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COMPUTATIONAL PREDICTIVE TOXICOLOGY MODELING FOR ASSESSING HUMAN HEALTH RISKS OF NOVEL PSYCHOACTIVE SUBSTANCES (NPS): A CASE STUDY

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ABSTRACT

The study aimed to identify the increasing health challenges posed by the rapid emergence of novel psychoactive substances (NPS) compounds. The research employed a multifaceted approach, integrating advanced computational techniques such as the Maestro Schrödinger 12.8 software suite, admeSAR, Protox II, Guasar toxicity modeling and molecular docking. A diverse dataset comprising chemical structures, physicochemical properties, and toxicity data for known NPS compounds was curated and used to develop predictive models for various adverse health effects, including neurotoxicity, cardiotoxicity, hepatotoxicity, and reproductive toxicity.

Key findings from the study revealed significant correlations between chemical structural features and toxicological endpoints, enabling the identification of structural alerts and toxicophores associated with NPS-induced adverse effects. Moreover, the study investigated the impact of metabolic pathways and bioactivation processes on NPS toxicity, providing insights into the potential formation of reactive metabolites and their contribution to adverse health outcomes.

Overall, this research contributes to advancing the field of predictive toxicology and provides valuable tools for assessing the health risks associated with NPS consumption. The findings underscore the importance of integrating computational approaches into regulatory decision-making processes and public health policies to effectively mitigate the adverse effects of NPS on individuals and communities.

Keywords: Novel Psychoactive Substances (NPS), Predictive Toxicology Modeling, Computational Approaches, Human Health Risks, Adverse Effects, Molecular Docking

INTRODUCTION

The European Monitoring Center for Drug and Drug Addictions (EMCDDA) in 2016 defined Novel Psychoactive Substances (NPS) as chemical substitutes for traditional drugs of abuse. To some extent, these new agents include very potent

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substances that may cause serious mental reactions and lethal poisonings. The World Health Organization (WHO) also defines psychoactive substances as substances that, when taken in or administered into one's system, affect mental processes. In other words, psychoactive substances can be referred to as chemical substances which when ingested, inhaled or injected into the body have the potential to alter mood, behavior, perception or mental functioning of an individual. Overall, novel psychoactive substances (NPS) are new narcotic or psychotropic drugs that are not regulated by the United Nations' 1961 Narcotic Drugs or 1971 Psychotropic Substances Conventions, but which may pose a public health threat (Zawilska et al., 2018). The term "novel" does not necessarily imply a recent development; rather, it refers to substances that have recently gained popularity or availability, thereby becoming a current or potential public health concern (Zawilska et al., 2018).

In 2011, Famuyiwa et al. conducted an epidemiological study on the use of psychoactive substances by adolescents in metropolitan Lagos, a city with a distinctive socio-economic profile. The study identified numerous issues related to substance misuse in Nigeria, including significant admissions to psychiatric institutions for drug-related health problems (Ohaeri & Odejide, 1993; Unaogu et al., 2017). High rates of substance abuse were noted across various groups, including the general population, secondary school pupils, prison inmates, university students, street children, and commercial transport workers. Additionally, data from the National Bureau of Statistics reported a significant 14.4% past-year prevalence of psychoactive substance use in Nigeria, especially cannabis and non-medical prescription opioids, with higher rates among men and those aged 25-39. There were notable regional differences, with the highest rates in the southern zones (13.8% to 22.4%) compared to the northern zones (10% to 14.9%). The prevalence of psychoactive drugs appears to be increasing in developing countries, as evidenced by the rising use of these substances in Nigeria. Incidents of thuggery, armed robbery, rape, cultism, violent demonstrations, religious crises, prostitution, and restlessness among the youth can be linked to the use and abuse of psychoactive substances.

NPS largely appears to mimic the psychological and/or pharmacological properties of the original drugs, which are designed to evade detection by the standard drug test and/or being classified as illegal (Corkery *et al.,* 2018; Schifano *et al.,* 2017). The intake of these substances is typically associated with the imbalance of a range of neurotransmitter pathways/receptors, and consequently with the risk of psychopathological disturbances. The occurrence of psychosis has been linked to several factors: a) increased central dopamine levels associated with the intake of novel psychoactive substances such as novel psychedelic phenethylamines, synthetic cathinones, and 4,4'-DMAR (Schifano *et al.,* 2023); b) cannabinoid CB1 receptor activation achieved with synthetic cannabimimetics (De Petrocellis & Di Marzo., 2010) 5-HT2A receptor activation reported with NBOMe compounds, latest tryptamine derivatives, lefetamine derivatives, DXM, and hallucinogenic plants (Schifano *et al.,* 2015); d) antagonist activity at NMDA receptors described with phencyclidine-like dissociatives (Martin, & Lodge., 1988); and e) k-opioid receptor activation typically associated with Salvia divinorum intake (Babu *et al.,* 2008).

According to the EMCDDA, since 2009, dozens of new compounds have been introduced to the market each year, encompassing a broad range of novel psychoactive substances (NPS). These substances include various drug classes, each with unique chemical structures and mechanisms of action, leading to diverse psychological and physiological effects. An overview of common NPS categories utilized in Nigeria, emphasizing their toxicity, mechanisms, addiction potential, dependency issues, and potential for therapeutic repurposing are provided below.

Synthetic Cannabinoids such as JWH-018, K2, and Spice are engineered to bind to cannabinoid receptors in the brain, mimicking THC, the active compound in cannabis. However, these substances can be significantly more potent and unpredictable, leading to severe toxicity and even fatalities (Zawilska, 2018). **Synthetic Cathinones**, often referred to as "Bath Salts" (e.g., Mephedrone, MDPV), are stimulants similar to amphetamines. They are notorious for their ability to induce powerful euphoric effects and are associated with acute behavioral disturbances, intense cravings, and severe neurological and cardiovascular complications (Prosser and Nelson, 2012). **Phenethylamines**, such as 2C-B and 2C-E, and **Tryptamines** like DMT and 5-MeO-DiPT, primarily affect the serotonin system, causing alterations in perception, mood, and cognition. These hallucinogens can lead to profound sensory distortions, posing risks of psychological distress and harm (Shulgin and Carter, 1980). **Piperazines** such as BZP and TFMPP, once legal substitutes for ecstasy, act as stimulants and have been linked to toxic effects like seizures, renal failure, and cardiovascular issues (Baumann et al., 2005). **Synthetic Opioids**, including fentanyl analogs like Carfentanil, represent one of the most dangerous classes of NPS due to their extreme potency and high risk of overdose and death (Armenian et al., 2018). **Kratom**, containing Mitragynine and 7-hydroxymitragynine, has gained attention for its dual opioid-agonistic and stimulant properties, leading to its use in self-managing pain and opioid withdrawal symptoms despite its addiction potential (Boyer et al., 2008). **Salvia divinorum**, driven by the active component Salvinorin A, is unique in its selective activation of kappa-opioid receptors, leading to intense hallucinations and dissociative experiences (MacLean et al., 2013). The use of **Nootropics** like Modafinil and Adrafinil is increasing, particularly for their off-label use to enhance cognitive function, despite potential side effects including dependence and withdrawal symptoms (Ballon and Feifel, 2006). Lastly, **Research Chemicals** such as 1P-LSD and ALD-52 continue to challenge public health frameworks with their untested psychotropic properties and unknown long-term health implications (Brandt et al., 2017).

