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Physico-chemical analysis, systematic benchmarking, and toxicological aspects of the JWH aminoalkylindole class-derived synthetic JWH cannabinoids

Catalina Mercedes Burlacu[1], Adrian Constantin Burlacu, Mirela Praisler[1,2]

[1] "Dunarea de Jos" University of Galati, Faculty of Sciences and Environment, 47 Domneasca Street, 800008, Galati, Romania, Catalina.Burlacu@ugal.ro, Mirela.Praisler@ugal.ro
[2] INPOLDE interdisciplinary research network, Dunarea de Jos University of Galati, 111 Domneasca St., 800201 Galati, Romania

Abstract
We are presenting the results obtained by computing different toxicity indices for some of the newest JWH synthetic cannabinoids, by using (Q)SAR models, ADME(T) predictions, simulations of NMR spectral techniques and other different computational dedicated software packages and forensic analytical tools. We have examined the main physical and chemical properties and evaluated the behavioral neurotoxicity and pharmacokinetic profile of 16 aminoalkylindole class-derived synthetic cannabinoids JWH as compared to the Delta-9-tetrahydrocannabinol (Δ9-THC), which was chosen as a standard compound. For this purpose, the geometries of the molecules have been optimized by using the AM1 semi-empirical quantum method. The conclusions of a comparative analysis of the toxicities of synthetic and natural cannabinoids are presented.

Keywords: JWH synthetic cannabinoids, QSAR descriptors, ADMET prediction.

1. INTRODUCTION

Commercial preparations containing synthetic cannabinoids are rapidly emerging as drugs of abuse. Although often assumed to be "safe" and "legal" alternatives to cannabis, reports indicate that synthetic cannabinoids induce toxicity not often associated with the primary psychoactive component of marijuana, Δ9-tetrahydrocannabinol (Δ9-THC).

This paper tried to summarize the evidence that the use of JWH synthetic cannabinoids poses greater health risks relative to marijuana and suggest that distinct pharmacological properties and metabolism of SCBs relative to Δ9-THC may contribute to this increased toxicity [1].

Physico-chemical analysis of chemicals both in vitro and in vivo is a major research direction, however, it is increasingly considered in recent times that in silico studies in medicine have the potential to accelerate the rate of discovery, while reducing the need for expensive laboratory work and lengthy clinical trials.

In this regard, physico-chemical analysis, biological and toxicological evaluation of synthetic compounds, precursors and derivatives, highlighting patterns of consumption of psychoactive substances, spectral methods of characterization and identification, artificial intelligence, and expert systems are among the most effective methods for the identification, research, and testing of new drugs or emerging chemical compounds, being, at the same time, formidable tools in the fight against trafficking networks of high-risk substance [2].

The present paper provides a review of the main toxic activities of Huffman synthetic cannabinoids probably due to strong CB1 receptor stimulation, as synthetic cannabinoids have a high affinity for the CB1 receptor.

2. MATERIALS AND METHODS

A number of 16 JWH synthetic cannabinoid compounds emerging as drugs of abuse have been characterized and analyzed in comparison with the phytocannabinoid THC (δ-9-tetrahydrocannabinol), most of them part of aminoalkylindole class:

- 7 compounds from naphthylindolines group : JWH-007, JWH-015, JWH-072, JWH-149, JWH-387 JWH-398 and JWH-424;
- 3 compounds from naphthoylpyrroles group: JWH-030, JWH-147 and JWH-370;
- 2 compounds from phenylacetylindoles group: JWH-251 andJWH-302;
- 1 compound from dibenzopyran group: JWH-133;
- 1 hybrid compound from dibenzopyran group: JWH-161;
- 1 compound from naphthylmethylindole group: JWH-184;
- 1 compound from hydrocarbon group: JWH-176.

Relevant literature available online was analyzed and were accessed and queried some of the main online databases, scientific platforms, servers, web benchmarking modules and software packages:

- Online chemical databases: PubChem, Spectrabase, Comptox, The National Institute of Standards and Technology (NIST) and Chemspider.
- Artificial Neural Network Modules and web applications: OCHEM, Comptox, Swiss Institute of Bioinformatics and ADMET Lab scientific platforms.
- QSAR and ADMET standalone Estimation Software Tools and Chemoinformatics Software Suite: HyperChem, Gaussian/GaussView, alvaMolecule, EPA Toxicity Estimation Software Tool (T.E.S.T.), OECD QSAR Toolbox(QSAR) [3].
- Open-Source Programs for Data Visualization and Analysis with Chemical Intelligence: JMol, Osiris Data Warrior.
- Data cleaning and transformation, numerical simulation, statistical modeling and machine learning software package: Python Jupyter Notebook.

