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STUDY REGARDING THE TOXICOLOGICAL ACTIVITY OF SOME JWH SYNTHETIC CANNABINOIDS

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Abstract
In the last years, the synthetic cannabinoids have become popular recreational drugs of abuse because of their psychoactive properties. It is known that some JWH synthetic cannabinoids are even more toxic than their natural counterpart, i.e. cannabis (tetrahydrocannabinol, THC). For this reason, their acute toxic effects may even lead to death under certain circumstances.
The aim of the research presented in this paper was to compute different toxicity measures for some of the newest JWH synthetic cannabinoids, by using dedicated software packages. The same toxicity measures have also been determined for THC, which was chosen as standard compound. For this purpose, the geometries of the molecules have been optimized by using the AM1 semi-empirical quantum method. The results of a comparative analysis of toxicities of synthetic and natural cannabinoids are presented.

Keywords: JWH synthetic cannabinoids, toxicity, AM1 semi-empirical quantum method.

1. INTRODUCTION

In the last years, the synthetic cannabinoids have become popular recreational drugs because of their psychoactive properties [1]. They are abused despite the fact that their acute toxic effects may even lead to death under certain circumstances [2].

John William Huffman, a pioneer in the development of these compounds, together with his team of researchers, discovered over 450 synthetic cannabinoids. Among these, the compound named JWH-018 is the most potent and easy to synthesize Huffman cannabinoid (see Fig. 1). Hence, this class of designer drugs is since identified with the "JWH" acronym, followed by a number, which indicates the order in which these synthetic cannabinoids have been identified.

As JWH-cannabinoids correspond to the two CB1 and CB2 cannabinoids receptors located in the cells of the nervous system and the cells of the immune system [3], they are responsible for toxic actions in the central nervous system and on the immunity of the consumers.

The toxic effects are probably due to strong CB1 receptor stimulation, as synthetic cannabinoids have high affinity for the CB1 receptor. These effects are characterized of symptoms such as agitation, hypertension and hypokalaemia [4]. The same toxicity indicators were also determined for cannabis (tetrahydrocannabinol, THC), which was chosen as the standard compound as it is the best-known natural cannabinoid. The results of a comparative analysis of toxicities of synthetic and natural cannabinoids are presented.

The present paper presents the main toxic activities of Huffman synthetic cannabinoids. The data found in the literature on the toxic activity of THC was corroborated with toxicity calculations

performed with two software packages, i.e. *ADME-tox* and *PROTOX*. These toxicity measures have been compared with those characterizing THC, which was chosen as standard compound. The results confirm that some JWH synthetic cannabinoids are even more toxic than their natural counterpart.

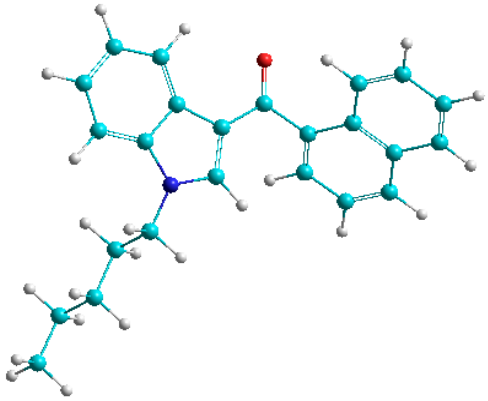


Fig. 1. Optimized molecular structure of the JWH-018 cannabinoid.

2. EXPERIMENTAL PART

A number of 32 compounds have been analyzed, 31 of which are JWH-cannabinoids. All are synthetic cannabinoids, with the exception of THC, which is the only natural compound. The 3D structures have been represented starting from the molecular structure of JWH-018 (see Fig. 2) and compared with THC (see Fig. 3). For this purpose, the geometries of the molecules have been optimized based on the AM1 semi-empirical quantum method, by using the *HyperChem* program. The toxicity calculations were performed with *ADME-Tox* and *PROTOX* software packages.