Bioinformatics has emerged as a pivotal and rapidly evolving field that greatly enhances biological research, particularly in areas like *in silico* toxicity studies (Mitra *et al.,* 2022). By integrating machine learning algorithms, Python programming, and an array of bioinformatics tools, researchers can now predict the toxicological profiles of compounds with high efficiency and accuracy. This approach leverages the power of computational methods to simulate biological interactions at the molecular level, significantly reducing the need for costly and time-consuming wet-lab experiments. Tools such as Maestro Schrödinger suite, admeSAR, Protox II, and SwissADME allow

for comprehensive assessments of chemical properties, interaction potentials, and ADMET profiles, thereby revolutionizing the prediction and understanding of compound toxicity. These advancements in bioinformatics not only accelerate the pace of toxicological screenings but also enhance the precision of drug safety evaluations, making significant contributions to the field of pharmacology and toxicology (Liu et al., 2013; Drwal et al., 2014; Schrödinger LLC, 2020). Therefore, this study explores the application of computational predictive toxicology modeling to assess the human health risks associated with novel psychoactive substances (NPS).

METHODS

This study employs a comprehensive *in silico* approach to assess the toxicity profiles of twenty selected NPS using advanced machine learning techniques, the Maestro Schrödinger 12.8 software suite, and various online toxicity prediction tools including admeSAR, Protox II, and Guasar toxicity.

Machine Learning Model Development

Machine learning models were developed using Python, a versatile programming language popular in scientific computing and machine learning. Key libraries utilized include Scikit-learn for model building and TensorFlow for more complex deep learning algorithms. The workflow involves the following steps:

Data Preprocessing: Data on chemical structures and known toxicity were obtained and preprocessed to convert chemical structural information (SMILES notation) into numerical descriptors that could be used as input for machine learning algorithms.

Feature Selection: Features influencing toxicity were selected using recursive feature elimination and other dimensionality reduction techniques to enhance model performance and interpretability.

Model Training and Validation: Models were trained using a dataset divided into training and validation subsets. Algorithms like Random Forest, Support Vector Machine, and Neural Networks were tested. K-fold cross-validation was employed to ensure the robustness and generalizability of the models.

Molecular Modeling and Simulations

Molecular docking and dynamics simulations were conducted using the Maestro Schrödinger 12.8 suite:

Ligand Preparation: Compounds were optimized for 3D geometry and ionization states using LigPrep.

Protein Preparation: Target protein structures, obtained from the Protein Data Bank (PDB), were prepared using the Protein Preparation Wizard in Maestro, which includes steps such as bond order assignment, addition of hydrogen atoms, and energy minimization.

Docking: Flexible ligand docking was performed using Glide, which provides high-accuracy docking modes. The best poses were selected based on docking scores and visual inspection.

TOXICITY PREDICTION USING ONLINE TOOLS

Additional toxicity predictions were conducted using several online tools:

admeSAR: Used for predicting a variety of ADMET properties. The SMILES of

compounds were input to assess their potential toxicity profiles.

Protox II: This tool predicts the toxicological risk based on the protein targets and mechanisms of toxicity, providing insights into potential adverse effects.

Guasar toxicity: Utilized to evaluate the NPS toxicity.

Integration and Analysis

Results from machine learning models, molecular docking, and online toxicity predictions were integrated to provide a comprehensive assessment of each compound's toxicity profile. Discrepancies and confirmations among different methods were analyzed to derive a consensus on the toxicological risks associated with each compound

RESULTS

In this study, we prioritize the toxicity profiles of various novel psychoactive substances (NPS) while utilizing computational tools, including QikProp, admeSAR, and ProTox II, we assessed the potential behaviors of NPS with targets such as neurotransmitter receptors which include serotonin, dopamine, and opioid receptors.

Table 1. Summary of Chemical Properties and Regulatory Parameters for the twenty (20) Selected Novel Psychoactive Substances. This table lists the Compound ID, Substance Name, Molecular Weight, Lipinski's Rule of Five (RO5) compliance, and Rule of Three (RO3) compliance for each substance. The table provides essential information for assessing the pharmacokinetic profiles and potential drug-likeness of these compounds, facilitating further research and regulatory evaluation.

Compound ID	Substance Name	Mol. Weight	RO5	RO3
C1	1-Acetyllysergic acid diethylamide	365.5	0	0
C2	1-Propionyl-lysergic acid diethylamide	379.5	0	0
C3	Adrafinil	289.4	0	1
C4	Modafinil	273.4	0	1
C5	Clonazolam	353.8	0	0
C6	Etizolam	342.8	0	0
C7	Deschloroketamine	203.28	0	0
C8	Flualprazolam	326.8	0	0
C9	Flubromazolam	371.2	0	0
C10	5-Methoxy-N,N-diisopropyltryptamine	274.4	0	0
C11	Dimethyltryptamine	188.27	0	0
C12	Salvinorin A	432.5	0	0
C13	7-Hydroxymitragynine	414.5	0	0
C14	Mitragynine	398.5	0	0
C15	Carfentanil	394.5	0	0
C16	1-[3-(Trifluoromethyl)phenyl]piperazine	230.23	0	0
C17	1-Benzylpiperazine	176.26	0	0
C18	Methylenedioxypyrovalerone	275.34	0	0
C19	Mephedrone	177.24	0	0
C20	1-Pentyl-3-(1-naphthoyl)indole	341.4	1	1

