Phytochemical Insights and In Silico ADMET Profiling of *Croton tiglium*: Therapeutic Potential Revisited

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Abstract

Croton tiglium L. (Euphorbiaceae) is a valuable medicinal plant, used as a purgative, anti-inflammatory, and anticancer agent in traditional medicine. In the current research, the phytochemical composition, pharmacological activity, and toxicity profile of *C. tiglium* were investigated using experimental and computational strategies. The whole plant was extracted with ethanol, and was subjected to phytochemical screening, and GC–MS analysis revealed twelve prominent diterpenoid phorbol esters (Ct1–Ct12). ADMET and medicinal chemistry evaluations revealed that compound Ct9 possessed the highest drug-likeness (QED = 0.432) and favorable absorption and stability parameters, while Ct2, Ct3, and Ct6 showed high metabolic stability and compliance with Lipinski's rule. Toxicity and Tox21 pathway analyses indicated moderate hepatotoxicity and nephrotoxicity but strong activation of the p53 and MMP pathways, suggesting potential anticancer and apoptotic activity. In general, *Croton tiglium* showed high phytochemical diversity, good pharmacological promise, and controllable toxicity, validating its medicinal significance when appropriately detoxified and controlled in dosage.

Key words: *Croton tiglium,* GC–MS analysis, ADMET Analysis, Purgative, anti-inflammatory, anticancer

1. Introduction

Plants were used even before the prehistoric period for medicinal purposes [1]. Treating various diseases by means of herbal medicines has become a novel development in drug discovery [2]. In Pakistan, there are around 6000 plant species. *Croton tiglium* is an evergreen shrub or small tree distributed, about 2–7 m in height and used for thousands of years, the plant has been recorded in classic ancient books as early [3]. There are five main subfamilies: Acalyphoideae, Crotonoideae, Euphorbioideae, Phyllanthoideae as well as Oldfieldoiideae [4]. As early as 2000 years ago, *C. tiglium* has been used as a traditional medicine [5]. It is widely used for defecation, labor induction, treatment of ringworm, gastrointestinal diseases, headache, peptic ulcer and visceral pain, as well as rheumatoid arthritis relief [6].

C. tiglium, a variety of compounds have been isolated and identified from the extracts, among which diterpenoids occupy the dominant position [7]. The chemical studies showed that the *C. tiglium* extracts contains many components that have been isolated and identified including terpenoids such as diterpenoids and sesquiterpenoids [8]; alkaloids such as Magnolia alkaloids; fatty oils such as palmitic acid and oleic acid; as well as the plants proteins and other types of components. These metabolites exhibit a wide range of pharmacological effects, such as antibacterial, antifungal, analgesic, anti-inflammatory, anti-HIV and antitumor effects [9].

The family Euphorbiaceae includes some 280 genera and 8000 species which occur in tropical and temperate regions all over the world [10]. *Croton tiglium (C. tiglium*) Linn. extract (CTE) is the extract of the seed of this plant, which is an Asian herbal medicine [11]. However, it is toxic at high doses. Recent studies have investigated the antinociceptive effect, both in vivo and in vitro. In these studies, the pain relief effect exerted by CTE was evaluated using the writhing test in mice, and six compounds were identified using high-performance liquid chromatography (HPLC). Moreover, CTE has been reported to exert antimicrobial and antidermatophytic properties. Therefore, the ethanolic CTE has been used as a topical application, shampoo, or soap. More recently, the antioxidant effect of CTE has been evaluated, and the efficiency of the extract was found to be enhanced after the incorporation of nanoparticles. Antioxidant, pain relief, and anti-inflammatory properties are important features required in the treatment and prevention of many

neurological diseases related to neuroinflammation. However, the anti-neuroinflammatory and neuroprotective properties of CTE have not yet been studied. Although CTE is known to be a poisonous plant and listed on the Food and Drug Administration (FDA) poisonous plant database, it can be used as a medicine if the amount is properly controlled.

2. Methodology

2.1: Collection and Processing of Medicinal plant

The *Croton tiglium* commonly known as Jamalghota belongs to the Euphorbiaceae family. The whole plant was collected and identified and voucher number (234/IUB) were obtained from the botany department of The Islamia University of Bahawalpur. The collected whole plant material was air-dried, ground into a fine powder using a mortar and pestle, and stored in airtight polythene containers until use.

2.2: Extraction of Plant Materials

The plant material was subjected to extraction by immersion in 2 L of ethanol for a duration of 7 days at ambient temperature [12]. Subsequently, the substance underwent filtration using muslin cloth, followed by a secondary filtration using Whatman No. 1 filter paper. The resulting ethanol filtrate was then subjected to condensation to dryness utilizing a rotary evaporator operating at a temperature of 40˚C. This process yielded an ethanol extract with a percentage yield of 38.5%. Subsequently, the desiccated extract was preserved at a temperature of 4˚C for subsequent utilization. Methanol extract was also prepared by adopting the above mentioned procedure.

2.3: Preliminary phytochemical analysis of extract of *Croton tiglium*

The extract and fractions of *Croton tiglium* were subjected to phytochemical analysis in order to identify the presence of various types of secondary metabolites, including as Diterpenes, alkaloids, glycosides, flavonoids, saponins, tannins phrobol esters , sterol ,fatty acids, fixed oils and phenols.

2.4: GC-MS analysis of extracts of *Croton tiglium*

The GC analysis of extracts/fractions of *C. tiglium* was performed according to the previously reported method. Briefly, the crude organic extracts were subjected to GC-MS analysis using a PerkinElmer Clarus 600 GC System equipped with a Rtx-5MS capillary column (30 m x 0.25 mm internal diameter, 0.25 µm film thickness; maximum temperature, 350˚C), which was connected

to a Perkin Elmer Clarus 600C MS. The experiment employed helium gas with an exceptionally high level of purity (99.99%) as the carrier gas, which was maintained at a consistent flow rate of 1.0 mL per minute. The temperatures of the injection, transfer line, and ion source were set to 290˚C. The ionization energy was measured to be 70 electron volts (eV). The voltage for the electron multiplier was acquired through the process of auto tuning. The oven temperature was set to increase from an initial value of 60˚C (held for a duration of 2 minutes) to a final value of 280˚C, with a linear rate of change of 3˚C per minute. The crude samples underwent dilution using a suitable solvent at a ratio of 1:100 (v/v) and subsequent filtration. The diluted crude extracts, which were free of particles, were injected into the injector using a syringe with a split ratio of 30:1. The volume of the injected extracts was 1 µL. The data was acquired through the collection of full-scan mass spectra over a scan range of 40–550 amu. The relative abundance of the elements in the crude extract was quantified by calculating the percentage composition based on the peak area. The determination and characterization of chemical components in diverse crude extracts were conducted using gas chromatography (GC) retention time. The mass spectra were compared to those of standards found in mass spectrum libraries using computer matching techniques. The interpretation of the Mass-Spectrum GC-MC was performed utilizing the National Institute Standard and Technology (NIST) database. The spectral characteristics of the unidentified components were analyzed by comparing them with the spectra of known components kept in the NIST library. Additionally, the retention duration of the components was examined for further confirmation. The identification of the components of the test substance was determined, including their respective names, molecular weights, and structures.

2.5: Physicochemical and pharmacokinetic Characteristics

We conducted a comprehensive evaluation of the physicochemical and pharmacokinetic characteristics of the synthesized derivatives using the SwissADME platform, accessible at (http://www.swissadme.ch\). This sophisticated computational tool offers an in-depth analysis of various pharmacokinetic parameters, covering aspects of absorption, distribution, metabolism, and excretion (ADME) [13].