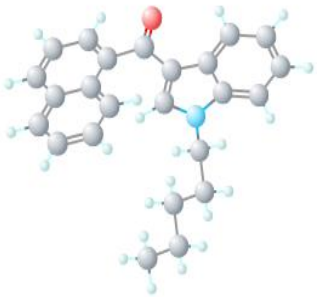


Fig. 2. 3D molecular structure of JWH-018

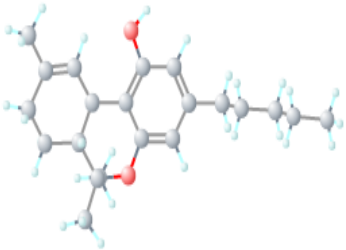


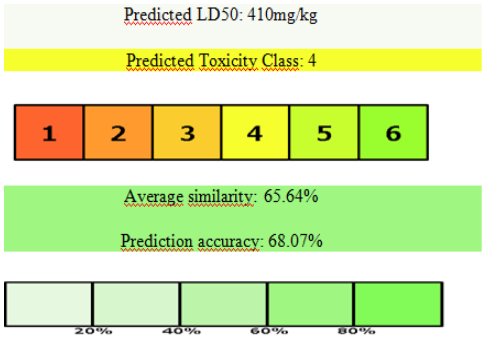
Fig. 3. 3D molecular structure of THC

ADME-tox provides parameters regarding the toxicity of JWH cannabinoids. The toxicity parameters indicate a variety of relevant data regarding the side effects of the analyzed substances. These parameters illustrate the way each of the compounds is absorbed, distributed and metabolized in the human body. Rat acute toxicity and *Tetrahymena Pyriformis* toxicity have also been analyzed. These two parameters are used for laboratory determinations of lethal doses of substances.

The second software, *PROTOX*, provides information on the immunotoxicity, mutagenicity and cytotoxicity of the analyzed compounds. Both software packages, *ADME-tox* and *PROTOX*, process the data starting with the SMILES molecular formula of the compounds. These formulas have been taken from the *PubChem* site [8].

Table 1. Oral toxicity prediction and toxicity model report obtained for JWH-018.

Oral toxicity prediction results for input compound



Name	JWH-018
Molweight	341.45
Number of hydrogen bond acceptors	1
Number of hydrogen bond donors	0
Number of atoms	26
Number of bonds	29
Number of rings	4
Number of rotable bonds	6
Total charge	0
Molecular Polar Surface Area	22

Toxicity Model Report

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	dili	Inactive	0.86
Toxicity end points	Carcinogenicity	carcino	Inactive	0.60
Toxicity end points	Mutagenicity	mutagen	Active	0.61
Toxicity end points	Cytotoxicity	cyto	Inactive	0.56
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	nr_ahr	Inactive	0.76
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	0.96
Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	nr_ar_lbd	Inactive	0.97
Tox21-Nuclear receptor signalling pathways	Aromatase	nr_aromatase	Inactive	0.90
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	Inactive	0.91
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligand Binding Domain (ER-LBD)	nr_er_lbd	Inactive	0.98
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	0.92
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	0.94
Tox21-Stress response pathways	Heat shock factor response element (HSE)	sr_hse	Inactive	0.94

3. RESULTS AND DISCUSSION

The quantitative predictions were performed with *ADME-tox* for all the studied compounds. Fig. 4 presents the aqueous solubility determined for the targeted compounds, a property that significantly affects the absorption and distribution characteristics of a drug. Most of the drugs on the market have an estimated *logS* value of more than -4 (see Fig. 5). Typically, a low solubility is associated with a bad absorption. Therefore, poorly soluble compounds are usually avoided. Fig. 4 indicates that in the case of JWH cannabinoids, only JWH-260 has an aqueous solubility comparable with THC. Many other JWH cannabinoids have an aqueous solubility of approximately -4, e.g. JWH-

046, JWH-098, JWH-148, JWH-153, JWH-159 and JWH-211. All studied JWH cannabinoids have an aqueous solubility of less than -3.