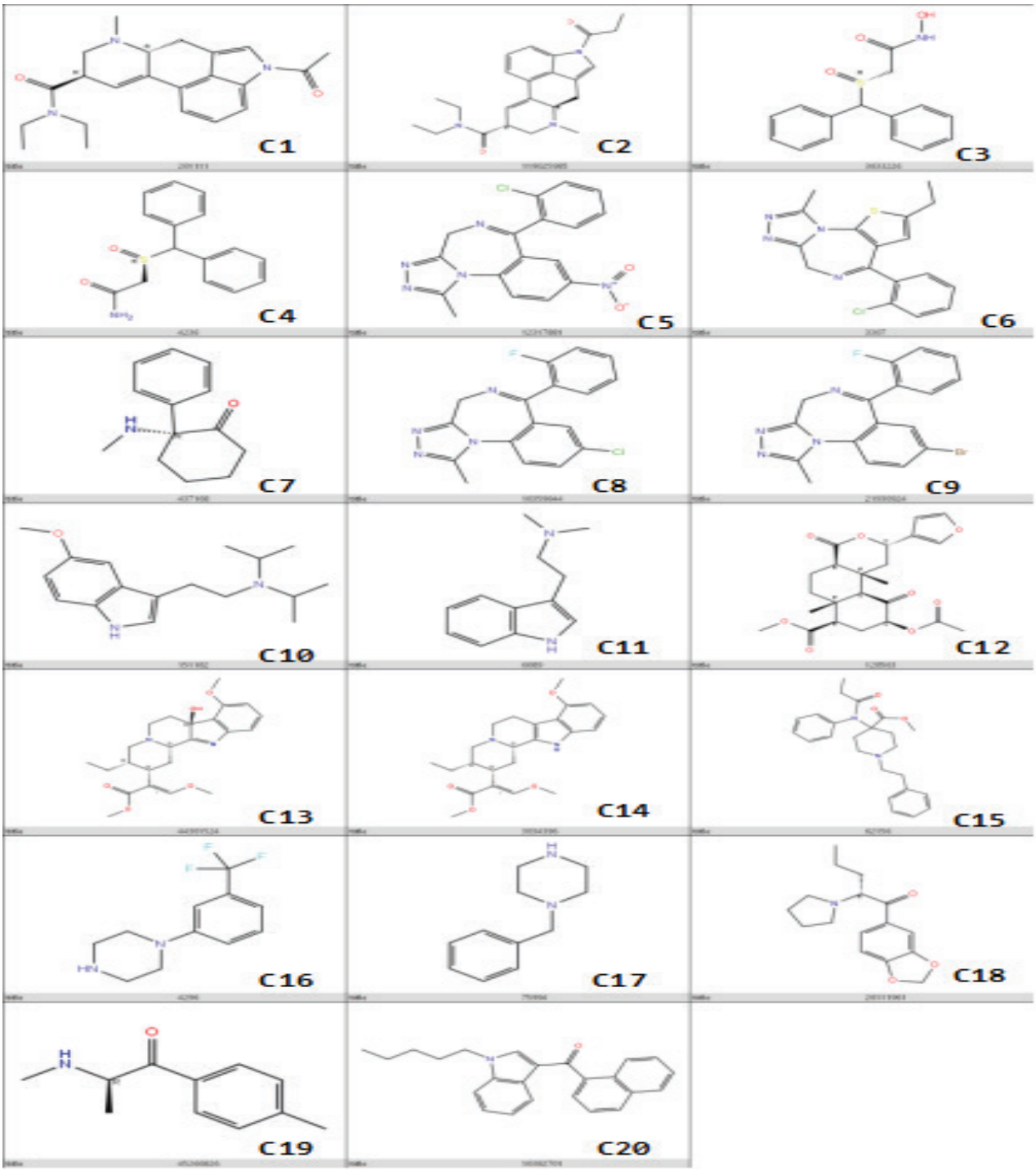

Figure 1. Illustration of the 2D molecular structures for twenty novel psychoactive substances, each denoted by a unique compound ID (C1 to C20). This figure provides a visual representation of structural variations across a range of psychoactive compounds, from classic psychedelics and synthetic cannabinoids to novel benzodiazepines and stimulants. The depicted structures highlight key molecular features and functional groups responsible for the pharmacological activities and binding properties of these substances.

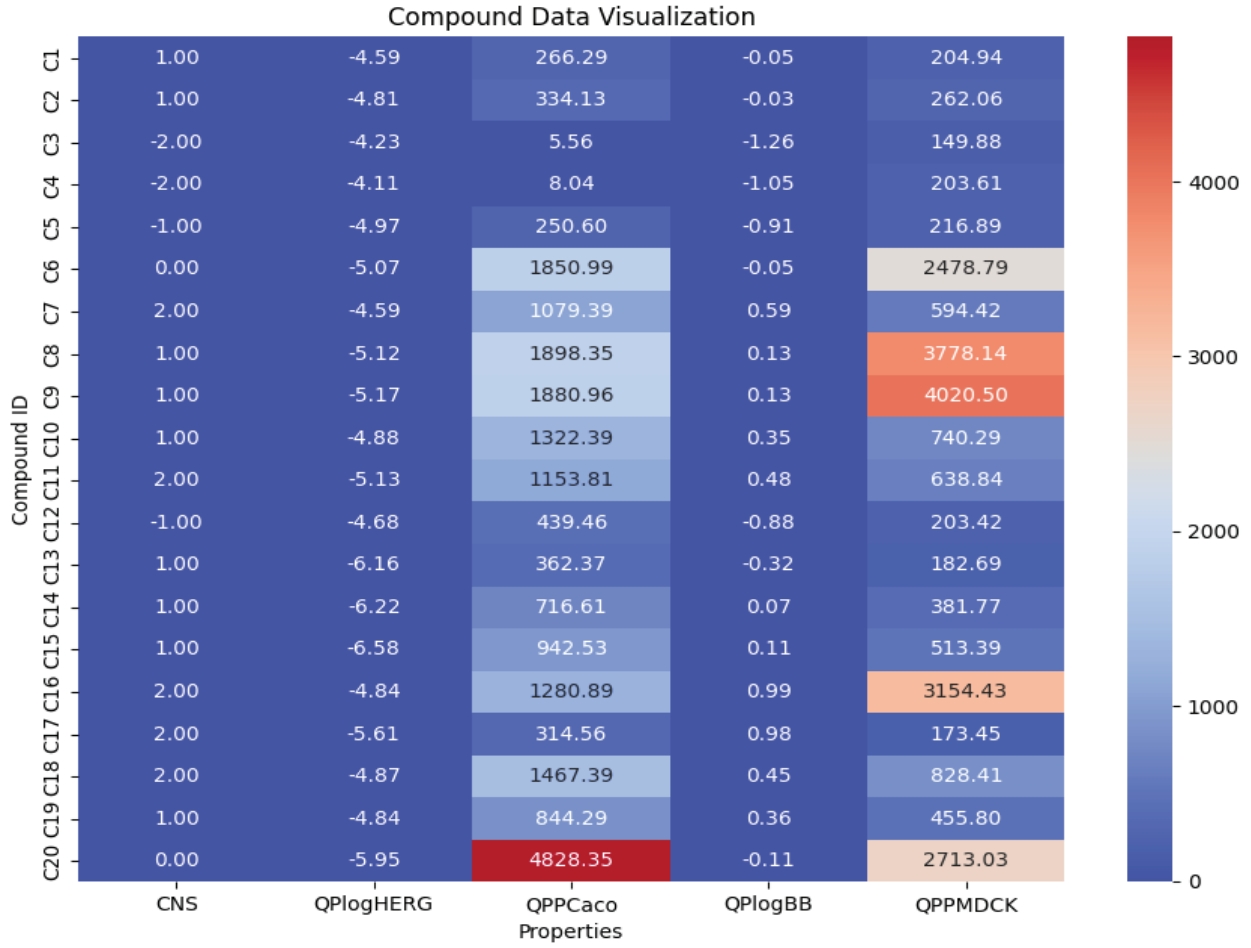

Figure 2. This table presents pharmacokinetic and toxicity parameters for various compounds, focusing on their effects within the central nervous system (CNS) and their pharmacological profiles. The table includes values for QPlogHERG (predicting drug-induced cardiotoxicity), QPPCaco (intestinal absorption potential), QPlogBB (blood-brain barrier permeability), and QPPMDCK (cell permeability in Madin-Darby Canine Kidney cells). These metrics are crucial for assessing the safety and efficacy of the compounds, providing a comprehensive overview of their pharmacokinetic behavior and potential biological impacts.

Hint:

1) QPlogHERG Predicted IC50 value for blockage of HERG K+ channels. Concern below –5.
2) QPPCaco Predicted apparent Caco-2 cell permeability in nm/sec. Caco-2 cells are a model for the gut-blood barrier. QikProp predictions are for non-active transport. <25 poor, >500 great.
3) QPlogBB Predicted brain/blood partition coefficient. Note: QikProp predictions are for orally delivered drugs so, for example, dopamine and serotonin are CNS negative because they are too polar to cross the blood-brain barrier –3.0 – 1.2.
4) QPPMDCK Predicted apparent MDCK cell permeability in nm/sec. MDCK cells are considered to be a good mimic for the blood brain barrier. QikProp predictions are for non-active transport. <25 poor, >500 great.