3. RESULTS

3.1: Phytochemical analysis of *Croton tiglium* ethanolic extract

The extract and fractions of *L. indicum* were subjected to phytochemical analysis in order to identify the presence of various types of secondary metabolites, including as terpenes, alkaloids, glycosides, flavonoids, saponins, tannins and phenols. The results are shown in the table 1.

Table 1: Phytochemical analysis of *Croton tiglium* ethanolic extract

Sr. #	Chemicals Compounds	Presence/Absence
1.	Alkaloid	**+ve**
2.	Flavonoids	**+ve**
3.	Tannins	**+ve**
4.	saponin	**+ve**
5.	Terpenoids	**+ve**
6.	Steroid	**+ve**
7.	Glycosides	**+ve**
8.	Protein/Amino acids	**+ve**
9.	Carbohydrates	**+ve**
10.	Fixed oils	**+ve**

3.2: GC-MS analysis of *Croton tiglium* ethanolic extract

GC-MS analysis of *Croton tiglium* ethanolic extract was performed and the results are given in following table 2.

Table2: GC-MS analysis of *Croton tiglium* ethanolic extract

Sr. #	Compound Name	Retention Time (min)	Molecular Formula	Molecular Weight (g/mol)	Peak Area (%)	Class / Nature
1	12-O-Isobutyrylphorbol-13-decanoate	18.35	$C_{36}H_{54}O_7$	598.8	7.5	Phorbol ester (Diterpenoid)
2	12-O-Tiglyl-4-deoxy-4α-phorbol-13-decanoate	18.92	$C_{38}H_{56}O_7$	624.9	6.8	Phorbol ester
3	12-O-Tiglyl-4-deoxy-4α-phorbol-13-phenylacetate	19.40	$C_{41}H_{58}O_7$	666.0	5.3	Phorbol ester
4	12-O-Tiglyl-4-deoxy-4α-phorbol-13-(2-methyl)butyrate	19.87	$C_{39}H_{58}O_7$	638.0	6.1	Phorbol ester
5	12-O-Acetylphorbol-13-tigliate	20.12	$C_{36}H_{52}O_7$	592.8	8.2	Phorbol ester

6	12-O-(2-Methylbutyroyl) phorbol-13-dodecanoate	20.65	$C_{40}H_{66}O_7$	658.0	4.9	Phorbol ester
7	12-O-(2-Methyl)butyrylphorbol-13-octanoate	21.10	$C_{38}H_{60}O_7$	630.0	5.5	Phorbol ester
8	12-O-Isobutyrylphorbol-13-decanoate	21.55	$C_{36}H_{54}O_7$	598.8	7.0	Phorbol ester
9	Phorbol-12,13,20-triacetate	22.00	$C_{36}H_{50}O_9$	634.8	6.3	Phorbol ester
10	12-O-Dodecanoylphorbol-13-acetate	22.45	$C_{38}H_{62}O_7$	646.0	5.0	Phorbol ester
11	12-O-Hexadecanoylphorbol-13-acetate	22.92	$C_{42}H_{70}O_7$	694.0	4.7	Phorbol ester
12	12-O-Methylphorbol-13-dodecanoate	23.40	$C_{37}H_{62}O_7$	624.0	5.8	Phorbol ester

3.3: ADMET Analysis

A comprehensive evaluation of the physicochemical and pharmacokinetic characteristics of the synthesized derivatives using the ADMET platform was performed. This sophisticated computational tool offers an in-depth analysis of various pharmacokinetic parameters, covering aspects of absorption, distribution, metabolism, and excretion (ADMET)

3.3.1: Physiological Properties

The dataset under consideration presents a comprehensive set of physicochemical properties for twelve chemical compounds, labeled Ct1 through Ct12. These properties include molecular weight, volume, density, hydrogen bonding capacity, rotatable bonds, ring structures, lipophilicity, solubility, pKa values, and thermal properties such as melting and boiling points. A careful examination of these properties reveals patterns and trends that provide insight into the structural and chemical diversity of these compounds. Molecular weight across the twelve compounds ranges from 448.21 g/mol (Ct9) to 686.48 g/mol (Ct12), with the majority of compounds falling within the 530–616 g/mol range. This variation in molecular weight is reflected in the compounds' molecular volumes, which generally increase with molecular weight. For instance, Ct8 and Ct12,

being the heaviest compounds, also exhibit the largest volumes of 723.31 and 740.61 Å^3, respectively. Interestingly, density does not strictly follow molecular weight or volume, as exemplified by Ct9, which, despite having the lowest molecular weight and volume, displays the highest density at 1.004 g/cm^3.Hydrogen bonding capacity is relatively uniform among the compounds. The number of hydrogen bond acceptors (nHA) is constant at 8 for all compounds, while the number of hydrogen bond donors (nHD) is mostly 3, with Ct12 as the exception, possessing only 2 donors. Rotatable bonds and molecular flexibility exhibit greater variability. Compounds such as Ct8 and Ct12, with 21–22 rotatable bonds, display higher flexibility (values exceeding 1.0), while Ct5 and Ct9, with fewer rotatable bonds, are considerably more rigid. This variation in flexibility is likely to influence both conformational dynamics and interactions with biological targets. Lipophilicity and aqueous solubility, represented by logP and logS respectively, show an inverse relationship. Ct9 is the most hydrophilic compound with a logP of 0.469 and relatively high solubility (logS -1.87), whereas Ct12 is highly lipophilic with a logP of 6.753 and correspondingly low solubility (logS -5.643). These properties are critical for predicting absorption, distribution, and bioavailability in pharmacological contexts. Acidic and basic pKa values vary moderately among the compounds. Acidic pKa values range from 7.168 (Ct9) to 9.955 (Ct12), and basic pKa values range from 3.646 (Ct9) to 5.414 (Ct4 and Ct7). This variation affects the ionization states of the compounds under physiological pH, which can influence both solubility and target interaction. Thermal properties reveal additional distinctions. Melting points vary significantly, with Ct12 melting at 125.88 °C, the lowest among the compounds, while Ct5 exhibits the highest melting point of 201.93 °C. Boiling points range from 288.79 °C (Ct4) to 399.70 °C (Ct12), reflecting differences in molecular interactions and structural stability as shown in Table 3.

Table 3: Physicochemical properties for drug-likeness of Ct1-Ct12

Physicochemical properties												
Properties	**Ct1**	**Ct2**	**Ct3**	**Ct4**	**Ct5**	**Ct6**	**Ct7**	**Ct8**	**Ct9**	**Ct10**	**Ct11**	**Ct12**
Molecular Weight	588.37	600.37	600.37	530.29	488.24	600.37	530.29	672.46	448.21	616.4	616.4	686.48
Volume	619.534	634.193	634.193	547.713	495.825	634.193	547.713	723.31	446.574	654.126	654.126	740.606
Density	0.95	0.947	0.947	0.968	0.985	0.947	0.968	0.93	1.004	0.942	0.942	0.927

nHA	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8..0	8.0	8.0	8.0
nHD	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	2.0
nRot	14.0	14.0	14.0	8.0	6.0	14.0	8.0	21.0	5.0	17.0	17.0	22.0
nRing	4.0	4.0	4.0	4.0	4.0	22.0	4.0	4.0	4.0	4.0	4.0	4.0
MaxRing	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
nHet	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
fChar	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
nRig	21.0	22.0	22.0	22.0	22.0	22.0	22.0	21.0	21.0	21.0	21.0	21.0
Flexibility	0.667	0.636	0.636	0.364	0.273	0.636	0.364	1.0	0.238	0.81	0.81	1.048
Stereo Centers	8.0	8.0	8.0	9.0	8.0	8.0	9.0	8.0	8.0	8.0	8.0	8.0
TPSA	130.36	130.36	130.36	130.36	130.36	130.36	130.36	130.36	130.36	130.36	130.36	119.36
logS	-4.374	-5.194	-5.194	-4.489	-3.218	-5.194	-4.489	-5.078	-1.87	-4.957	-4.957	-5.643
logP	4.352	5.0	5.0	2.925	1.482	5.0	2.925	6.459	0.469	5.108	5.108	6.753
logD7.4	4.151	4.144	4.144		1.747	4.144	3.02	4.771	0.812	4.155	4.155	4.711
pka (Acid)	8.113	8.883	8.883	7.938	7.268	8.883	7.938	9.518	7.169	8.807	8.807	9.955
pka (Base)	4.812	5.016	5.016	5.414	4.066	5.016	5.414	4.449	3.646	4.306	4.306	4.707
Melting point	140.336	146.549	146.549	156.97	201.933	146.549	156.97	132.5	170.817	146.604	146.604	125.881
Boilingpoint	336.708	347.527	347.527	288.789	301.491	347.527	288.789	383.509	304.797	356.195	356.195	399.696

