (NTCP) receptor protein with PDB ID (SYAX) and the ligand is our taurochotine receptor inhibitor predicted drug, Figure (5).

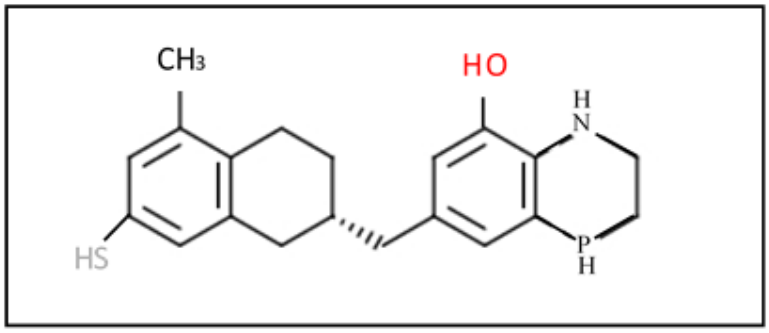

Figure 4: Showings structure of taurochotine ($C_{20}H_{24}NOPS$)

Figure 5: Showing the docking result

Pharmacokinetics

In this study, using SwissADME server the pharmacological effect of taurochotine shows that its water is moderately soluble in both classes with log S(ESOL)=-5.09 with solubility =2.90e.03mg/ml;8.10e.06mol and logS(Ali)= -5.94 with solubility =4.14emg/ml;1.16e.06mol, and the third class is poorly soluble with log S(SILICOSE.(T)=-6.79 with solubility =5.81e.05mg/ml;1.63e.07mol.

Water solubility	
Log S (ESOL)	-5.09
Solubility	2.90e-03 mg/ml, 8.10smol/l
Class	Moderately soluble
Log S(All)	-5.94
Solubility	4.14e.04mg/ml ; 1.16e-05mol/l
Class	moderately soluble
Log S (SILICOSIS-IT)	-6.79
Solubility	5.81e-05mg/ml; 1.63e-07mol/l
Class	poorly soluble

Figure 6: Shows the result of water solubility

The taurochotine may have high GI absorption, and it cannot pass the blood-brain barrier (BBB). The interaction of molecules with cytochrome p450 (CYP)is also essential as these superfamilies of isoenzymes participate in a fundamental way in the metabolism, Biotransformation and elimination of drugs [16]. It is estimated that 50 to 90% of therapeutic molecules can be substrates of the five major isoforms

(CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4) and P-gp which is essential in drug assimilation [23]. The drug taurochotine inhibits all p450 (CYP) enzymes except CYP2C19, inhibition of these isoenzymes is one of the main causes of drug or its metabolites in the body [17]. The skin permeation is logk=-6.04cm/s which can be delivered using water, on other substances in the body. The prediction of permeability coefficient (Kp) for the absorption of molecules by the epidermis of mammals is based on the linear model built by Potts and Guy, thus the more negative log Kp the less the molecule permeates. taurochotine is P-glycoprotein inhibitor but not as substrate [18]. This result is vital to assume active efflux or secretion transporters that act as a barrier to absorption in numerous compartments such as in the gastrointestinal membranes and the brain. An important role of p-gp is to protect the CNS from Xenobiotic and if the molecule can act as an inhibitor, it reinforces the possibility of crossing the blood-brain barrier [19].

Pharmacokinetics	
GI absorption	**high**
BBB	**NO**
P-gp substrate	**Yes**
CYP1A2 inhibitor	**Yes**
CYP2C19 inhibitor	**NO**
CYP2C9 inhibitor	**Yes**
CYP2D6 inhibitor	**Yes**
CYP3A4 inhibitor	**Yes**
Log K^p (skin permeation)	**-5.33cm/s**

Figure 7: Showing the results of pharmacokinetics

The Lipinski drug likeness is recorded as 1 violation MOLGP >4.15. which can be a good drug to use also is good in Ghose, Veber, Egan, and Muegg, and these are evaluated drug-likeness in different parameters; Ghose evaluation by computing physiochemical properties, presence of functional groups and important substructures, Egan predict the drug absorption based on physical processes involved in membrane permeability, Viber model characteristics molecules as drug-like if they have 10 or fewer rotatable bonds and a PSA (Polar Surface Area); Muegg model is a database independent pharmacophore point filter that discriminate between drug like and nondrug like chemical matter ketone, hydroxyl, sulfonyl and amine groups are considered the most important four functional motifs in drug like molecule included in all mentioned drug like parameters [20]. The taurochotine with 0.55 bioavailability score which means is a good permeable compound as a drug, that is, F< 10 [21]. The compounds don't give a false result in PAINS (Pan-assay Interference Compounds) which are chemical compounds that often gives false positive results in high throughout screens tend to react nonspecifically to numerous biological targets rather than specifically affecting one desired target [22].

Druglikness	
Lipinski	**Yes; 1 violation; MLOGP> 4.15**
Ghose	**Yes**
Veber	**Yes**
Egan	**Yes**
Muegge	**Yes**
Bioavailability Score	**0.55**

Figure 8: Showing the results of Drug likeness

The objective of medicinal chemistry is to support the daily effort in drug discovery and help to find any problematic fragments in the molecule and taurochotine drug has no such fragment [23]. Moreover, the Brenk shows 3 compounds with alerts, that is, catechol, phosphor, and thiol_2. The structural alert indicated by Brenk is purely based on the knowledge of a compilation of chemical parts known to be toxic, metabolic unstable, or with properties responsible for poor pharmacokinetics [24]. The taurochotine compound with lead-likeness follows a normal rule of drug discovery which uses nowadays by scientists and chemists in industry but has a molecular weight of less than 350. With 4.16 synthetic bioavailability score, it shows that taurochotine is not a difficult drug to synthesize molecule [25]. This value is a score based on the fragmented analysis of the structures of more than 13 million compounds to hypothesis that the more a molecular fragment is frequent, the easier to obtain the molecule [26]. The score is defined between 1 (easy synthesis) and 10 (very difficult to synthesize).