The analytical reference methods, the interpretation of the data sets, and the physicochemical and toxicological information available on web databases were in accordance with the regulations and records in the European REACH (Regulation on the Registration, Evaluation, Authorization, and Restriction of Chemicals) database.

The experimental part of the study presented examples and methods applicable in the screening of chemical compounds as an in silico alternative to in vitro and in vivo tests.

For our research, we used the familiar methods of machine learning and QSAR (quantitative structure-activity relationship) activity, Artificial Neural Network (ANN), and two simulated methods of spectral identification 1H-NMR and 13C-NMR [4].

The geometries of the molecules of the 16 target synthetic cannabinoids and the THC compound downloaded in the MOL file format have been optimized with HyperChem, and Gaussian/ GaussView software packages by using the AM1 semi-empirical quantum method (e.g. Figure 1), starting from the canonical SMILES molecular identifiers from the PubChem scientific platform and the molecules' structures downloaded in the MOL file format(.mol).

The small molecules obtained after optimization were saved again in MOL format (.mol) and then were compiled into output files MDL (.sdf) and in a tabular format CSV (.csv) with alvaMolecule for extended portability to other software packages.

Most of the 75 QSAR and the 40 ADMET molecular descriptors calculated and tested in our study have been selected from three blocks: constitutional descriptors, molecular properties, and indices drug likeness (the complete list of calculated and tested molecular descriptors can be downloaded on request). We prepared a synopsis, tables, and comparative diagrams for each of the 16 synthetic cannabinoids and THC containing physico-chemical and toxicological data and information obtained from online databases, validated and scientifically accepted [5, 6].

3. RESULTS AND DISCUSSION

The ADMET predictions were performed for all the studied compounds.

Table 1. AQUEOUS SOLUBILITY(logS Descriptor) EVALUATION REPORT

AQUEOUS SOLUBILITY (LOGS DESCRIPTOR) EVALUATION REPORT		
COMPOUND	**IDENTIFIER**	**Log S(SILICOS-IT) log mol/ L**
01	THC	-3.500
02	JWH 007	-6.346
03	JWH 015	-6.264
04	JWH 030	-6.177
05	JWH 072	-6.414
06	JWH 133	-6.086
07	JWH 147	-6.446
08	JWH 149	-6.410
09	JWH 161	-6.191
10	JWH 176	-7.319
11	JWH 184	-6.941
12	JWH 251	-6.444
13	JWH 302	-6.357
14	JWH 370	-6.427
15	JWH 387	-6.495
16	JWH 398	-6.490
17	JWH 424	-6.458

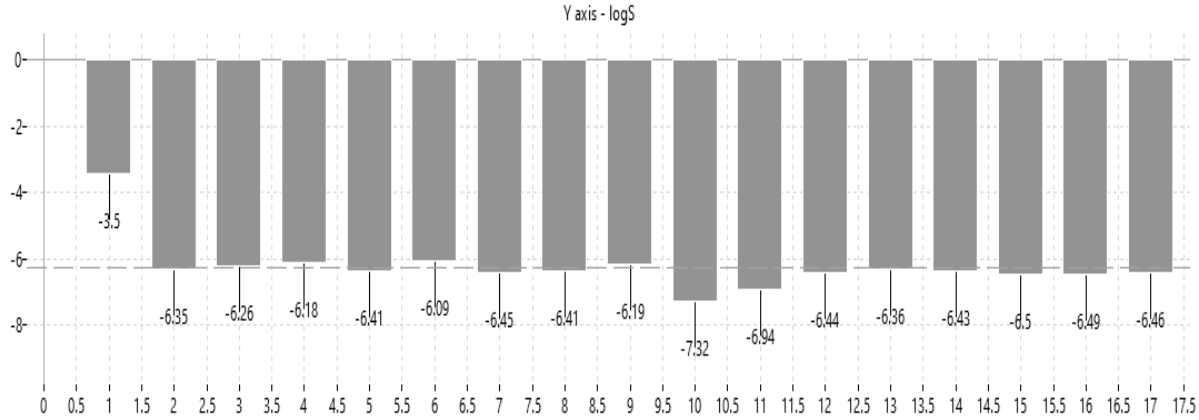

Figure 1. AQUEOUS SOLUBILITY (logS) descriptor (chart drafted with alvaMolecule software package)

Table 1 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. Typically, a low solubility is associated with bad absorption. Therefore, poorly soluble compounds are usually avoided [7].