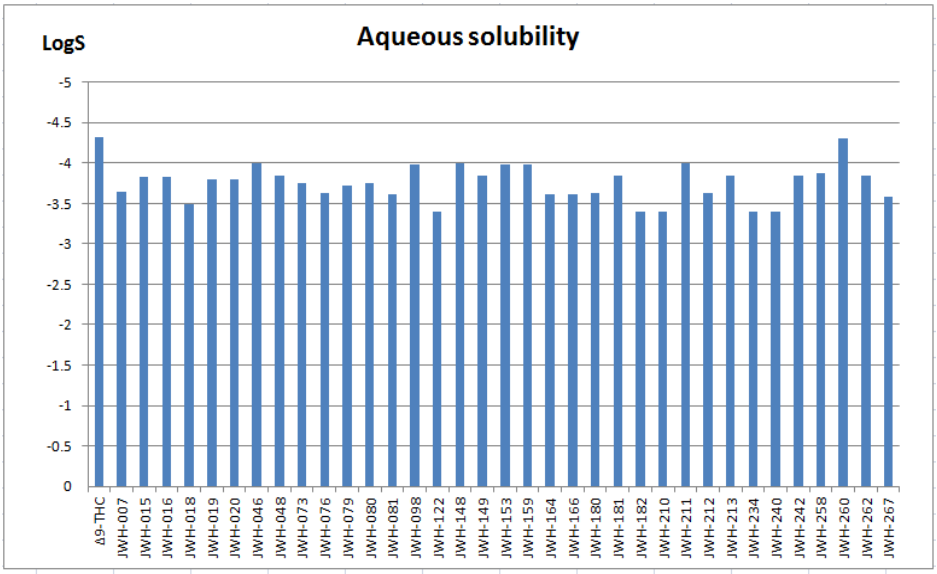


Fig. 4. Aqueous solubility of the studied JWH cannabinoids in comparison with THC.

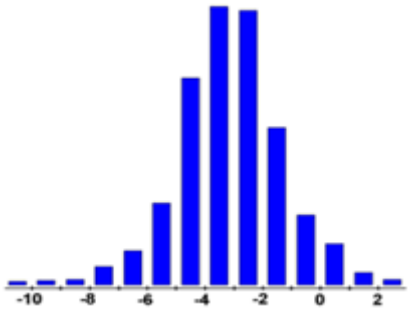


Fig. 5. Distribution of logS in traded drugs [5].

Caco-2 promiscuity parameter is used for toxicity assessments and refers to a human colon epithelial cancer cell line that are used to model human intestinal absorption of drugs. Enzyme promiscuity refers to the ability of an enzyme to catalyze a fortuitous side reaction besides its main reaction. This parameter is evaluated based on the *logPapp* parameter, i.e. the *in vitro* passive membrane permeability across the *Caco-2* cell.

The results obtained regarding the *Caco-2* promiscuity of the studied JWH cannabinoids are presented in Fig. 6. The largest *LogPapp* value, second only to that determined for the natural THC compound, was obtained for JWH-267. Slightly smaller values have been obtained for JWH-098, JWH-153 and JWH-159.