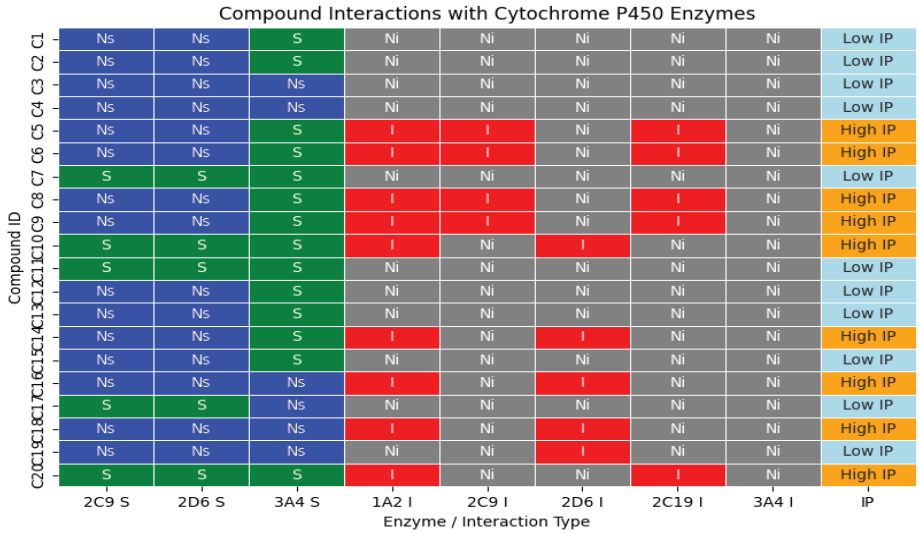

Figure 3. This table categorizes various compounds based on their interaction with cytochrome P450 enzymes, indicating whether each compound acts as a Substrate (S), Inhibitor (I), Non-Substrate (Ns), or Non-Inhibitor (Ni). Additionally, the table includes a measure of CYP Inhibitory Promiscuity (IP), which quantifies each compound's ability to inhibit multiple P450 enzymes. This data is essential for understanding the metabolic processing of these compounds and their potential to cause drug-drug interactions.

Table 2. Summary of Docking score and Molecular Mechanics, General Born surface area (MM/GBSA) for the twenty (20) Selected Novel Psychoactive Substances against opioid receptor. The table provides essential information for assessing the bond interaction and complex stability of the twenty (20) Selected Novel Psychoactive Substances with opioid receptor.

	Opioid Receptor (8EF5)	
Compounds	**Docking Score (kcal/mol)**	**MM/GBSA (kcal/mol)**
Modafinil	-7.19	-48.22
1-Pentyl-3-(1-naphthoyl)indole	-6.859	-30.37
Adrafinil	-6.763	-44.95
Salvinorin A	-6.739	-35.45
Flubromazolam	-6.233	-44.17
Methylenedioxypyrovalerone	-5.68	-33.47
Mitragynine	-5.635	-41.82
Etizolam	-5.347	-42.19
5-Methoxy-N,N-diisopropyltryptamine	-5.324	-29.36
7-Hydroxymitragynine	-5.223	-39.44
1-[3-(Trifluoromethyl)phenyl]piperazine	-5.217	-33.32
Deschloroketamine	-5.125	-32.42
Flualprazolam	-5.114	-39.5
Clonazolam	-5.073	-29.88
1-Propionyl-lysergic acid diethylamide	-5.034	-37.09
Dimethyltryptamine	-4.785	-17.08
Mephedrone	-4.712	-27.7
1-Acetyllysergic acid diethylamide	-4.613	-33.35
1-Benzylpiperazine	-4.121	-24.39
Carfentanil	-3.139	-46.76

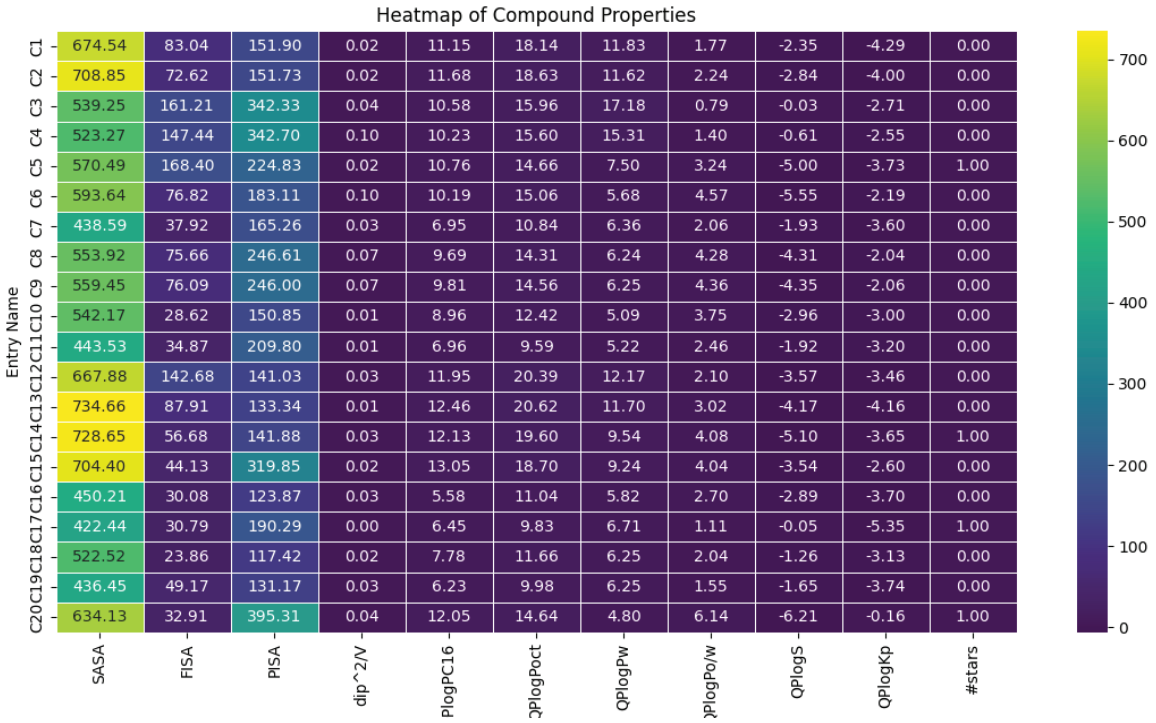

Figure 4. Comprehensive ADME Profile Heatmap of Twenty NPS This heatmap illustrates the complex physicochemical and pharmacokinetic properties of twenty novel psychoactive substances, ranging from C1 to C20.

#stars: Number of property or descriptor values that fall outside the 95% range of similar values for known drugs. Outlying descriptors and predicted properties are denoted with asterisks (*) in the out file. A large number of stars suggests that a molecule is less drug-like than molecules with few stars. The following properties and descriptors are included in the determination of #stars: MW, dipole, IP, EA, SASA, FOSA, FISA, PISA, WPSA, PSA, volume, #rotor, donorHB, accptHB, glob, QPpolrz, QPlogPC16, QPlogPoct, QPlogPw, QPlogPo/w, logS, QPLogKhsa, QPlogBB, #metabol. (0 - 5).