From the examination of the charts and the ADMET evaluation report regarding the approximate logS parameter for the 16 JWH synthetic cannabinoids presented and for the standard reference phytocannabinoid THC, we draw the following conclusions:

- The approximate value, in silico, of the logS parameter for the THC phytocannabinoid, is -3,500 and is, therefore, higher than the general average of the experimentally calculated values, of -4, the phytocannabinoid having a high solubility in water, being, therefore, optimal.

• None of the approximate values for the 16 synthetic cannabinoids analyzed was in the optimal range of water solubility, which was much lower compared to both the approximate value, in silico, for THC and the general average calculated experimentally in around -4.

• The highest value of the 16 synthetic cannabinoids JWH had the compound JWH 133 (-6,086), thus having a higher water solubility than the other chemical compounds analyzed, and the lowest value was approximated for JWH 176 (-7,319).

LOGD (DISTRIBUTION COEFFICIENT D AT PH=7.4) (LOGD DESCRIPTOR) EVALUATION REPORT		
COMPOUND	**IDENTIFIER**	**logD (OCTANOL/WATER)**
01	THC	2.007
02	JWH 007	3.470
03	JWH 015	3.245
04	JWH 030	3.052
05	JWH 072	3.061
06	JWH 133	2.448
07	JWH 147	3.563
08	JWH 149	3.533
09	JWH 161	2.190
10	JWH 176	3.553
11	JWH 184	3.443
12	JWH 251	3.433
13	JWH 302	2.033
14	JWH 370	3.538
15	JWH 387	3.364
16	JWH 398	3.188
17	JWH 424	3.282

Table 2. logD (DISTRIBUTION COEFFICIENT D AT PH=7.4) (LogD Descriptor)

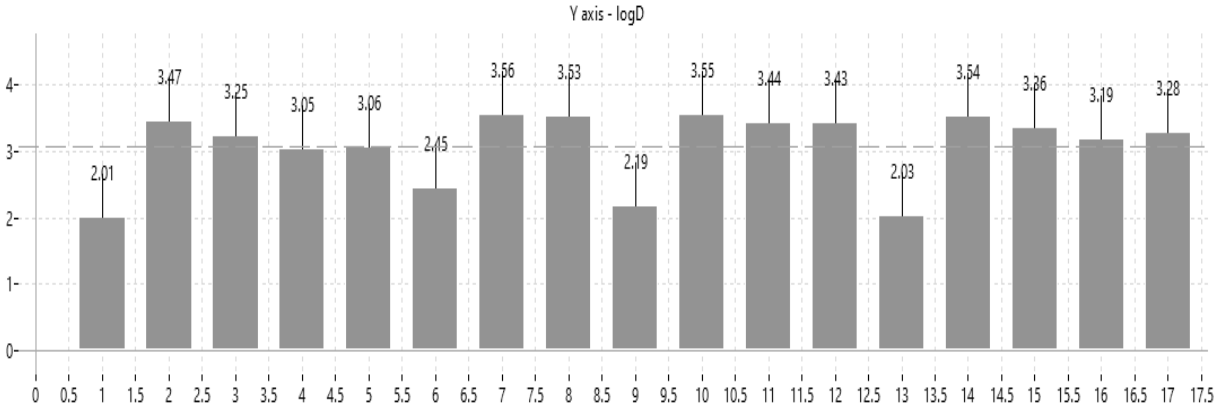

Figure 2. LogD (DISTRIBUTION COEFFICIENT D AT PH=7.4) (LogD) descriptor (chart drafted with alvaMolecule software package) EVALUATION REPORT

LogD, the distribution constant is a better descriptor of the lipophilicity of a molecule. This can be determined in a similar way to LogP, but instead of using water, the aqueous phase is adjusted to a specific pH using a buffer [8].