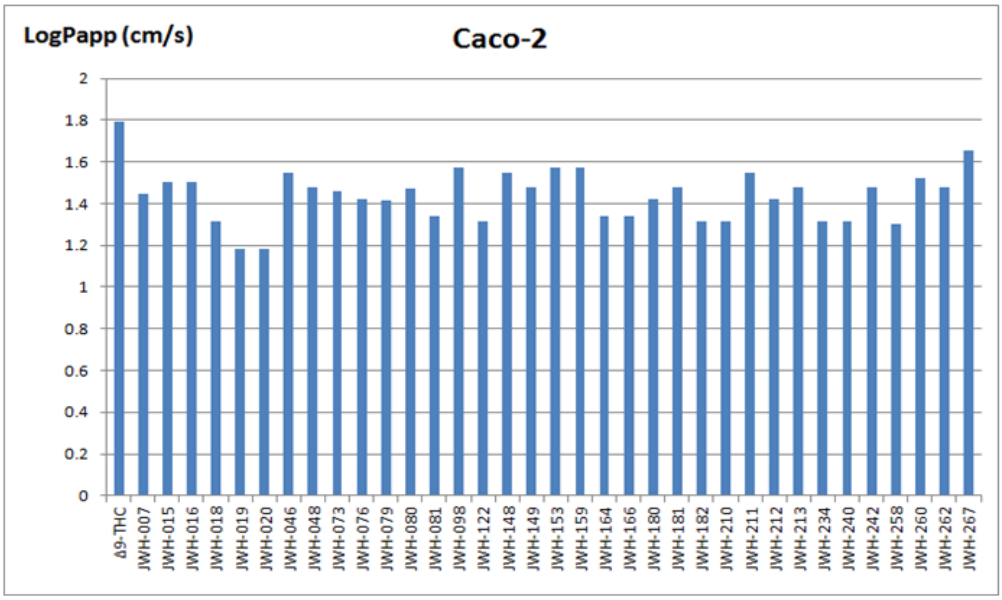


Fig. 6. Caco-2 promiscuity of the studied JWH cannabinoids in comparison with THC.

Other *ADME-tox* qualitative predictions were also obtained for the studied compounds. Fig. 7 presents the results obtained regarding the blood brain barrier BBB+. This semipermeable membrane barrier is highly selective and separates the circulating blood from the brain and extracellular fluid in the central nervous system (CNS).

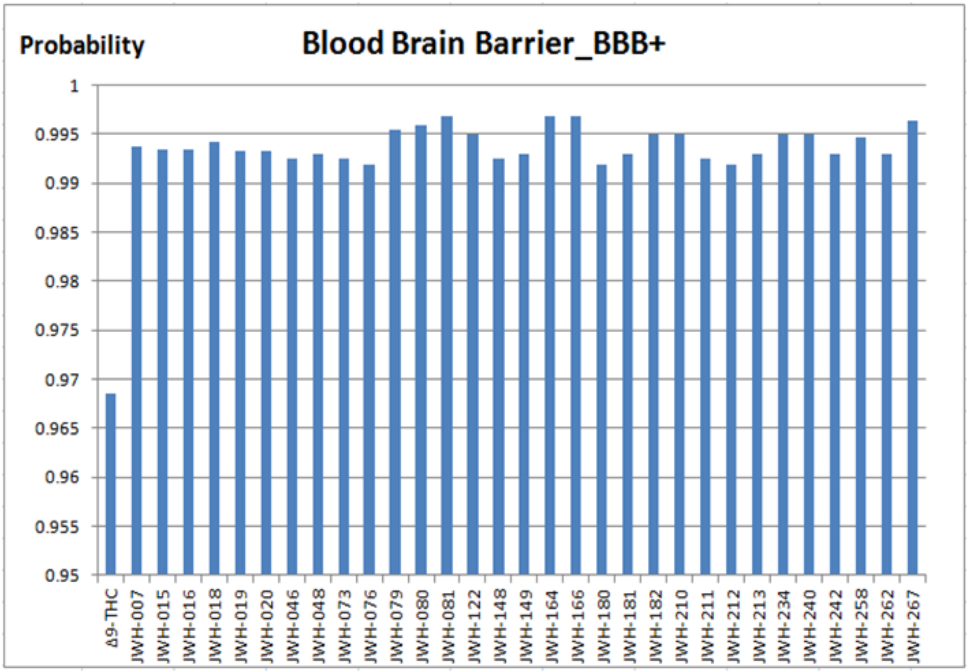


Fig. 7. Probability of passing through the blood brain barrier BBB+ of the studied JWH cannabinoids in comparison with THC.

The blood-brain barrier works effectively to protect the brain from the circulation of pathogens. Consequently, infections caused by blood of the brain are rare, and when they are very difficult to treat. The blood-brain barrier becomes more permeable during inflammation. In some cases, a drug should be given directly into the cerebrospinal fluid (CSF), where it can enter the brain by crossing the blood-fluid barrier cerebrum.

From this point of view, Fig. 7 indicates that all the studied JWH cannabinoids have a significantly higher probability than THC, their natural counterpart. Hence, we may conclude that all these compounds are indeed more toxic for the brain than the latter compound. The largest probabilities have been obtained for JWH-081, JWH-164 and JWH-166.