SASA Total solvent accessible surface area (SASA) in square angstroms using a probe with a 1.4 Å radius. 300.0 – 1000.0

FISA Hydrophilic component of the SASA (SASA on N, O, and H on heteroatoms). 7.0 – 330.0

PISA π (carbon and attached hydrogen) component of the SASA. 0.0 – 450.0

dip^2/V† Square of the dipole moment divided by the molecular volume. This is the key term in the Kirkwood-Onsager equation for the free energy of solvation of a dipole with volume V. 0.0 – 0.13

QPlogPC16 Predicted hexadecane/gas partition coefficient. 4.0 – 18.0

QPlogPoct‡ Predicted octanol/gas partition coefficient. 8.0 – 35.0

QPlogPw Predicted water/gas partition coefficient. 4.0 – 45.0

QPlogPo/w Predicted octanol/water partition coefficient. –2.0 – 6.5

QPlogS Predicted aqueous solubility, log S. S in mol dm–3 is the concentration of the solute in a saturated solution that is in equilibrium with the crystalline solid. –6.5 – 0.5

QPlogKp Predicted skin permeability, log Kp. –8.0 – –1.0

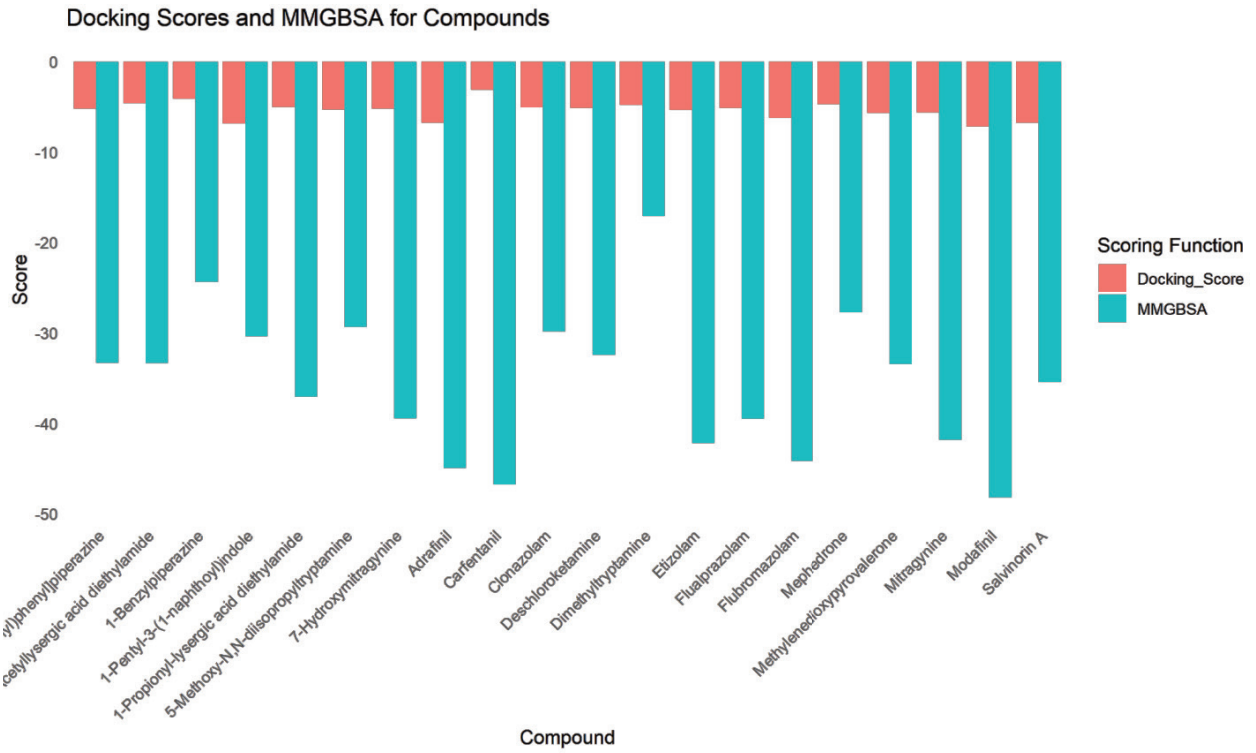

Figure 5. A bar chart illustration of the twenty (20) Selected Novel Psychoactive Substances against opioid receptors. The bond interactions (Docking Score) are indicated by the orange stick, and the bond energy interaction is indicated by the blue stick signifying MMGBSA and complex stability.

DISCUSSION

Table 1 summarizes the chemical properties and regulatory parameters of the twenty selected novel psychoactive substances (NPS) which are structurally displayed in Figure 1, and are framed from several perspectives, primarily focusing on the implications of these properties for pharmacokinetics, potential therapeutic applications, and regulatory scrutiny. The compounds listed in the table (Table 1) have a broad range of molecular weights, from relatively small molecules like Mephedrone (177.24 g/mol) to larger ones such as Salvinorin A (432.5 g/mol). The molecular weight of a drug can influence its absorption, distribution, metabolism, and excretion (ADME) properties. Generally, molecules with a higher molecular weight might have poorer absorption and could be more challenging to distribute across biological membranes unless they have specific structural properties that facilitate their transport (Olugbogi et al., 2022).

Lipinski's Rule of Five and Rule of Three Compliance

The compliance with Lipinski's Rule of Five (RO5) and Rule of Three (RO3) is a fundamental aspect to consider. Lipinski's RO5 predicts the drug-likeness of compounds based on their physical and chemical properties, including molecular weight, logP (octanol-water partition coefficient), and the number of hydrogen bond donors and acceptors. In Table 1, most compounds show a "0" under RO5, suggesting that they potentially comply

Table 3. Comprehensive Assessment of Acute Rodent Toxicity: A Comparative Analysis of LD50 Values Across Multiple Administration Routes for Compounds C1-C20. This indicates acute rodent toxicity, and specifies the method of analysis (LD50 values), and covers the range of compounds included (C1-C20).