Therefore, Log D is pH dependent, so the pH at which log D was measured must be specified. Of particular interest is log D at pH = 7.4 (physiological pH of blood serum).

According to Table 2, the approximate values can be interpreted as follows:

- 1 to 3: Moderate solubility; Moderate permeability; Low metabolism.
- 3 to 5: Low solubility; High permeability; Moderate to high metabolism.

From the examination of the graphs and the ADME(T) evaluation report regarding the approximate logD parameter, we draw the following conclusions:

- The approximate value, in silico, of the logD parameter for the THC phytocannabinoid is 2.007 and is therefore between 1 and 3, this aspect can be interpreted as having moderate solubility, moderate permeability and low metabolism, the phytocannabinoid being within the values of other abusers / drugs on the market and having the lowest value of all JWH synthetic cannabinoids analyzed in this paper.
- Only 3 of the 16 synthetic cannabinoids JWH analyzed comparatively still have a value between 1-3, so moderate solubility, moderate permeability, and low metabolism, JWH 133 (2,448), JWH 161 (2,190), and JWH 302 (2,033).
- The remaining 13 JWH synthetic cannabinoids analyzed having values between 3-5, so low solubility, high permeability, moderate to high metabolism, the highest value having JWH 147 (3,563).

The logP value of a compound, which is the logarithm of its partition coefficient between n-octanol and log water (c octanol / c water), is a well-established measure of the hydrophilicity of the compound.

High logP values result in low absorption or permeability. It has been shown that the compounds have a reasonable property of being well absorbed, their logP value should not exceed 5.0.

Table 3. logP (DISTRIBUTION COEFFICIENT P)(logP Descriptor) EVALUATION REPORT

OGP (DISTRIBUTION COEFFICIENT P) (LOGP DESCRIPTOR) EVALUATION REPORT			
COMPOUND	**IDENTIFIER**	**LOGP**	**TOTAL MOL WEIGHT**
01	THC	5.736	314.467
02	JWH 007	6.524	355.480
03	JWH 015	5.744	327.426
04	JWH 030	5.063	291.393
05	JWH 072	5.436	313.399
06	JWH 133	6.375	312.495
07	JWH 147	7.120	381.517
08	JWH 149	6.833	369.506
09	JWH 161	7.301	403.564
10	JWH 176	7.358	324.466
11	JWH 184	6.884	341.496
12	JWH 251	5.565	319.447
13	JWH 302	5.266	335.446
14	JWH 370	7.038	381.517
15	JWH 387	6.978	420.349
16	JWH 398	6.869	375.898
17	JWH 424	6.978	420.349

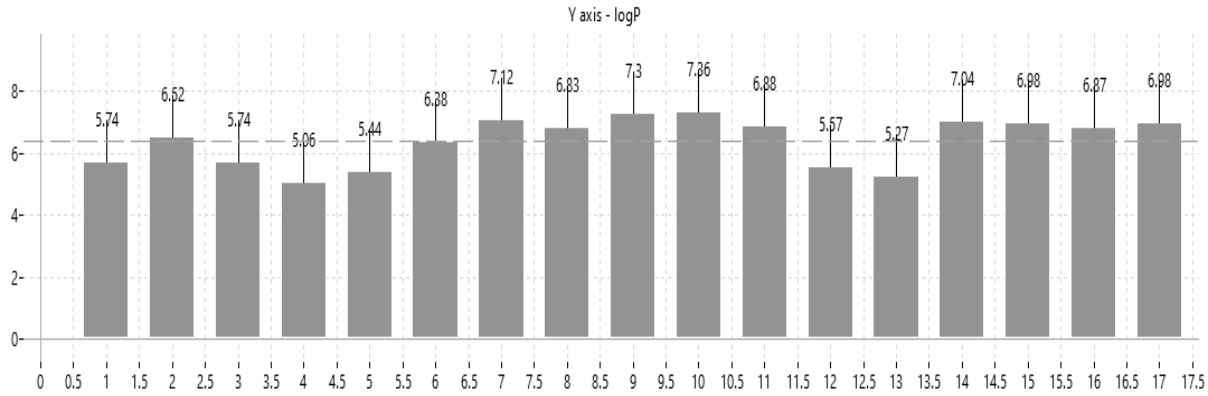

Figure 3. logP (DISTRIBUTION COEFFICIENT P) (LogP) descriptor (chart drafted with alvaMolecule software package)

Lipophilicity is probably the most important physicochemical property of a potential drug, it plays a role in solubility, absorption, membrane penetration, plasma protein binding, distribution, CNS penetration, and partitioning into other tissues or organs such as the liver and has an impact on escape routes [8].