3.3.2: Medicinal Chemistry

The dataset provided encompasses a comprehensive analysis of twelve chemical compounds (Ct1–Ct12) based on various physicochemical, medicinal chemistry, and drug-likeness parameters.

These properties collectively inform the potential of each compound as a viable drug candidate, assessing factors such as chemical stability, biological activity, synthetic accessibility, and the likelihood of assay interference.A key metric evaluated is the **Quantitative Estimate of Drug-likeness (QED)**, which provides a holistic measure of a compound's suitability as a drug. Among the compounds, Ct9 exhibits the highest QED (0.432), suggesting it is the most drug-like, whereas Ct8 scores lowest (0.074), indicating potential deficiencies in its pharmacokinetic or structural properties. Complementing this measure, **Lipinski's Rule of Five**—a cornerstone in predicting oral bioavailability—reveals that half of the compounds, including Ct2, Ct3, and Ct6, satisfy all criteria, while others such as Ct1 and Ct9 do not, highlighting areas where modifications may be necessary to enhance permeability or solubility.Structural complexity and natural-product likeness are also integral to evaluating these compounds. The **Fsp³ fraction**, indicating saturation and three-dimensionality, varies from 0.667 in Ct5 to 0.829 in Ct12. Higher Fsp³ values are generally associated with improved solubility, lower promiscuity, and favorable pharmacokinetic profiles. Similarly, the **NPscore**, which quantifies similarity to natural products, is highest for Ct5 (3.326) and Ct9 (3.207), implying that these compounds possess structural motifs often found in bioactive natural molecules. **MCE-18**, a medicinal chemistry efficiency metric, further highlights Ct4 and Ct7 as structurally optimized, suggesting a balance between potency and molecular complexity.Chemical reactivity and potential assay interference are critical in drug discovery, as reactive groups or fluorescent properties can confound biological testing. Encouragingly, all compounds show zero **PAINS alerts**, indicating a low likelihood of nonspecific interactions with biological targets. Nevertheless, some compounds, such as Ct2–Ct7, have multiple **Alarm_NMR alerts**, signaling the presence of potentially reactive moieties. **Colloidal aggregation**, which can artifactually inhibit proteins in assays, is notably high in Ct12 and Ct8, while Ct9 exhibits elevated blue fluorescence, potentially interfering with fluorescence-based assays. Such factors are crucial to consider when prioritizing compounds for experimental validation.Interestingly, **synthetic accessibility (SAscore)** is uniformly zero across all compounds, implying that they are predicted to be easy to synthesize. This is advantageous for medicinal chemistry efforts, although it is somewhat unusual, as typical datasets usually exhibit some variation. Compliance with other medicinal chemistry rules, such as Pfizer and GSK guidelines, is consistently favorable, suggesting these compounds generally adhere to recognized safety and drug-likeness standards as shown in Table 4.

Table 4: Medicinal properties of Ct1-Ct12

Properties	Ct1	Ct2	Ct3	Ct4	Ct5	Ct6	Ct7	Ct8	Ct9	Ct10	Ct11	Ct12
QED	0.168	0.122	0.122	0.271	0.311	0.122	0.271	0.074	0.432	0.12	0.12	0.075
SAscore	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
GASA	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Fsp3	0.794	0.743	0.743	0.7	0.667	0.743	0.7	0.825	0.708	0.806	0.806	0.829
MCE-18	81.475	81.475	81.475	86.118	85.556	81.475	86.118	77.315	84.585	78.462	78.462	77.067
NPscore	2.542	2.766	2.766	3.163	3.326	2.766	3.163	2.121	3.207	2.378	2.378	2.151
Lipinski Rule	0.0	1.0	1.0	0.0	0.0	1.0	0.0	1.0	0.0	1.0	1.0	1.0
Pfizer Rule	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
GSK Rule	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
GoldenTriangle	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0
PAINS	0 alerts	0 alerts	0 alerts	0 alerts	0 alerts	0 alerts	0 alerts	0 alerts	0 alerts	0 alerts	0 alerts	0 alerts
Alarm_NMR Rule	1 alerts	2 alerts	2 alerts	2 alerts	2 alerts	2 alerts	2 alerts	1 alerts	1 alerts	1 alerts	1 alerts	1 alerts
BMS Rule	1 alerts	1 alerts	1 alerts	0 alerts	0 alerts	1 alerts	0 alerts	1 alerts	0 alerts	1 alerts	1 alerts	1 alerts
Chelating Rule	0 alerts	0 alerts	0 alerts	0 alerts	0 alerts	0 alerts	0 alerts	0 alerts	0 alerts	O alerts	0 alerts	0 alerts
Colloidal aggregators	0.326	0.19	0.19	0.106	0.019	0.19	0.106	0.43	0.047	0.384	0.384	0.489
FLuc inhibitors	0.001	0.001	0.001	0.0	0.0	0.001	0.0	0.001	0.0	0.001	0.001	0.001
Blue fluorescence	0.004	0.002	0.002	0.003	0.005	0.002	0.003	0.002	0.292	0.003	0.003	0.002
Green fluorescence	0.0	0.241	0.241	0.02	0.013	0.241	0.02	0.0	0.0	0.0	0.0	0.0
Reactive compounds	0.222	0.186	0.186	0.278	0.255	0.186	0.278	0.217	0.292	0.218	0.218	0.207

3.3.3: Absorption

The dataset represents a comprehensive profile of twelve chemical compounds (Ct1 through Ct12) evaluated for their permeability, absorption, and interaction with efflux transporters. These properties are crucial for understanding the pharmacokinetic behavior of compounds, particularly their potential oral bioavailability and transport across biological membranes.The first two

properties, **Caco-2 Permeability** and **MDCK Permeability**, measure the ability of compounds to pass through cellular monolayers that mimic the intestinal and renal epithelium, respectively. The values are presented in logarithmic form, with more negative values indicating lower permeability. Across the twelve compounds, the Caco-2 permeability ranges from approximately -5.284 to -4.943, while MDCK permeability spans from -4.893 to -4.713. These results suggest that while all compounds show moderate permeability, subtle differences exist that could influence absorption rates.**PAMPA (Parallel Artificial Membrane Permeability Assay)** provides an estimate of passive transcellular diffusion. Values range from 0.276 to 0.978, indicating significant variability in passive permeability among the compounds. High PAMPA values suggest a strong ability to diffuse across lipid membranes, whereas lower values indicate limited passive absorption potential.The dataset also includes **P-glycoprotein (Pgp) interaction properties**, namely Pgp inhibitor and Pgp substrate. Pgp is an efflux transporter that can limit drug absorption and contribute to multidrug resistance. Compounds with high Pgp substrate probability are likely to be pumped out of cells, reducing their effective absorption, while those acting as Pgp inhibitors may block this efflux, potentially increasing bioavailability. The dataset shows that most compounds are predicted to be Pgp substrates, with varying probabilities of being Pgp inhibitors. One inconsistency exists in the Pgp inhibitor value for Ct8, which appears to be a typographical error and requires clarification .**Human Intestinal Absorption (HIA)** predictions range from 0.353 to 0.987, reflecting differences in the likelihood of each compound being absorbed through the human gut. Compounds with higher HIA values are more likely to achieve significant systemic exposure following oral administration.Finally, the dataset presents fractional absorption estimates at different thresholds: **F20%, F30%, and F50%**, representing the fraction of the compound predicted to be absorbed. Notably, all compounds exhibit very high predicted absorption, with values close to or equal to 1, indicating excellent oral bioavailability potential as shown in Table 5.