Compound ID	Rat IP LD50 Log10 (mmol/kg)	Rat IV LD50 log10 (mmol/kg)	Rat Oral LD50 log10 (mmol/kg)	Rat SC LD50 log10 (mmol/kg)	Rat IP LD50 (mg/kg)	Rat IV LD50 (mg/kg)	Rat Oral LD50 (mg/kg)	Rat SC LD50 (mg/kg)	Acute Rodent Toxicity Classification of Chemicals by OECD Project	Rat IP LD50 Classification	Rat IV LD50 Classification	Rat Oral LD50 Classification	Rat SC LD50 Classification
C1	-0,331 out of AD	-1,033 in AD	0,063 in AD	-0,454 out of AD	170,500 out of AD	33,870 in AD	422,700 in AD	128,400 out of AD		Class 4 out of AD	Class 3 in AD	Class 4 in AD	Class 3 out of AD
C2	-0,376 out of AD	-1,071 in AD	0,169 in AD	-0,529 in AD	159,700 out of AD	32,260 in AD	559,900 in AD	112,300 in AD		Class 4 out of AD	Class 3 in AD	Class 4 in AD	Class 3 in AD
C3	0,081 in AD	-0,541 in AD	0,659 in AD	0,229 in AD	349,000 in AD	83,230 in AD	1321,000 in AD	489,900 in AD		Class 4 in AD	Class 4 in AD	Class 4 in AD	Class 4 in AD
C4	0,270 in AD	-0,146 in AD	1,003 in AD	0,356 in AD	509,600 in AD	195,300 in AD	2752,000 in AD	619,900 in AD		Class 5 in AD	Class 4 in AD	Class 5 in AD	Class 4 in AD
C5	0,531 in AD	-0,457 in AD	0,811 in AD	0,806 in AD	1202,000 in AD	123,600 in AD	2290,000 in AD	2263,000 in AD		Class 5 in AD	Class 4 in AD	Class 5 in AD	Class 5 in AD
C6	0,544 in AD	-0,551 in AD	0,754 in AD	1,072 in AD	1199,000 in AD	96,440 in AD	1945,000 in AD	4050,000 in AD		Class 5 in AD	Class 4 in AD	Class 4 in AD	Non Toxic in AD
C7	-0,020 in AD	-1,082 in AD	0,506 in AD	0,587 in AD	194,300 in AD	16,840 in AD	652,000 in AD	785,400 in AD		Class 4 in AD	Class 3 in AD	Class 4 in AD	Class 4 in AD
C8	0,567 in AD	-0,357 in AD	0,854 in AD	1,078 in AD	1207,000 in AD	143,500 in AD	2334,000 in AD	3912,000 in AD		Class 5 in AD	Class 4 in AD	Class 5 in AD	Non Toxic in AD
C9	0,528 in AD	-0,425 in AD	0,740 in AD	1,042 in AD	1253,000 in AD	139,600 in AD	2041,000 in AD	4087,000 in AD		Non Toxic in AD	Class 4 in AD	Class 5 in AD	Non Toxic in AD
C10	-0,154 in AD	-0,700 in AD	0,664 in AD	0,287 in AD	192,300 in AD	54,800 in AD	1265,000 in AD	531,000 in AD		Class 4 in AD	Class 4 in AD	Class 4 in AD	Class 4 in AD
C11	-0,066 in AD	-0,769 in AD	0,680 in AD	-0,172 in AD	161,900 in AD	32,040 in AD	902,000 in AD	126,800 in AD		Class 4 in AD	Class 3 in AD	Class 4 in AD	Class 3 in AD
C12	-0,433 in AD	-2,004 in AD	-0,019 in AD	-0,612 in AD	159,600 in AD	4,282 in AD	414,400 in AD	105,700 in AD		Class 4 in AD	Class 2 in AD	Class 4 in AD	Class 3 in AD
C13	-0,501 in AD	-1,431 in AD	0,065 in AD	-1,156 out of AD	130,900 in AD	15,380 in AD	480,900 in AD	28,950 out of AD		Class 4 in AD	Class 3 in AD	Class 4 in AD	Class 3 out of AD
C14	-0,369 in AD	-1,465 in AD	0,019 in AD	-0,210 in AD	170,500 in AD	13,650 in AD	416,600 in AD	245,600 in AD		Class 4 in AD	Class 3 in AD	Class 4 in AD	Class 4 in AD
C15	-0,252 out of AD	-1,855 in AD	0,119 out of AD	-1,012 out of AD	220,800 out of AD	5,507 in AD	518,800 out of AD	38,400 out of AD		Class 4 out of AD	Class 2 in AD	Class 4 out of AD	Class 3 out of AD
C16	0,013 in AD	-0,740 in AD	0,264 in AD	0,222 in AD	237,300 in AD	41,870 in AD	422,700 in AD	383,800 in AD		Class 4 in AD	Class 4 in AD	Class 4 in AD	Class 4 in AD
C17	-0,244 in AD	-0,783 in AD	0,469 in AD	0,127 in AD	100,500 in AD	29,070 in AD	518,500 in AD	236,300 in AD		Class 4 in AD	Class 3 in AD	Class 4 in AD	Class 4 in AD
C18	-0,195 in AD	-1,283 in AD	0,452 in AD	0,102 in AD	175,700 in AD	14,330 in AD	779,300 in AD	348,100 in AD		Class 4 in AD	Class 3 in AD	Class 4 in AD	Class 4 in AD
C19	-0,132 in AD	-0,873 in AD	0,533 in AD	0,292 in AD	130,800 in AD	23,740 in AD	604,100 in AD	346,900 in AD		Class 4 in AD	Class 3 in AD	Class 4 in AD	Class 4 in AD
C20	0,363 in AD	-1,086 in AD	0,611 in AD	0,402 out of AD	788,200 in AD	28,020 in AD	1393,000 in AD	862,500 out of AD		Class 5 in AD	Class 3 in AD	Class 4 in AD	Class 4 out of AD

with the parameters that make them likely to have acceptable absorption and permeability. However, compounds like 1-Pentyl-3-(1-naphthoyl) indole, which deviate from RO5, might face challenges in these areas.

Rule of Three (RO3) is generally applied for assessing the suitability of compounds for permeability and solubility in the context of designing small molecule "fragment" compounds in drug discovery. Notable in the table (Table 1) is that only Adrafinil and Modafinil show an RO3 compliance, indicating these compounds might have advantages in molecular fragmentation approaches aimed at developing new pharmaceutical agents.

Regulatory Implications

From a regulatory perspective, the data provided in Table 1 offers a starting point for evaluating the safety and efficacy of these substances as potential therapeutic agents. Regulatory bodies often require detailed pharmacokinetic profiles and compliance with drug-likeness rules as part of the drug approval process. Substances like Carfentanil, despite its potential therapeutic applications, pose significant safety risks due to their potency, impacting their regulatory approval and control measures. Table 1 serves as an essential tool for researchers and regulatory agencies alike in assessing the potential of these novel psychoactive substances.

The data visualization provided in Figure 2 offers a detailed examination of pharmacokinetic and toxicity parameters for various compounds, emphasizing the interplay between their pharmacological properties and potential toxic effects, particularly in terms of cardiotoxicity, absorption, brain accessibility, and cell permeability.

Cardiotoxicity and Blood-Brain Barrier Permeability

The parameter QPlogHERG is pivotal for assessing the risk of drug-induced cardiotoxicity, specifically through the blockage of HERG K+ channels, a critical concern for drug safety. Negative values closer to or below -5 suggest a lower risk of cardiotoxicity, making these drugs safer for human use in terms of cardiac effects. For instance, compounds with a QPlogHERG value significantly lower than -5 could be considered as having a lower potential for causing dangerous arrhythmias, an essential factor in the early stages of drug development (Witchel, 2018). The QPlogBB, which measures blood-brain barrier permeability, is equally crucial. Drugs with a QPlogBB between -3.0 and 1.2 are deemed capable of crossing the blood-brain barrier, which is vital for CNS-targeted therapies but could pose risks if unintended CNS exposure occurs, leading to potential neurotoxicity (Pajouhesh & Lenz, 2005). For example, compound C1 has a QPlogHERG of -4.59, suggesting a moderate risk of cardiotoxicity due to its proximity to the threshold of -5, beyond which the risk significantly increases. In contrast, compound C10, with a QPlogHERG value of -4.88, might be considered safer from a cardiotoxicity standpoint. It is essential to note that drugs with higher QPlogHERG values (closer to zero) require careful evaluation and potential modification to reduce the risk of adverse cardiac events, such as arrhythmias, which can be a common concern in drug development (Poluzzi et al., 2012).

As earlier mentioned, the QPlogBB values provide insights into the potential CNS impact of these compounds. Compound C9, with a QPlogBB of 0.13, is likely to cross the blood-brain barrier, suggesting

suitability for CNS-targeted therapies but requiring careful assessment of potential CNS-related side effects. In contrast, compound C3 with a QPlogBB of -1.26 may have limited ability to affect the CNS, making it potentially safer regarding neurotoxicity but less useful for treating CNS disorders. These values are essential for determining a compound's therapeutic application and its safety profile, especially for drugs intended to act within the central nervous system (Summerfield & Read, 2015).

Intestinal and Cellular Permeability

QPPCaco and QPPMDCK are indicators of a compound's permeability through intestinal and cellular barriers, respectively. QPPCaco values reflect how well a compound can be absorbed in the gastrointestinal tract, with higher values indicating better absorption potential. A QPPCaco value greater than 500 nm/sec is considered excellent, suggesting that the compound has effective passive absorption properties, which is beneficial for oral drugs (Artursson & Karlsson, 1991). Conversely, QPPMDCK provides insight into how well a compound permeates through cellular membranes, specifically mimicking the blood-brain barrier. High values (>500 nm/sec) suggest good permeability, which is desirable for drugs intended to treat central nervous system disorders but might raise concerns about systemic exposure and associated toxic effects. Focusing on QPPCaco and QPPMDCK, the data varies significantly across compounds, indicating their differing abilities to permeate biological membranes. Compound C7, with a QPPCaco of 1079.39 nm/sec, shows excellent intestinal absorption potential, which is ideal for oral administration. However, its QPPMDCK value of 594.42 nm/sec, while high, suggests a significant degree of permeability through cellular barriers, including the blood-brain barrier. This dual high permeability can be advantageous for targeted CNS drugs but might pose risks of systemic exposure. Conversely, compound C6's lower QPPCaco of 250.60 nm/sec indicates poorer absorption, which might limit its oral bioavailability but reduce the risk of unwanted systemic effects (Bergström et al., 2003).