From the examination of the charts and the ADMET evaluation report regarding the approximate logP parameter, we draw the following conclusions:

- The approximate value, in silico, of the logP parameter for the THC phytocannabinoid is 5,736 and therefore, is higher than the overall average of the experimentally calculated values of 5,0, the phytocannabinoid having an absorption or a low permeability to the average of the other abuse / medicines on the market.
- Of the 16 synthetic cannabinoids JWH analyzed in comparison, 4 compounds have a lower logP value than THC and therefore have a better absorption or permeability than THC, namely, in order, JWH 030 (5,063), JWH 302 (5,266), JWH 072 (5,436) and JWH 251 (5,565).
- None of the approximate values for the 16 synthetic cannabinoids analyzed were in the optimal range of absorption, or of reduced permeability having higher values, compared to the general average calculated experimentally around 5.0.
- The lowest value of the 16 JWH synthetic cannabinoids was compound JWH 030 (5,063), thus having a good absorption or permeability than the other chemical compounds analyzed, and the highest value was approximated for JWH 176.

Caco-2 promiscuity parameter is used for toxicity assessments and refers to a human colon epithelial cancer cell line that is 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 [9].

This parameter is evaluated based on the logPAPP parameter, i.e. the in vitro passive membrane permeability across the Caco-2 cell.

Table 4. logPAPP (CACO-2 PERMEABILITY)(Caco-2 Descriptor) EVALUATION REPORT

LOGPAPP (CACO-2 PERMEABILITY) (CACO-2 DESCRIPTOR) EVALUATION REPORT		
COMPOUND	**IDENTIFIER**	**CACO2(CM/S)**
01	THC	-4.746
02	JWH 007	-4.628
03	JWH 015	-4.547
04	JWH 030	-4.452
05	JWH 072	-4.539
06	JWH 133	-4.548
07	JWH 147	-4.718
08	JWH 149	-4.616
09	JWH 161	-4.966
10	JWH 176	-4.500
11	JWH 184	-4.598
12	JWH 251	-4.572
13	JWH 302	-4.641
14	JWH 370	-4.727
15	JWH 387	-4.635
16	JWH 398	-4.622
17	JWH 424	-4.635

The results obtained regarding the Caco-2 promiscuity of the studied JWH cannabinoids are presented in Table 4. The largest logPapp value, second only to that determined for the natural THC compound, was obtained for JWH-161.

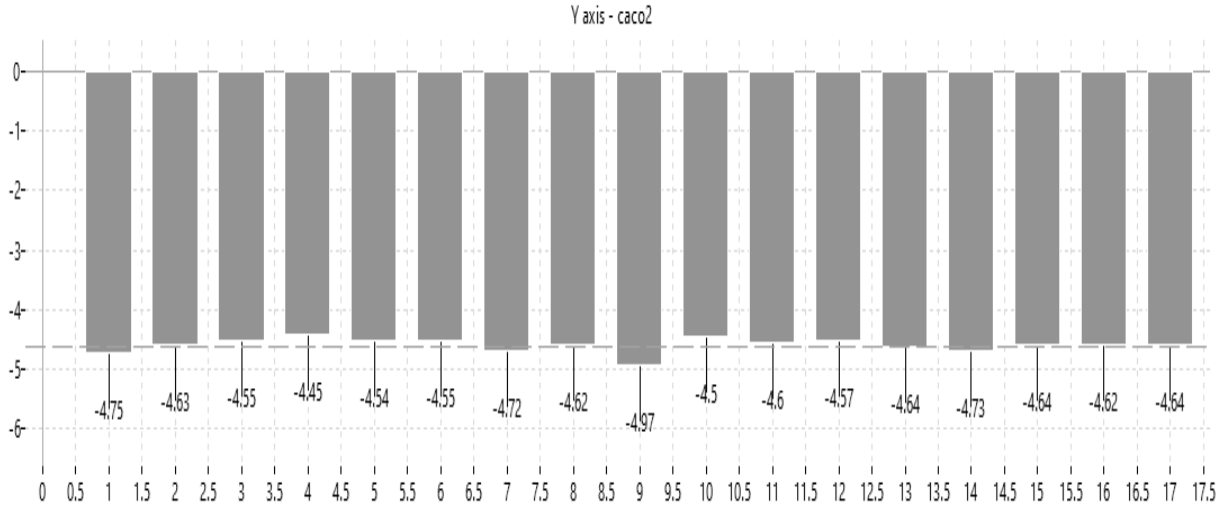

Figure 4. logPAPP (CACO-2 PERMEABILITY) (Caco-2) descriptor (chart drafted with alvaMolecule software package)