Table 5: Absorption profile of Ct1-Ct12

Properties	Ct1	Ct2	Ct3	Ct4	Ct5	Ct6	Ct7	Ct8	Ct9	Ct10	Ct11	Ct12
Caco-2 Permeability	-5.049	-5.147	-5.147	-4.989	-4.943	-5.147	-4.989	-5.182	-5.284	-5.19	-5.19	-5.172
MDCK Permeability	-4.739	-4.891	-4.891	-4.744	-4.743	-4.891	-4.744	-4.732	-4.893	-4.713	-4.713	-4.727

PAMPA	0.604	0.963	0.963	0.978	0.952	0.963	0.978	0.47	0.976	0.426	0.426	0.276
Pgp inhibitor	0.069	0.001	0.001	0.581	0.155	0.001	0.581	0.007	0.979	0.137	0.137	0.115
Pgp substrate	0.989	0.87	0.87	0.99	0.865	0.87	0.99	0.833	0.966	0.973	0.973	0.977
HIA	0.353	0.987	0.987	0.478	0.47	0.987	0.478	0.833	0.606	0.932	0.932	0.952
F20%	0.995	0.995	0.995	0.999	0.988	0.995	0.999	1.0	0.997	1.0	1.0	1.0
F30%	0.998	1.0	1.0	1.0	0.999	1.0	1.0	1.0	1.0	1.0	1.0	1.0
F50%	1.0	1.0	1.0	1.0	0.999	1.0	1.0	1.0	0.999	1.0	1.0	1.0

3.3.4: Distribution

The dataset presents pharmacokinetic and transporter-related properties for twelve compounds, labeled Ct1 through Ct12, providing a comprehensive overview of their potential behavior in the human body. The properties analyzed include plasma protein binding (PPB), volume of distribution at steady state (VDss), blood–brain barrier (BBB) permeability, fraction unbound in plasma (Fu), and inhibitory potential against various transporters including OATP1B1, OATP1B3, BCRP, MRP1, and BSEP.**Plasma Protein Binding (PPB)** values among the compounds exhibit a generally high degree of protein association, ranging from 55.443% in Ct9 to 98.307% in Ct12. This indicates that most compounds are predominantly bound to plasma proteins, which has direct implications for their free drug concentration and pharmacological activity. Notably, Ct5 and Ct9 are outliers with relatively lower PPB values, suggesting a higher proportion of free, pharmacologically active drug in plasma.The **volume of distribution (VDss)** values show considerable variation, from a low of -0.76 in Ct9 to a high of 0.959 in Ct12. These values, likely log-transformed, reflect the extent to which each compound distributes beyond the plasma into tissues. Ct9, in particular, with its negative VDss, indicates a strong preference for remaining in the plasma, aligning with its lower PPB and higher fraction unbound. **Blood–brain barrier (BBB) permeability** across all compounds is remarkably low, with values ranging from 0.0 to 0.034. This suggests that these compounds are unlikely to penetrate the central nervous system effectively, indicating limited potential for central pharmacological or toxicological effects.The **fraction unbound (Fu)** in plasma varies widely, from 2.2% in Ct12 to 41.918% in Ct9. As expected, Fu is inversely correlated with PPB; compounds with lower protein binding, such as Ct5 and Ct9, show substantially higher unbound fractions. This unbound fraction is critical for determining the pharmacologically active portion of the drug.Transporter inhibition profiles reveal distinct patterns. **OATP1B1 inhibition** shows considerable variability, with Ct5 and Ct9–Ct10 exhibiting

near-maximal inhibition (0.997–0.999), whereas Ct2, Ct3, and Ct6 demonstrate minimal inhibitory potential (~0.194). In contrast, **OATP1B3 inhibition** is uniformly high across most compounds, exceeding 0.9 for the majority, suggesting these compounds may significantly influence hepatic uptake of co-administered drugs. **BCRP inhibition** is minimal for nearly all compounds, indicating low potential for interactions via this transporter. Meanwhile, **MRP1 inhibition** is generally strong, with most compounds scoring above 0.97, highlighting the possibility of substantial interaction with this efflux transporter. Interestingly, **BSEP inhibition** is universally absent (0.0), suggesting no expected interference with bile salt export as shown in Table 6.

Table 6: Distribution profile of Ct1-Ct12

Properties	Ct1	Ct2	Ct3	Ct4	Ct5	Ct6	Ct7	Ct8	Ct9	Ct10	Ct11	Ct12
PPB	95.132	97.52	97.52	84.88	64.452	97.52	84.88	97.487	55.443	96.926	96.926	98.307
VDss	0.565	0.383	0.383	0.121	-0.263	0.383	0.121	0.82	-0.76	0.682	0.682	0.959
BBB	0.004	0.001	0.001	0.034	0.017	0.001	0.034	0.0	0.001	0.0	0.0	0.0
Fu	5.464	3.354	3.354	18.394	32.464	3.354	18.394	3.311	41.918	4.182	4.182	2.2
OATP1B1 inhibitor	0.337	0.194	0.194	0.964	0.997	0.194	0.964	0.888	0.999	0.983	0.983	0.965
OATP1B3 inhibitor	0.912	0.998	0.998	0.812	0.955	0.998	0.964	1.0	0.946	1.0	1.0	1.0
BCRP inhibitor	0.026	0.001	0.001	0.0	0.001	0.001	0.0	0.003	0.001	0.004	0.004	0.002
MRP1 inhibitor	0.984	0.99	0.99	0.972	0.939	0.99	0.972	0.986	0.872	0.982	0.982	0.992
BSEP inhibitor	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

3.3.5: Metabolism

The dataset presented provides an in-depth analysis of twelve chemical compounds (Ct1–Ct12) with respect to their interactions with various cytochrome P450 (CYP) enzymes and their human liver microsomal (HLM) stability. Cytochrome P450 enzymes are essential in drug metabolism,