The pharmacokinetic behaviors elucidated by these parameters are crucial for predicting the safety profiles of these compounds. Compounds like those with high QPPMDCK values might show excellent potential for CNS applications but require careful evaluation to mitigate risks like neurotoxicity or off-target effects elsewhere in the body. Continued research and development guided by standard metrics are imperative to refine drug candidates and advance them through the pipeline, ensuring they meet safety standards while addressing therapeutic needs (Di et al., 2003).

Cytochrome P450 Interaction and Drug Metabolism

The table in Figure 3 provides a detailed analysis of the interactions between NPS compounds and cytochrome P450 enzymes, categorizing each compound based on its role as a Substrate (S), Inhibitor (I), Non-Substrate (Ns), or Non-Inhibitor (Ni). Additionally, the figure assesses CYP Inhibitory Promiscuity (IP), which is pivotal in understanding the metabolic processing and potential for drug-drug interactions among these compounds. These interactions can significantly influence the toxicity profile of a compound, with consequences that can affect both efficacy and safety in clinical settings.

Cytochrome P450 enzymes play a critical role in drug metabolism, predominantly in the liver, where they modify drugs and other xenobiotics to facilitate their elimination from the body. When a compound acts as a substrate (S) for a specific P450 enzyme, it is directly metabolized by this enzyme, which can influence both its pharmacokinetic properties and potential toxicity. For instance, if a drug is a substrate for a highly expressed P450 enzyme like CYP3A4, it may be rapidly metabolized, potentially requiring higher doses to achieve therapeutic effects, or it could produce metabolites that might be toxic (Zanger & Schwab, 2013). Conversely, non-substrates (Ns) are not directly affected by these enzymes, which might lead to longer half-lives or less predictable pharmacokinetics.

CYP Inhibition and Drug-Drug Interactions

Inhibition of cytochrome P450 enzymes is a critical concern, particularly for compounds classified as inhibitors (I) in the table. Inhibitors can cause significant drug-drug interactions by blocking the metabolism of other drugs that are substrates of the same enzyme, potentially leading to increased plasma levels of these drugs and heightened risk of adverse effects or toxicity. For example, a high CYP inhibitory promiscuity (High IP) indicates that a compound can inhibit multiple P450 enzymes, increasing the risk of widespread drug-drug interactions. Such interactions are particularly dangerous in polypharmacy scenarios, where patients are taking multiple medications simultaneously, which is common in elderly populations and those with chronic diseases (Rendic & Di Carlo, 2017).

For compounds like those in rows 6 and 10 of Figure 3, marked as Substrates and Inhibitors with High IP, there's a significant risk of both being affected by and affecting other drugs through metabolic pathways. These compounds can inhibit the enzymes responsible for their own metabolism, potentially leading to increased plasma levels and prolonged effects, which could enhance therapeutic efficacy but also risk toxicity. For example, if compound C6 is a substrate and also an inhibitor for the same enzyme (like CYP3A4), it may slow down its own metabolism, increasing the risk of accumulation and potential toxicity. Additionally, if taken with other drugs metabolized by CYP3A4, it could inhibit their breakdown, leading to adverse drug reactions.

Compounds in rows 18 and 20, identified only as Inhibitors with High IP, pose a risk primarily through their interaction with other drugs rather than direct effects. Such compounds can significantly interfere with the metabolism of other drugs that are substrates for the same enzymes, potentially leading to severe toxicities if those drugs have narrow therapeutic windows. In clinical settings, if a patient is prescribed any of these compounds, clinicians must review all other medications the patient is taking to adjust dosages and prevent toxic buildup.

Compounds as Substrates Only (Low IP)

For compounds like those in rows 1, 2, and 3, identified as Substrates but with Low IP, the primary concern is their own metabolism which might be rapid and extensive, leading to a need for higher dosing to maintain therapeutic levels. These compounds are less likely to interfere significantly with the metabolism of other drugs, which is advantageous in patients

receiving multiple therapies. These compounds may be safer options in terms of drug-drug interactions but may require careful pharmacokinetic profiling to ensure effective dosing.

Compounds with No Interaction (Low IP)

Compounds in rows 5 and 7, marked as Non-substrates and Non-inhibitors with Low IP, are unlikely to interact with the cytochrome P450 system, suggesting minimal risk of drug-drug interactions. These compounds could be considered safer from a metabolic interaction perspective, but their efficacy and metabolism might rely on other pathways or enzymes. Their non-involvement with CYP450 enzymes could imply alternative metabolic routes or excretion processes, potentially involving conjugation mechanisms or renal and biliary excretion.

Clinical Implications and Management of Risks

The identification of CYP interactions is crucial for the clinical management of drugs, particularly in determining appropriate dosing regimens and avoiding potential adverse interactions. For drugs with high inhibitory promiscuity, careful monitoring and possibly adjusting dosages of concomitant medications that are metabolized by the same enzymes are necessary. Clinicians must be aware of these interactions and manage patient medication regimens accordingly to mitigate the risk of toxicity. Furthermore, the development of new drugs must consider these interactions to optimize therapeutic efficacy and minimize harm. Regulatory bodies like the FDA often require detailed CYP interaction profiles before approving new drugs to ensure that they can be used safely across diverse patient populations (FDA, 2020).

Docking Score and MM/GBSA Analysis

Table 2 and Figure 5 provides insightful data on the docking scores and Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) calculations for twenty NPS when bound to the opioid receptor. These scores are fundamental for understanding how these compounds might interact with the opioid receptor, affecting their potential efficacy and safety profiles as therapeutic agents or drugs of abuse.

The docking scores, expressed in kcal/mol, give an initial estimate of the binding affinity between each compound and the opioid receptor (Shoichet & Kobilka, 2012). A more negative score indicates a stronger predicted affinity, suggesting that the compound might bind more effectively to the receptor. For instance, Modafinil shows the highest affinity with a docking score of -7.190 kcal/mol, followed closely by 1-Pentyl-3-(1-naphthoyl) indole and Adrafinil (-6.859 kcal/mol and -6.763 kcal/mol, respectively). These high scores suggest that these compounds could have significant interactions with the opioid receptor, potentially influencing analgesic effects or addictive properties. The MM/GBSA values further refine these predictions by considering more detailed aspects of the binding interaction, such as solvation effects and entropic contributions. Modafinil again shows a very strong interaction with an MM/GBSA value of -48.22 kcal/mol, indicative of a stable and potentially highly effective binding (Kollman et al., 2000).

Implications of Binding Affinity

The binding affinities indicated by both docking and MM/GBSA scores can have significant implications for the pharmacological and toxicological profiles of these compounds. For example, compounds

with higher binding affinities to opioid receptors, such as Modafinil and Adrafinil, might exhibit stronger central nervous system effects, which could translate into higher efficacy in therapeutic contexts or greater risks in terms of abuse and dependency. Furthermore, the detailed MM/GBSA scores can help predict the likelihood of off-target effects, which are crucial for the safety profiles of these substances (Kollman et al., 2000). For instance, a compound with a strong and selective binding profile (e.g., Carfentanil with an MM/GBSA of -46.76 kcal/mol) might be an effective pain reliever but also poses high risks for overdose and addiction due to its potent opioid receptor interaction (Proschak et al., 2019).