Table 5. BBB (Blood–Brain Barrier) (BBB+ Descriptor) EVALUATION REPORT

BBB (BLOOD–BRAIN BARRIER) (BBB+ DESCRIPTOR) EVALUATION REPORT			
COMPOUND	**IDENTIFIER**	**CATEGORY 1-PERMEABLE/0- NONPERMEABLE**	**PROBABILITY**
01	THC	1	0.878
02	JWH 007	1	0.969
03	JWH 015	1	0.978
04	JWH 030	1	0.882
05	JWH 072	1	0.950
06	JWH 133	1	0.974
07	JWH 147	1	0.882
08	JWH 149	1	0.980
09	JWH 161	1	0.950
10	JWH 176	1	0.973
11	JWH 184	1	0.990
12	JWH 251	1	0.904
13	JWH 302	1	0.909
14	JWH 370	1	0.857
15	JWH 387	1	0.918
16	JWH 398	1	0.959
17	JWH 424	1	0.854

Table 5 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).

The blood-brain barrier works effectively to protect the brain from the circulation of pathogens. Consequently, infections caused by the blood of the brain are rare, and when they occur, they are very difficult to treat.

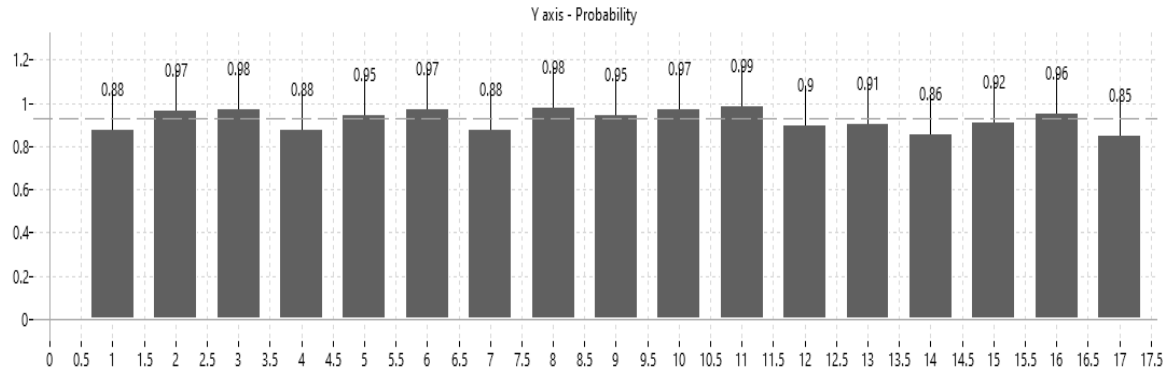

Figure 5. BBB (Blood–Brain Barrier) (BBB+) descriptor (chart drafted with alvaMolecule software package)

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, Table 5 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. 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.

Table 6 indicates that all the studied JWH cannabinoids have a higher LD50 than THC with the exception of JWH-161 and JWH-424. A lower LD50 is indicative of increased toxicity.

Table 6. LD50 (LD50 of acute toxicity) (LD50 Descriptor) EVALUATION REPORT

LD50 (LD50 OF ACUTE TOXICITY) (LD50 DESCRIPTOR) EVALUATION REPORT		
COMPOUND	**IDENTIFIER**	**LD50 (-LOG MOL / KG)**
01	THC	2.666
02	JWH 007	2.547
03	JWH 015	2.604
04	JWH 030	2.441
05	JWH 072	2.519
06	JWH 133	2.306
07	JWH 147	2.592
08	JWH 149	2.560
09	JWH 161	2.861
10	JWH 176	2.652
11	JWH 184	2.525
12	JWH 251	2.549
13	JWH 302	2.478
14	JWH 370	2.574
15	JWH 387	2.629
16	JWH 398	2.672
17	JWH 424	2.706

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 Table 7.