Another parameter that was studied was the rat acute toxicity. Acute toxicity describes the adverse effects of a substance. This effect may arise from a single or from multiple exposures in less than 24 hours. The toxicity is considered acute if the adverse effects occur in less than 14 days after the administration of the compound. Acute toxicity data may be obtained from animal testing, as well as from *in vitro* testing or from inference from data obtained for similar substances.

The rat acute toxicity is measured by the *LD50* parameter, which is frequently used as a general indicator of the acute toxicity of a substance. A lower *LD50* is indicative of increased toxicity. Fig. 8 indicates that all the studied JWH cannabinoids have a lower *LD50* than THC, with the exception of JWH-098, JWH-153, JWH-159 and JWH-267. The lowest JWH-166 is characterizing JWH-258. Very low *LD50* values are also recorded for JWH-018, JWH-019 and JWH-020.

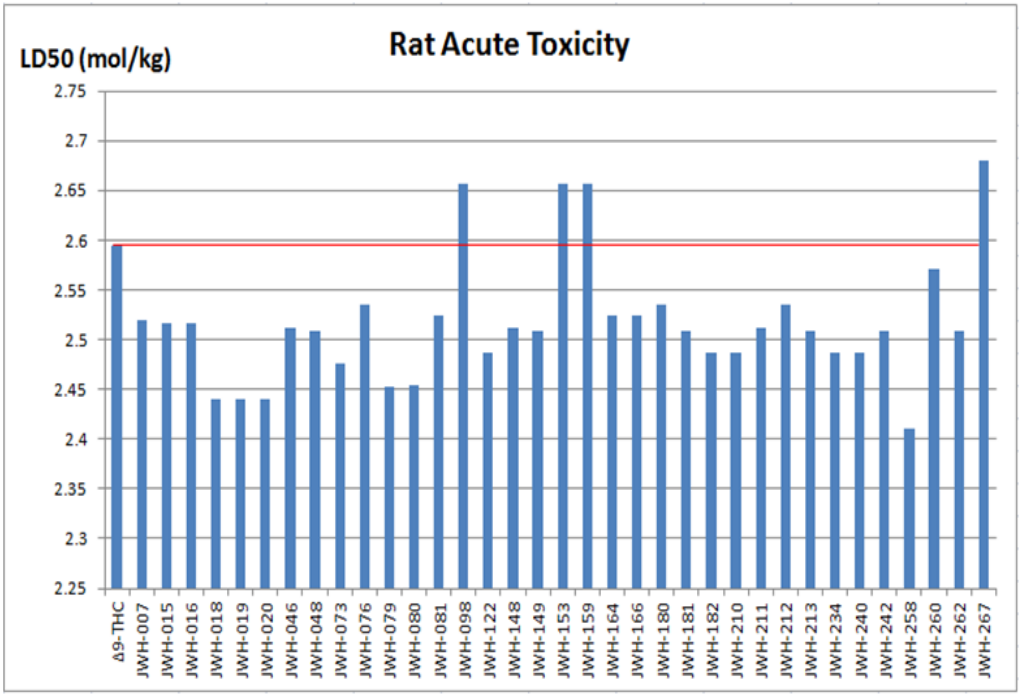


Fig. 8. Rat acute toxicity of the studied JWH cannabinoids in comparison with THC.

Tetrahymena pyriformis toxicity (TPT) is the most commonly ciliated model used as a toxicology endpoint. The results obtained for the targeted JWH cannabinoids are presented in Fig. 9. The toxicity data is expressed as the negative logarithm of 50% growth inhibitory concentration (*pIGC50*) values, the threshold value being *pIGC50* = -0.5. In other words, the compounds having *pIGC50* > -0.5 are assigned as TPT and the others as non-TPT.