influencing the pharmacokinetics, efficacy, and potential toxicity of compounds. Understanding whether a compound acts as an inhibitor or substrate of these enzymes is crucial in predicting drug–drug interactions and metabolic behavior.In the context of CYP1A2, most compounds display minimal inhibitory activity, with the notable exceptions of Ct8 and Ct12, which show relatively high inhibitory probabilities of 0.793 and 0.788, respectively. This indicates that these compounds may potentially interfere with CYP1A2-mediated metabolism of other drugs. Conversely, CYP1A2 substrate activity is generally low across all compounds, suggesting that these compounds are unlikely to be metabolized extensively by this enzyme.CYP2C19 shows a contrasting pattern. Ct1 and Ct8 stand out as strong inhibitors (0.716 and 1.0), whereas most other compounds exhibit weak or negligible inhibition. Substrate activity is low overall, with minor activity observed in compounds Ct2–Ct7, indicating limited metabolism via CYP2C19. Similarly, CYP2C9 inhibition is generally weak, but substrate activity varies significantly: Ct2, Ct3, and Ct6 show extremely high substrate probabilities (0.992), implying that these compounds are likely metabolized primarily by CYP2C9.For CYP2D6, both inhibition and substrate activity are largely negligible across all compounds, suggesting minimal involvement of CYP2D6 in the metabolism or interaction potential of these compounds. On the other hand, CYP3A4, one of the most important drug-metabolizing enzymes, shows strong inhibitory potential across many compounds. Ct1–Ct8 consistently exhibit high probabilities of inhibition (0.971–1.0), indicating a high likelihood of significant drug–drug interactions if co-administered with other CYP3A4 substrates. Substrate activity for CYP3A4 varies more widely, with Ct2–Ct7 showing strong substrate potential (0.984–1.0), while other compounds such as Ct8 and Ct12 exhibit negligible activity.CYP2B6 and CYP2C8 also show notable inhibition patterns. Ct1, Ct8, Ct10–Ct12 are potent CYP2B6 inhibitors, while several compounds (Ct1–Ct12) are consistently strong CYP2C8 inhibitors, suggesting that these enzymes may play a role in both potential interactions and metabolic modulation for these compounds. Substrate activity for these two enzymes is largely absent, indicating limited metabolism via CYP2B6 and CYP2C8.Finally, HLM stability provides insight into the metabolic resilience of the compounds. Compounds such as Ct2, Ct3, Ct6, Ct4, and Ct5 exhibit very high stability (>0.93), suggesting slower metabolism and potentially longer half-lives in the human liver. In contrast, Ct1, Ct10, Ct11, and Ct12 show moderate to low stability (0.12–0.536), implying they may be metabolized more rapidly, which could affect bioavailability and dosing considerations as shown in Table 7.

Table 7: Metabolism profile of Ct1-Ct12

Properties	Ct1	Ct2	Ct3	Ct4	Ct5	Ct6	Ct7	Ct8	Ct9	Ct10	Ct11	Ct12
CYP1A2 inhibitor	0.032	0.0	0.0	0.0	0.0	0.0	0.0	0.793	0.0	0.0	0.0	0.788
CYP1A2 substrate	0.0	0.0	0.0	0.058	0.022	0.0	0.058	0.0	0.0	0.0	0.0	0.0
CYP2C19 inhibitor	0.716	0.063	0.063	0.002	0.003	0.063	0.002	1.0	0.0	0.027	0.027	1.0
CYP2C19 substrate	0.0	0.015	0.015	0.014	0.014	0.015	0.014	0.0	0.014	0.001	0.001	0.0
CYP2C9 inhibitor	0.0	0.001	0.001	0.008	0.001	0.001	0.008	0.0	0.003	0.0	0.0	0.0
CYP2C9 substrate	0.001	0.992	0.992	0.057	0.055	0.992	0.057	0.428	0.003	0.015	0.015	0.835
CYP2D6 inhibitor	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.011	0.003	0.003	0.0
CYP2D6 substrate	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CYP3A4inhibitor	0.971	1.0	1.0	0.998	0.654	1.0	0.998	0.999	0.142	0.623	0.623	0.999
CYP3A4 substrate	0.186	0.984	0.984	1.0	0.637	0.984	1.0	0.001	0.588	0.012	0.012	0.015
CYP2B6 inhibitor	1.0	0.0	0.0	0.028	0.002	0.0	0.028	1.0	0.003	0.988	0.988	1.0
CYP2B6 substrate	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CYP2C8 inhibitor	0.995	1.0	1.0	0.922	0.924	1.0	0.922	1.0	0.993	1.0	1.0	1.0
HLM Stability	0.12	0.99	0.999	0.974	0.934	0.999	0.974	0.576	0.646	0.189	0.189	0.536

3.3.6: Excretion

The dataset presented comprises pharmacokinetic properties of twelve compounds, labeled Ct1 through Ct12, specifically focusing on plasma clearance (CLplasma) and half-life (T1/2). These parameters are critical in understanding the absorption, distribution, metabolism, and excretion characteristics of each compound, providing insight into their behavior within a biological system.**Plasma clearance (CLplasma)**, measured in appropriate units, represents the volume of plasma from which a compound is completely removed per unit time. In the dataset, CLplasma values range from a low of 3.703 for compounds Ct3 and Ct6 to a high of 8.491 for Ct5. This

variation indicates that certain compounds are cleared from the bloodstream more efficiently than others. Notably, some compounds share identical clearance values, such as Ct3 and Ct6, Ct4 and Ct7, and Ct10 and Ct11, suggesting structural or functional similarities that may influence their metabolic rate.**Half-life (T1/2)**, which denotes the time required for the plasma concentration of a compound to reduce by half, ranges from 0.819 in Ct1 to 1.655 in Ct5. Interestingly, the data reveals that T1/2 does not strictly inversely correlate with CLplasma, as might be expected from basic pharmacokinetic principles. For example, Ct5 exhibits both the highest clearance and the longest half-life, an anomaly that could reflect unique metabolic or distribution dynamics. Other compounds, such as Ct1, demonstrate the shortest half-life paired with moderate clearance, highlighting the complex interplay between elimination processes and compound-specific properties.Several patterns emerge upon closer examination. Duplication of values across compounds indicates potential structural similarities or experimental repetition. For instance, Ct3 and Ct6, as well as Ct4 and Ct7, have identical clearance and half-life values, while Ct10 and Ct11, and Ct2 and Ct12, also mirror each other. These redundancies may simplify comparative analyses but also warrant attention when interpreting variability and trends as shown in Table 8.

Table 8: Excretion profile of Ct1-Ct12

Properties	Ct1	Ct2	Ct3	Ct4	Ct5	Ct6	Ct7	Ct8	Ct9	Ct10	Ct11	Ct12
CLplasma	5.51	5.167	3.703	8.327	8.491	3.703	8.327	4.413	5.701	4.154	4.154	5.167
T1/2	0.819	1.001	1.152	1.374	1.655	1.152	1.374	1.131	1.416	1.123	1.123	1.001

3.3.7: Environmental Toxicity

The dataset provided presents an analysis of **twelve chemical compounds (Ct1 to Ct12)** across four key environmental and toxicological properties: **Bioconcentration Factor (BCF), IGC50, LC50 for Fathead Minnow (LC50FM), and LC50 for Daphnia Magna (LC50DM)**. Each of these parameters provides insight into the potential ecological impact and bioaccumulative nature of the compounds.The **Bioconcentration Factor (BCF)** measures the tendency of a compound to accumulate in living organisms relative to its concentration in the surrounding environment. In the data, BCF values vary from as low as **0.462** (Ct9) to as high as **1.924** (Ct3 and Ct6), indicating a wide spectrum of bioaccumulation potential. Compounds such as Ct3 and Ct6 exhibit higher

bioconcentration tendencies, suggesting a greater likelihood of persistence within biological systems. Conversely, compounds like Ct5 and Ct9 show minimal accumulation, implying a lower ecological persistence.The **IGC50**, representing the concentration at which 50% growth inhibition occurs, serves as an indicator of general toxicity. Here, IGC50 values range from **3.112** (Ct9) to **5.685** (Ct2 and Ct12). Higher values denote lower toxicity, suggesting that compounds like Ct2 and Ct12 are relatively safer in terms of growth inhibition effects, whereas compounds such as Ct9 are more toxic.For **LC50FM**, the lethal concentration for Fathead Minnow, the data exhibits a range from **3.8** (Ct9) to **6.258** (Ct3 and Ct6). Similarly, **LC50DM**, which indicates the lethal concentration for Daphnia Magna, ranges from **4.601** (Ct9) to **6.987** (Ct2). These metrics are crucial for assessing aquatic toxicity. Lower LC50 values suggest higher toxicity to aquatic organisms, while higher values reflect safer profiles. Notably, Ct9 consistently shows lower LC50 values, signaling higher toxicity, whereas compounds like Ct2 and Ct6 demonstrate relatively lower ecological risk as shown in Table 9.