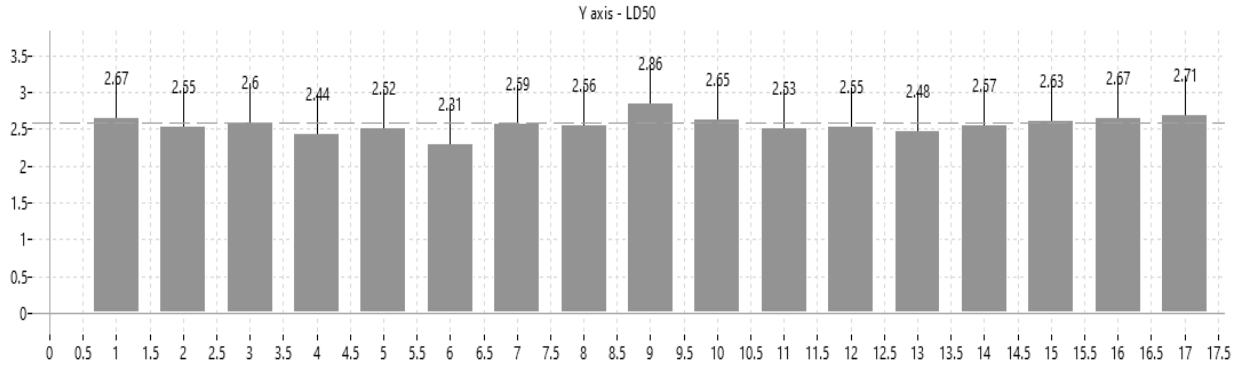

Figure 6. LD50 (LD50 of acute toxicity) (LD50) descriptor (chart drafted with alvaMolecule software package)

Table 7. TETRAHYMENA PYRIFORMIS TOXICITY (logIGC50-1 Descriptor) EVALUATION REPORT

LOGIGC50-1 TETRAHYMENA PYRIFORMIS TOXICITY (LOGIGC50-1 DESCRIPTOR) EVALUATION REPORT		
COMPOUND	**IDENTIFIER**	**LOG(IGC50-1) (MODEL 3)**
01	THC	2.3
02	JWH 007	2.8
03	JWH 015	2.3
04	JWH 030	2.5
05	JWH 072	2.1
06	JWH 133	1.9
07	JWH 147	2.9
08	JWH 149	2.8
09	JWH 161	2.8
10	JWH 176	2.7
11	JWH 184	3.1
12	JWH 251	2.6
13	JWH 302	2.6
14	JWH 370	2.9
15	JWH 387	2.5
16	JWH 398	2.8
17	JWH 424	2.4

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

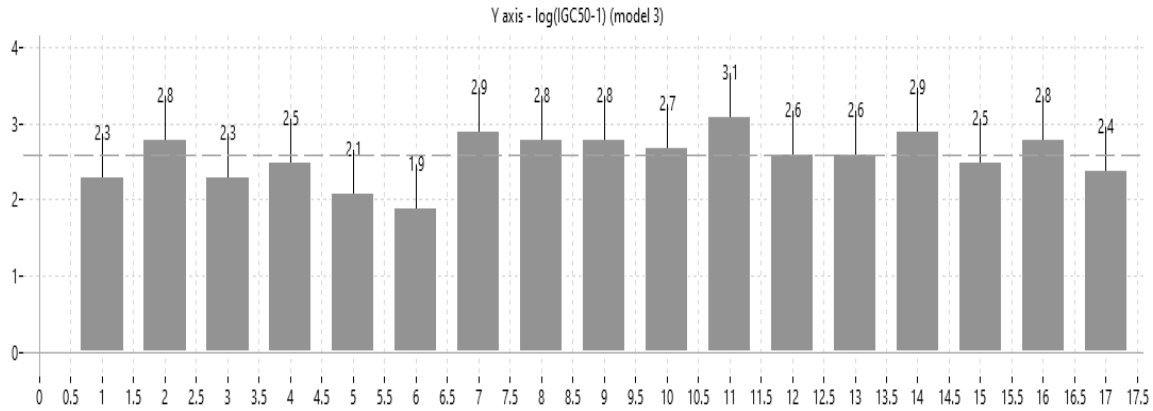

Figure 7. logIGC50-1 TETRAHYMENA PYRIFORMIS TOXICITY descriptor (chart drafted with alvaMolecule software package)

pIGC50 > -0.5 are assigned as TPT and the others as non-TPT. The results obtained for the AMES mutagenicity are presented in Table 8.