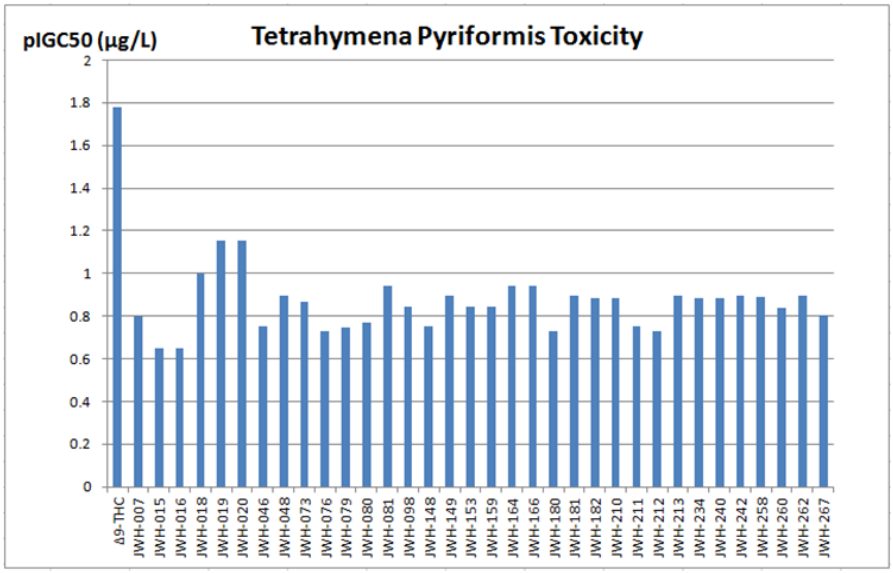


Fig. 9. Tetrahymena pyriformis toxicity (TPT) results obtained for the studied JWH cannabinoids in comparison with THC.

The results obtained with the PROTOX software are presented in Fig. 10. They clearly indicate that only THC exhibits an important mutagenic activity, while most of the JWH cannabinoids exhibit immunotoxicity activity as a priority action.

	PREDICTION TOXICITY		
Compound	Immunotoxicity	Mutagenicity	Cytotoxicity
THC	Inactive	active	Inactive
JWH-007	Active	active	Inactive
JWH-015	Inactive	active	Inactive
JWH-016	Active	active	Inactive
JWH-018	Active	active	Inactive
JWH-019	Active	active	Inactive
JWH-020	Active	active	Inactive
JWH-046-	Inactive	active	Inactive
JWH-048	Active	active	Inactive
JWH-073	Inactive	active	Inactive
JWH-076	Inactive	active	Inactive
JWH-079	Inactive	active	Inactive
JWH-080	Active	active	Inactive
JWH-081	Active	active	Inactive
JWH-098	Active	active	active
JWH-120	Inactive	active	Inactive
JWH-122	Inactive	active	Inactive
JWH-148	Inactive	active	Inactive
JWH-149	Active	active	Inactive
JWH-153	Active	active	Active
JWH-159	Active	Active	Active
JWH-164	Active	active	Inactive
JWH-166	Active	active	Inactive
JWH-180	Inactive	active	Inactive
JWH-181	Active	active	Inactive
JWH-182	Active	Active	Inactive
JWH-210	Active	active	Inactive
JWH-211	Inactive	active	Inactive
JWH-212	Inactive	active	Inactive
JWH-213	Active	active	Inactive
JWH-234	Active	active	Inactive
JWH-240	Active	active	Inactive
JWH-242	Active	active	Inactive
JWH-258	Active	active	Inactive
JWH-260	Active	active	Active
JWH-262	Active	active	Inactive
JWH-267	Active	active	Active

4. CONCLUSION

A number of 31 JWH synthetic cannabinoids has been estimated and compared with THC from the point of view of their absorption, distribution, metabolism and toxicity. The estimations have been computed by using two specialized software packages, i.e. *ADME-tox* and *PROTOX*. The results indicate that the JWH cannabinoids identified as having high human intestinal absorption will cross easier the blood-brain barrier, as high toxicity is due to good absorption. The *LD50* values obtained for JWH cannabinoids are all higher than that of THC. The same behavior was noticed in the case of *Tetrahymena pyriformis* toxicity assessments.

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