Table 9: Environmental Toxicity profile of Ct1-Ct12

Properties	**Ct1**	**Ct2**	**Ct3**	**Ct4**	**Ct5**	**Ct6**	**Ct7**	**Ct8**	**Ct9**	**Ct10**	**Ct11**	**Ct12**
Bioconcentration Factors	1.641	1.724	1.924	0.624	0.519	1.924	0.64	1.777	0.462	1.889	1.889	1.724
IGC50	4.683	5.685	5.058	3.456	3.367	5.058	3.456	5.609	3.112	5.559	5.559	5.685
LC50FM	5.514	6.126	6.258	4.575	4.446	6.258	4.575	5.861	3.8	6.156	6.156	6.126
LC50DM	6.249	6.987	6.872	5.431	5.259	6.872	5.431	6.847	4.601	6.814	6.814	6.987

3.3.8: Toxicophore Rules

The table presents a comprehensive assessment of twelve chemical compounds (labeled Ct1 to Ct12) across various toxicological and chemical screening rules. These rules are designed to evaluate the potential hazards, environmental impact, and safety profiles of chemical substances, providing valuable insight into their suitability for industrial, pharmaceutical, or environmental applications.The **Aquatic Toxicity Rule** highlights the potential harm these compounds may pose

to aquatic organisms. Notably, compounds Ct3 through Ct7 exhibit the highest alert levels, indicating significant toxicity to aquatic life, whereas Ct2 has no reported alerts, suggesting it is less harmful in aquatic environments. This suggests that careful management and possibly remediation strategies are necessary when handling highly aquatic-toxic compounds.The **Genotoxic, Carcinogenicity, and Mutagenicity Rule** evaluates the likelihood of compounds causing genetic mutations or cancer. Most compounds exhibit moderate alert levels, with Ct4 showing no alerts. Compounds with repeated alerts across this rule, such as Ct1, Ct2, and Ct5–Ct12, could potentially pose long-term health risks, emphasizing the importance of thorough safety testing before use in consumer products or pharmaceuticals.Interestingly, the **Non-Genotoxic Carcinogenicity Rule** registers zero alerts for all compounds, indicating that none of the chemicals are predicted to induce cancer via non-genotoxic mechanisms. This may be considered a positive aspect in the overall safety profile of these substances.The **Skin Sensitization Rule** uniformly reports two alerts for nearly all compounds, signaling a moderate potential for causing allergic reactions upon skin contact. This suggests that protective measures, such as gloves or safety equipment, may be necessary during handling.Regarding **Acute Toxicity**, only Ct2 and Ct9 show alerts, indicating a heightened risk of immediate toxic effects. This is critical for laboratory and industrial settings, where exposure to such compounds must be minimized.The **Non-Biodegradability Rule** suggests that most compounds are persistent in the environment, with Ct2 and Ct12 showing slightly higher alerts. This persistence raises concerns about environmental accumulation and the potential long-term ecological impact of these chemicals.The **SureChEMBL Rule**, which often serves as a reference for database filtering, reports zero alerts across all compounds, indicating no structural alerts flagged in this specific screening tool. Meanwhile, the **FAF-Drugs4 Rule** shows minor alerts for all compounds, which may reflect structural properties that warrant additional scrutiny for drug development purposes as shown in Table 10.

Table 10: Toxicophore Rules of Ct1-Ct12

Properties	Ct 1	Ct2	Ct3	Ct4	Ct5	Ct6	Ct7	Ct8	Ct9	Ct10	Ct11	Ct12

Aquatic Toxicity Rule	3	0	4 alerts	4 alerts	4 alerts	4 alerts	4 alerts	3 alerts	3 alerts	3 alerts	3 alerts	3 alerts
Genotoxic Carcinogenicity Mutagenicity Rule	2	2 alerts	2 alerts		2 alerts	2 alerts	2 alerts	2 alerts	2 alerts	2 alerts	2 alerts	2 alerts
NonGenotoxic Carcinogenicity Rule	0	0	0	0	0	0	0	0	0	0	0	0
Skin Sensitization Rule	2	2 alerts	2 alerts	2 alerts	2 alerts	2 alerts	2 alerts	2 alerts	2 alerts	2 alerts	2 alerts	2 alerts
Acute Toxicity Rule	0	3 alerts	0	0	0	0	0	0	3 alerts	0	0	0
NonBiodegradable	1	2 alerts	1 alerts	1 alerts	1 alerts	1 alerts	1 alert	1 alerts	1 alerts	1 alerts	1 alerts	2 alerts
SureChEMBL Rule	0	0	0	0	0	0	0	0	0	0	0	0
FAF-Drugs4 Rule	1	1 alerts	1 alerts	1 alerts	1 alerts	1 alerts	1 alert	1 alerts	1 alerts	1 alerts	1 alerts	1 alerts

3.3.9: Toxicity

The dataset provided represents a comprehensive toxicity and safety profile for twelve compounds, labeled Ct1 through Ct12, across multiple pharmacological and toxicological endpoints. Each property in the dataset corresponds to a specific biological or chemical safety parameter, with values ranging from 0 to 1, likely representing predicted probabilities or normalized risk scores. A higher value indicates a greater likelihood of the compound exhibiting that particular toxicity or adverse effect.Among the evaluated properties, **cardiac toxicity** is assessed through hERG Blockers and hERG Blockers (10 μM). These endpoints are crucial in drug development, as blockade of the hERG potassium channel can lead to fatal arrhythmias. The dataset reveals variability across compounds; for instance, Ct2 and Ct12 exhibit higher probabilities of hERG inhibition, while Ct4 and Ct7 show lower risks.**Hepatic and liver-related**

toxicities are also prominent in the dataset. Properties such as DILI (drug-induced liver injury), Human Hepatotoxicity, and FDAMDD provide insight into potential liver damage risks. While some compounds, such as Ct5, show relatively high risk for liver toxicity, others, such as Ct8, exhibit more moderate values, indicating differential safety profiles among the compounds. The dataset further includes **genotoxicity and carcinogenicity parameters**, namely AMES Toxicity, Carcinogenicity, and Genotoxicity. These properties are critical for evaluating the mutagenic potential of compounds. Notably, Ct5 demonstrates elevated AMES toxicity and genotoxicity, suggesting caution in its development. **Other organ-specific toxicities** are also documented, including Drug-induced Nephrotoxicity, Drug-induced Neurotoxicity, Ototoxicity, and Hematotoxicity. Ct8 shows particularly high nephrotoxicity, while Ct3 demonstrates a pronounced ototoxic effect. Respiratory toxicity is generally high across several compounds, with Ct1, Ct2, and Ct3 showing significant risk .**Sensitization and irritation potential** are covered through Skin Sensitization, Eye Corrosion, Eye Irritation, and Respiratory endpoints. The dataset reveals almost universally high values for skin sensitization, indicating that these compounds may induce strong allergic responses upon contact. Eye irritation and corrosion risks vary among compounds but are generally lower than skin sensitization.Finally, **cytotoxicity** is assessed through endpoints like A549 Cytotoxicity and Hek293 Cytotoxicity, providing insight into the compounds' effect on specific human cell lines. Ct8 demonstrates relatively high cytotoxicity against the A549 cell line, whereas other compounds like Ct4 and Ct5 show minimal effects as shown in Table 11.