Table 8. AMES MUTAGENICITY(Ames Mutagenicity Descriptor) EVALUATION REPORT

AMES MUTAGENICITY (AMES MUTAGENICITY DESCRIPTOR) EVALUATION REPORT			
COMPOUND	**IDENTIFIER**	**CATEGORY 1-ACTIVE/ 0-INACTIVE**	**PROBABILITY**
01	THC	0	0.090
02	JWH 007	1	0.554
03	JWH 015	1	0.560
04	JWH 030	1	0.504
05	JWH 072	1	0.628
06	JWH 133	0	0.084
07	JWH 147	0	0.498
08	JWH 149	1	0.530
09	JWH 161	0	0.316
10	JWH 176	0	0.484
11	JWH 184	1	0.700
12	JWH 251	0	0.480
13	JWH 302	1	0.508
14	JWH 370	1	0.506
15	JWH 387	0	0.488
16	JWH 398	0	0.494
17	JWH 424	0	0.488

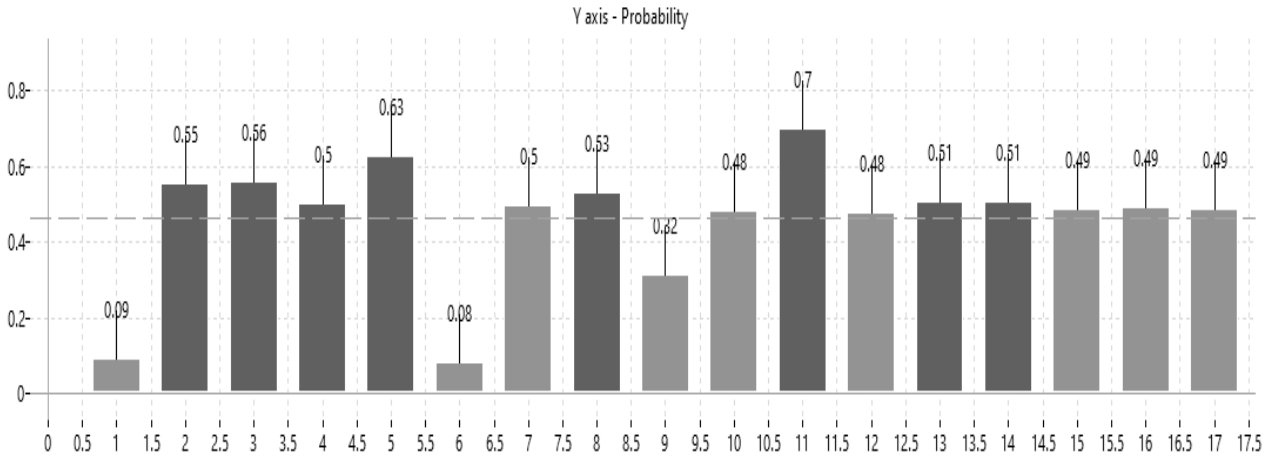

Figure 8. AMES MUTAGENICITY(Ames Mutagenicity) descriptor descriptor (chart drafted with alvaMolecule software package)

They clearly indicate that THC does not exhibit any important mutagenic activity, while some of the JWH cannabinoids exhibit immunotoxicity activity as a priority action. Many other parameters were studied in this research with important results [9].

4. CONCLUSIONS

The comparative results of the in silico tests confirm that some JWH synthetic cannabinoids are even more toxic than their natural counterpart. All JWH synthetic cannabinoids analyzed have much more toxic potential, having a high affinity for the CB1 receptor. Some have a particularly low value of the Ki constant binding to more than 100 times more closely to the CB1 receptor than THC. JWH synthetic cannabinoids can be confirmed and analyzed using artificial intelligence, expert systems, and spectral techniques such as GC-MS, GC-IR, GC-FTIR, FT-IR, ATR-FTIR, and RAMAN which are among the most efficient and formidable tools in the fight against high-risk trafficking networks [10].

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