Medicinal Chemistry	
PAINS	**0 alert**
Brenk	**3 alerts; catechol, phosphor, thiol_2**
Leadikeness	**No: 2 violation : MW > 350, XLOGP3 > 3.5**
Synthetic accessibility	**4.16**

Figure 9: Results of Medicinal chemistry

The lipophilicity or lipo-solubility (logP) is a property of great significance and is used as an indicator of the oral bioavailability of drug candidate molecules also constituting one of the main parameters of ADME/Tox. In general, the optimization of the gastrointestinal absorption profile, through the rough passive diffusion after oral administration of a prototype candidate drug is achieved through the balance of its permeability and water solubility profile known as logP or log D [27]. Taurochotine showed presented average of 4.13 for logP classified as optimal for good intestinal absorption due to the balance between water solubility and permeability rate by passive diffusion. Extreme values result in unbalance in these profiles with the capacity to negatively impact the oral bioavailability profile. In addition, the increase in lipophilicity values is involved in toxic properties such as blocking of CYP 450 and hERG as well as phospholipids induction [28]. Thus, there is relevant evidence suggesting that controlling lipophilicity among all physiochemical properties within a defined ideal range improves the quality of a molecule and consequently the probability of therapeutic success.

Lipophilicity	
Log P $_{o}/^{w}$ (ILOGP)	**3.36**
Log p $_{o}/^{w}$ (XLOGP3)	**4.44**
Log P $_{o}/^{w}$ (WLOGP)	**3.49**
Log P $_{o}/^{w}$ (MLOGP)	**4.27**
Log P $_{o}/^{w}$ (SILICOSIS-IT)	**4.93**
Consensus Log p $_{o}/^{w}$	**4.10**

Figure 10: Show the result of lipophilicity

This study predicted that physiochemical properties are important and necessary to understand and design new pharmacological compounds with the ability to bind to various biological targets and present beneficial effects to the body leading to the discovery of new treatments for diseases of more complex origin such as HBV virus [34]. Some properties such as electronic distribution, size of the molecules, hydrophobicity, binding characteristics, and presence of groups responsible for the biological activity of the molecules in a biological organism including transport properties, bioavailability affinity for proteins, metabolic stability and toxicity and other properties.[29] One of the physiochemical properties is molecular weight which can be a great differential in relation to intracellular processes such as intestinal absorption, penetration in the blood brain barrier (BBB) elimination rate and interaction with molecular targets the analyzed molecule of taurochotine showed molecular weight with acceptable variability (357.45g/mol). The acid base of a character determined by the ability to accept and donate protons H^{+} [21], inferred that molecules that exhibit a lower number of hydrogen bond donor atoms O-H and N-H and a higher number of hydrogen bond accepter atoms – sum of hydrogen bond accepter atoms O and N have the most favorable ADME/Tox profile [22-24].

The molecule's topological polar surface area (TPSA) is frequently linked to the structures' ability to form bonds, and it's also linked to changes in oral permeability [30] This metric is also utilized in conjunction with the counting of rotational bonds and enables for the investigation of molecular flexibility as it affects the molecule's drug likeness profile. Because the vast majority of active medications taken orally are passively absorbed through a transposed lipidic layer, the hydrophilic environment of biological membranes is maintained [31].

Physiochemical properties	
Formula	**$C_{20}H_{24}NOPS$**
Molecular weight	**357.45 g/mol**
Num heavy atoms	**24**
Num atom heavy atoms	**12**
Fractions Csp3	**0.40**
Num rotatable bonds	**2**
Num H-bond acceptors	**1**
Num H-bond donors	**2**
Molar refractivity	**111.35**
TPSA	**84.65 A^{2}**

Figure 11: Showing the results of physiochemical properties

As far as we know, this this the first computational research that suggest a taurochotine drug for sodium NTCP receptor inhibitor as treatment for hepatitis B virus while other previous studies were clinically-applied studies [32]; they identify 5 diverse novel NTCP inhibitors (Chicago sky blue 6B, rosiglitazone, sulfasalazine, TRIAC, and zafirlukast) [33], while we identify Taurochotine as NTCP inhibitor and HBV Treatment. Finally, wet lab studies are recommended to conform our findings.

CONCLUSION

With these predicted pharmacokinetics and chemical properties of NTCP inhibitor, a new emerging drug to treat hepatitis B virus disease, which affects the majority of people around the world, especially in poor countries, can be developed, and this can be achieved with more research and laboratory procedures. According to the predicted software's results, the drug

seems to be safe and without any side effects. This work needs Clinical Research Trial (CRT) and more laboratory checking so as to be released into the market. This way of drug design becomes more applicable in pharmaceutical companies and in drug and chemical laboratories especially after discovery of efficient and affordable software had been designed and available.

Data Availability

All data underlying the results are available as part of the article, and no additional source data were required.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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