Table 11: Toxicity profile of Ct1-Ct12

Properties	Ct1	Ct2	Ct3	Ct4	Ct5	Ct6	Ct7	Ct8	Ct9	Ct10	Ct11	Ct12
hERG Blockers	0.158	0.364	0.156	0.031	0.22	0.156	0.031	0.329	0.02	0.175	0.175	0.364
hERG Blockers (10um)	0.444	0.788	0.426	0.151	0.111	0.426	0.151	0.76	0.207	0.64	0.64	0.788
DILI	0.407	0.357	0.486	0.35	0.499	0.486	0.35	0.341	0.575	0.451	0.451	0.357
AMES Toxicity	0.142	0.243	0.35	0.0	0.596	0.0	0.0	0.189	0.776	0.325	0.325	0.243

Rat Oral Acute Toxicity	0.191	0.221	0.492	0.314	0.298	0.492	0.314	0.216	0.375	0.256	0.256	0.221
FDAMDD	0.163	0.339	0.63	0.443	0.235	0.63	0.443	0.406	0.404	0.4	0.4	0.339
Skin Sensitization	0.978	0.999	0.774	0.857	0.792	0.774	0.857	0.999	0.968	0.998	0.998	0.999
Carcinogenicity	0.256	0.611	0.323	0.748	0.681	0.323	0.748	0.579	0.737	0.575	0.575	0.611
Eye Corrosion	0.044	0.105	0.0	0.002	0.001	0.0	0.002	0.106	0.039	0.062	0.062	0.105
Eye Irritation	0.666	0.794	0.022	0.299	0.414	0.022	0.299	0.106	0.797	0.839	0.839	0.794
Respiratory	0.812	0.895	0.875	0.467	0.42	0.875	0.467	0.852	0.514	0.873	0.873	0.895
Human Hepatotoxicity	0.705	0.538	0.65	0.741	0.714	0.65	0.741	0.598	0.653	0.654	0.654	0.538
Drug-induced Nephrotoxicity	0.64	0.864	0.411	0.742	0.574	0.411	0.742	0.879	0.736	0.78	0.78	0.864
Drug-induced Neurotoxicity	0.05		0.298	0.235	0.097	0.298	0.235	0.039	0.288	0.057	0.057	0.067
Ototoxicity	0.715	0.498	0.906	0.801	0.746	0.906	0.801	0.5	0.42	0.434	0.434	0.498
Hematotoxicity	0.627	0.449	0.298	0.357	0.318	0.298	0.357	0.402	0.724	0.507	0.507	0.449
Genotoxicity	0.006	0.0	0.084	0.778	0.938	0.084	0.778	0.0	0.948	0.002	0.002	0.0
RPMI-8226 Immunitoxicity	0.096	0.18	0.214	0.146	0.093	0.214	0.146	0.161	0.095	0.123	0.123	0.18
A549 Cytotoxicity	0.174	0.539	0.336	0.087	0.061	0.336	0.087	0.702	0.102	0.613	0.613	0.539
Hek293 Cytotoxicity	0.132	0.297	00.375	0.262	0.186	0.375	0.262	0.294	0.288	0.238	0.238	0.297

3.3.10: Toxic Pathway

The dataset presents a detailed profile of twelve compounds, labeled Ct1 through Ct12, across various nuclear receptor (NR) and stress response (SR) pathways. Each pathway represents a potential biological target for assessing the interaction and activity of these compounds, with values ranging from 0.0 (no activity) to higher decimal numbers indicating measurable activity.Among the nuclear receptor pathways, **NR-AhR** and **NR-PPAR-gamma** show no detectable activity for any of the twelve compounds, suggesting that these compounds are either inactive or non-interactive with these specific receptors. In contrast, **NR-AR** and **NR-ER** exhibit moderate activity across multiple compounds. For instance, Ct9 shows relatively higher activity (0.301 for NR-AR and 0.087 for NR-ER), highlighting its potential interaction with androgen and estrogen receptors. Similarly, **NR-Aromatase**, responsible for estrogen biosynthesis, displays varying levels of activity, with compounds Ct4, Ct7, and Ct9 showing notable engagement. A closer examination of the **ligand-binding domains (LBD)**, such as **NR-AR-LBD** and **NR-ER-LBD**, indicates minimal activity except for a slight increase in Ct9 for NR-AR-LBD (0.004) and NR-ER-LBD (0.102), suggesting selective binding tendencies in this compound.The stress response pathways (SR) display more pronounced variability among the compounds. **SR-MMP**, a marker for matrix metalloproteinase activity, shows the highest activity among all tested pathways, particularly for Ct9 (0.837), followed closely by Ct10 and Ct11, indicating a strong potential for modulating extracellular matrix remodeling. Similarly, **SR-p53**, a crucial tumor suppressor pathway, shows extreme sensitivity to Ct9 (0.964), reflecting a potent activation that could have significant implications in cell cycle regulation and apoptosis. Other SR pathways, including **SR-ARE**, **SR-ATAD5**, and **SR-HSE**, show low to moderate activation, with sporadic peaks for specific compounds such as Ct9, which consistently exhibits higher activity across multiple pathways as shown in Table 12.

Table 12: Tox21 pathway profile of Ct1-Ct12

Properties	Ct1	Ct2	Ct3	Ct4	Ct5	Ct6	Ct7	Ct8	Ct9	Ct10	Ct11	Ct12
NR-AhR	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
NR-AR	0.224	0.017	0.0	0.024	0.023	0.0	0.024	0.051	0.301	0.107	0.107	0.017
NR-AR-LBD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.004	0.0	0.0	0.0
NR-Aromatase	0.006	0.001	0.0	0.093	0.027	0.0	0.093	0.003	0.106	0.074	0.074	0.001
NR-ER	0.074	0.028	0.01	0.109	0.038	0.01	0.109	0.109	0.087	0.047	0.047	0.028

NR-ER-LBD	0.0	0.0	0.0	0.0	0.004	0.0	0.0	0.0	0.102	0.007	0.007	0.0
NR-PPAR-gamma	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
SR-ARE	0.002	0.0	0.0	0.002	0.039	0.0	0.002	0.001	0.33	0.028	0.028	0.0
SR-ATAD5	0.0		0.0	0.0	0.003	0.0	0.0	0.0	0.329	0.005	0.005	0.0
SR-HSE	0.0	0.0	0.0	0.0	0.001	0.0	0.0	0.0	0.005	0.002	0.002	0.0
SR-MMP	0.057	0.005	0.0	0.001	0.046	0.0	0.001	0.023	0.837	0.468	0.468	0.005
SR-p53	0.017	0.01	0.0	0.014	0.33	0.0	0.014	0.039	0.964	0.0	0.507	0.01

The twelve diterpenoid phorbol esters (Ct1–Ct12) identified from *Croton tiglium* through GC–MS and ADMET profiling exhibit diverse biological and pharmacological activities [14], aligning with both traditional and modern medicinal uses of the plant.

Ct1 (12-O-Isobutyrylphorbol-13-decanoate) – Exhibits strong anti-inflammatory and irritant properties by modulating protein kinase C (PKC) signaling. It may contribute to C. tiglium's purgative and local stimulant activity.

Ct2 (12-O-Tiglyl-4-deoxy-4α-phorbol-13-decanoate) – Demonstrates cytotoxic [15] and anticancer potential, showing good metabolic stability and compliance with Lipinski's rule, indicating suitability for oral administration.

Ct3 (12-O-Tiglyl-4-deoxy-4α-phorbol-13-phenylacetate) – Known for antitumor and apoptotic activity, with excellent HLM stability and moderate toxicity, making it a promising anticancer lead compound.

Ct4 (12-O-Tiglyl-4-deoxy-4α-phorbol-13-(2-methyl)butyrate) – Displays anti-inflammatory and antimicrobial effects through inhibition of inflammatory mediators [16] such as TNF-α and NO.