Clinical and Regulatory Considerations

The data from Table 2 must be carefully considered in clinical and regulatory frameworks. For clinicians, understanding the binding affinities of these NPS can aid in predicting therapeutic potentials and side effects, essential for managing treatment plans, especially in pain management or psychiatric settings. From a regulatory perspective, compounds with high docking and MM/GBSA scores may require stricter controls to prevent misuse and manage therapeutic use (Kollman et al., 2000). Moreover, these computational predictions need to be complemented by clinical trials and pharmacokinetic studies to fully understand the implications of these interactions in human populations. As such, the computational models serve as an initial step in the drug development and approval process, guiding more detailed experimental investigations. The analysis of docking and MM/GBSA scores provides a valuable tool in the early stages of drug discovery and development for novel psychoactive substances. It allows researchers and regulators to gauge the potential effects of these compounds on the opioid receptor, aiding in the design of safer and more effective therapeutic agents while also informing policy regarding the control and monitoring of NPS usage (Shoichet & Kobilka, 2012).

Impact of Physicochemical Properties on Drug Behavior

The heatmap presented in Figure 4 offers a detailed overview of the physicochemical and pharmacokinetic properties of twenty NPS, providing essential insights into their potential therapeutic and toxicological profiles. By analyzing properties such as solvent accessible surface area (SASA), hydrophilic surface area (FISA), hydrophobic surface area (PISA), and various partition coefficients, we can infer the likelihood of these compounds to exhibit bioavailability, membrane permeability, and potential toxicity.

The physicochemical properties displayed in the heatmap such as SASA, FISA, and PISA play critical roles in determining a compound's interaction with biological systems. SASA, which represents the total surface area accessible to a solvent, can influence the solubility and overall bioavailability of a drug (Veber et al., 2002; Artursson & Karlsson, 1991). Compounds like C3 and C4, with relatively high SASA values, may exhibit better solubility in physiological environments, potentially enhancing their absorption rates. However, a high FISA as seen in C5 and C6 could imply increased solubility but reduced permeability across hydrophobic biological membranes like the blood-brain barrier, affecting their distribution and efficacy in targeting central nervous system disorders (Artursson & Karlsson, 1991).

Partition Coefficients and Toxicity Potential

Partition coefficients such as QPlogPC16, QPlogPoct, QPlogPw, and QPlogPo/w provide insights into the lipophilicity and hydrophobicity of these compounds, which are crucial for predicting their behavior in biological systems. A high lipophilicity, indicated by higher values in QPlogPoct and QPlogPC16, often correlates with better membrane permeability, which can enhance a drug's effectiveness in reaching its target tissues. For instance, compounds C1 and C2 show higher values in these metrics, suggesting strong membrane crossing potential which could lead to effective drug action but also increase the risk of accumulating in non-target tissues, potentially leading to toxicity (Lipinski, 2004). Furthermore, extreme values in QPlogPw and QPlogPo/w, as observed in C9 and C10, might indicate issues with aqueous solubility and could lead to poor absorption or rapid elimination, affecting the drug's therapeutic window (Veber et al., 2002; Artursson & Karlsson, 1991).

Predictive Toxicology and Risk Assessment

The #stars parameter, which indicates the number of physicochemical properties of a compound that fall outside the typical range for known drugs, is a critical metric for assessing a compound's "drug-likeness" and potential toxicity. Compounds such as C19 and C20, with low #stars values, tend to align more closely with the property profiles of known drugs, suggesting a lower risk of adverse effects. In contrast, compounds with higher #stars values might possess atypical properties that could result in unpredictable pharmacokinetics and potential toxicity, necessitating further investigation and careful monitoring in therapeutic use(Veber et al., 2002; Artursson & Karlsson, 1991). These analyses highlight the complex relationship between a compound's physicochemical properties and its potential impact on human health. These properties allow researchers and healthcare providers to better predict which compounds might be safely developed into therapeutic agents and which might pose significant risks, guiding both clinical and regulatory decision-making processes.

Interpretation of LD50 Values and Toxicity Profiles

Table 3 presents a detailed overview of the acute rodent toxicity profiles for the twenty novel psychoactive substances, ranging from C1 to C20. By examining the LD50 values across multiple administration routes; intraperitoneal (IP), intravenous (IV), oral, and subcutaneous (SC), and their corresponding classifications under the OECD guidelines, we can infer the relative toxicity and safety margins of these compounds. This table not only highlights the dose-dependent lethal outcomes but also classifies these substances into different toxicity categories, providing a comprehensive understanding of their potential hazards.

The LD50 values, expressed in both logarithmic and milligram per kilogram terms, serve as a quantitative measure of a substance's toxicity, indicating the dose required to cause death in 50% of the test population. Substances like C1 and C2, which exhibit lower LD50 values across multiple routes, especially in their log10 (mmol/kg) formats, indicate higher toxicity (Gupta, 2011). For example, C1 has an intraperitoneal LD50 of -0.331 log10(mmol/kg) and an oral LD50

of 0.063 log10(mmol/kg), positioning it as moderately toxic but with significant variations depending on the route of administration. These values are crucial for determining safe handling practices and exposure limits, especially in research and clinical settings where dosages near the lower LD50 values can pose serious health risks (Olson et al., 2000).

Toxicity Variations by Administration Route

The data reflects significant variation in toxicity depending on the administration route. Compounds like C3 and C4, which show higher oral LD50 values, suggest lower toxicity when administered by this route, potentially allowing for safer oral administration in therapeutic contexts. In contrast, the same compounds may exhibit higher toxicity intravenously or intraperitoneally, as indicated by their lower LD50 values in these routes (Gupta, 2011). This underscores the importance of route-specific toxicity assessments in drug development, as the route of administration can significantly influence a drug's safety profile. For instance, C6 and C8, both classified as non-toxic subcutaneously, may still present risks when administered through other routes, highlighting the complex interplay between compound properties, administration routes, and resultant toxicological effects (Olson et al., 2000).

Regulatory and Clinical Implications

The acute rodent toxicity data, particularly the classifications provided (e.g., Class 4, Class 3), are instrumental for regulatory and safety evaluations. Regulatory bodies utilize such data to set guidelines and restrictions on the use of these substances (Gupta, 2011). Clinicians and pharmacologists can use this data to anticipate adverse effects and adjust dosages accordingly. Furthermore, compounds with consistent high toxicity across all routes, such as C19 and C20, might require additional risk management strategies or could be candidates for restricted use, thereby ensuring patient and user safety in clinical and non-clinical environments. In summary, the comprehensive assessment provided in Table 3 is essential for understanding the acute toxicity potential of novel psychoactive substances. This data aids in the development of safe usage guidelines, helps in the design of further toxicological studies, and supports regulatory and clinical decision-making processes (Olson et al., 2000).

CONCLUSION

The comprehensive assessment of novel psychoactive substances (NPS) presented in this study underscores the significant toxicity risks these compounds pose to patient health, particularly in terms of neuronal impact and addiction potential. The diverse physicochemical properties, interaction with critical neurotransmitter systems, and variable toxicity profiles highlighted by the LD50 values and pharmacokinetic parameters indicate that these substances can profoundly affect neuronal function, potentially leading to serious neurotoxic effects and psychological disturbances. Furthermore, the addictive nature of these compounds, driven by their mechanisms of action at key receptor sites such as opioid and serotonin receptors, presents a substantial risk for abuse. These findings emphasize the urgent need for rigorous regulatory oversight, informed clinical management, and

comprehensive public health strategies to mitigate the risks associated with the use and abuse of these potent substances, ensuring safety and minimizing the potential for addiction and long-term harm.

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