Ct5 (12-O-Acetylphorbol-13-tigliate) – The most abundant phorbol ester identified, responsible for the purgative [17] and analgesic activity of croton oil; however, it requires detoxification before safe use.

Ct6 (12-O-(2-Methylbutyroyl)phorbol-13-dodecanoate) – Exhibits antibacterial and antifungal potential with high metabolic stability, supporting its role in treating skin and gastrointestinal infections[18].

Ct7 (12-O-(2-Methyl)butyrylphorbol-13-octanoate) – Shows moderate anti-inflammatory activity and interacts favorably with P-glycoprotein transporters, aiding bioavailability.

Ct8 (12-O-Isobutyrylphorbol-13-decanoate) – Displays antioxidant and cytoprotective activity, though its high lipophilicity suggests potential accumulation and toxicity.

Ct9 (Phorbol-12,13,20-triacetate) – Emerged as the most pharmacologically active compound, activating p53 and MMP pathways, leading to apoptosis and anticancer effects. It also exhibited the highest drug-likeness (QED = 0.432) and balanced ADMET properties, marking it as a lead candidate for anticancer drug development.

Ct10 (12-O-Dodecanoylphorbol-13-acetate) – Exhibits anti-HIV and tumor-suppressive potential through PKC modulation and inhibition of abnormal cell proliferation [19].

Ct11 (12-O-Hexadecanoylphorbol-13-acetate) – Possesses antiviral [20] and wound-healing activity, consistent with reports of croton oil being used for topical treatment [21] in traditional medicine.

Ct12 (12-O-Methylphorbol-13-dodecanoate) – Displays strong lipophilicity and anticancer potential, but also exhibits higher predicted hepatotoxicity, indicating a need for detoxification or derivatization before clinical use.

4. Discussion

The current research extensively assessed the phytochemical, pharmacokinetic, and toxicological profiles of *Croton tiglium* L., which is a renowned traditional medicine plant in the Euphorbiaceae family. The results are in agreement with conventional uses and recent pharmacological information that indicate the plant's bioactive diterpenoid constituents as the most important elements responsible for its curative action and toxicity.

4.1: Phytochemical and GC–MS Findings

The initial phytochemical screening also verified the existence of alkaloids, flavonoids, terpenoids, saponins, glycosides, tannins, and phenolics, consistent with previous works done by, who established that *C. tiglium* is composed of over 150 compounds, which are mainly diterpenoids, fatty acids, and alkaloids. GC–MS profiling in this work revealed twelve dominant phorbol ester

diterpenoids (Ct1–Ct12), with 12-O-acetylphorbol-13-tigliate (Ct5) and 12-O-isobutyrylphorbol-13-decanoate (Ct1) being the most predominant compounds. The results corroborate & reports that phorbol esters are the most prevalent compounds in Croton oil composition, and are responsible for both its pharmacological activity and irritant nature.

4.2: Pharmacokinetic (ADMET) Evaluation

The ADMET and physicochemical information indicated that the diterpenoids of *C. tiglium* have lipophilic and relatively flexible molecular characteristics. Ct9 and Ct12 compounds had optimal lipophilicity (logP = 0.469–6.75) and moderate solubility, which is desirable for membrane permeability and oral absorption. Ct9 compound's high drug-likeness score (QED = 0.432) suggests that this compound could have good pharmacokinetic compatibility for drug development. These features are consistent with earlier pharmacokinetic studies of diterpenes from nature demonstrating that moderate lipophilicity supports biological uptake with selective bioavailability.

Furthermore, compounds Ct2, Ct3, and Ct6 demonstrated high human liver microsomal (HLM) stability (>0.99), suggesting a slower metabolic degradation and prolonged systemic half-life. This is supported by the reported metabolic resistance of phorbol esters due to their stable ester linkages and high molecular weights (Blumberg, 1988). Their compliance with Lipinski's rule of five further reinforces their potential as orally active therapeutic leads.

4.3: Distribution and Metabolism

The high plasma protein binding (PPB) percentages (84–98%) for the majority of the compounds suggest that they will be sustained in plasma, although with diminished free drug levels. The compounds showed low blood–brain barrier (BBB) permeability, which reduces the possibility of central nervous system side effects — a desirable feature for systemics.

Cytochrome P450 enzyme interaction profiles indicated that several of the diterpenoids are potent CYP3A4 inhibitors, with the potential for drug–drug interactions. This is supported by reports demonstrating that phorbol esters influence protein kinase C (PKC) and have the ability to modify hepatic enzyme activity.

4.4: Toxicity and Safety Evaluation

Toxicological findings revealed that although *C. tiglium* compounds have moderate hepatotoxic and nephrotoxic activity, they are not mutagenic and non-genotoxic carcinogenic alerts. This corroborates historical Ayurvedic use where detoxification (Śodhana) of C. tiglium seeds is essential prior to medicinal application to minimize toxicity. A lack of non-genotoxic carcinogenicity and low scores of acute toxicity suggest that controlled dosing of detoxified preparations may have therapeutic efficacy with acceptable safety risks.

Skin sensitization and eye irritation scores were high for the majority of the compounds, indicating their reported irritant nature due to croton oil. Nevertheless, the significant activation of SR-p53 (0.964) and SR-MMP (0.837) pathways by Ct9 indicates that the compound can potentially act with anticancer effects through p53-dependent apoptosis induction and matrix metalloproteinase modulation. These findings corroborate previous studies which accounted for phorbol esters of Croton species with high cytotoxic activity that cause apoptosis in cancer cell lines A549 and HepG2.

4.5: Environmental and Ecological Toxicity

Environmental toxicity profiling had mild to moderate bioconcentration (BCF = 0.46–1.92) and comparatively low aquatic toxicity, showing that although *C. tiglium* metabolites are bioactive, they are not severe environmental threats at therapeutic levels. Ct2 and Ct6 were the least toxic environmentally, whereas Ct9 had greater aquatic toxicity, which is characteristic of highly lipophilic bioactive diterpenes.

4.6: Medicinal Implications

Together, these results highlight that *Croton tiglium* is a rich source of biologically active diterpenes with potential pharmacological activity. Of all the compounds tested, Ct9 was the most prospective lead with high drug-likeness, moderate toxicity, and robust anticancer activity. The findings justify folk uses of *C. tiglium* in inflammatory and while highlighting the importance of detoxification to reduce irritant and hepatotoxic activities. The combination of in-silico ADMET modeling with phytochemical and GC–MS information presents a contemporary pharmacologic basis for the rational design of *C. tiglium*.

5. Conclusion

The outcome of this research confirms *Croton tiglium* as an effective medicinal plant with high bioactive diterpenoid compound content. Among the twelve constituents identified, Ct9 showed the greatest drug-likeness activity, while Ct2, Ct3, and Ct6 showed good pharmacokinetic and safety profiles. These findings affirm the ancient therapeutic applications of *C. tiglium* and indicate the potential of *C. tiglium* as a source of new bioactive molecules. Nonetheless, owing to its innate irritant and hepatotoxic properties, controlled detoxification and regulation of dosage are still vital for safe medicinal application. On the whole, *C. tiglium* is of great potential for plant-based purgative [22] [23] and anti-inflammatory [24] drug development.

Authors' contributions

The study plan was designed and supervised by Tahira Shamim. The whole experimental work was carried out by Laila Sumreen, Jafir Hussain Shirazi and Nadir Hussain following the directions of Tahira Shamim. The manuscript write-up was carried out by Muhammad Arshad, Rida Tanveer, Iqra Samreen, Syeda Abida Hussain under the guidance of Tahira Shamim. All authors read and approved the manuscript for publications.

Availability of data and materials

Data will be available on request by the corresponding author.

Competing interests

There is no any financial or personal competing interest among authors.

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