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Non-psychotropic cannabinoids as inhibitors of TET1 protein

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ABSTRACT

Non-psychotropic cannabinoids (e.g., cannabidiol, cannabinol and cannabigerol) are contained in numerous alimentary and medicinal products. Therefore, predicting and studying their possible side effects, such as changes in DNA methylation, is an important task for assessing the safety of these products. Interference with TET enzymes by chelating ferrous ions can contribute to the altered methylation pattern.

All tested cannabinoids displayed a strong affinity for Fe(II) ions. Cannabidiol and cannabinol exhibited potent inhibitory activities (IC_{50} = 4.8 and 6.27 μM, respectively) towards the TET1 protein, whereas cannabigerol had no effect on the enzyme activity. An *in silico* molecular docking study revealed marked binding potential within the catalytic cavity for CBD/CBN, but some affinity was also found for CBG, thus the total lack of activity remains unexplained. These results imply that cannabinoids could affect the activity of the TET1 protein not only due to their affinity for Fe(II) but also due to other types of interactions (e.g., hydrophobic interactions and hydrogen bonding).

1. Introduction

It is well known that cannabinoid applications can modulate a wide spectrum of physiological processes by modulating specific cannabinoid receptors. They have a high potential for many medicinal applications, including the alleviation of multiple sclerosis symptoms (e.g., chronic pain, spasticity), recovery of memory deficits [1], induction of myeloid-derived suppressor cells [2], suppression of the side effects of chemotherapy (e.g., nausea and vomiting), depression, anxiety, sleep disorder, psychosis and intraocular pressure. Moreover, products from cannabinoids, such as oil, cosmetics, foods and food supplements, including pets, E-liquids and beverages, are widely used, and the market is expanding. For these reasons, studies of their biological properties and mechanisms of potential side effects are urgently needed. The side effects of cannabinoids and their chronic exposure are mainly associated with psychoactive (−)-Δ^9-tetrahydrocannabinol (THC). However, this compound is legislatively restricted in the majority of Cannabis products. However, chronic exposure to non-psychoactive cannabinoids may

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also have a significant impact on users.

Cannabidiol (CBD) is one of the main non-psychotropic constituents of *Cannabis sativa*, constituting approximately 40% of typical extracts [3]. CBD has various therapeutically important properties, such as antioxidant, antiapoptotic, anti-inflammatory, and neuroprotective properties [4,5]. Other major non-psychoactive cannabinoids include cannabinol (CBN) and cannabigerol (CBG). Since the number of biological and pharmacological studies that focus on these cannabinoids is limited, their properties are not as well-known as those of CBD. Nonetheless, some studies have shown their potential use for medicinal applications. For example, CBG application led to a decrease in nitrotyrosine levels and the activity of inducible nitric oxide synthase, poly [ADP-ribose] polymerase 1, IL-1β, TNF-α and INF-γ, as well as to the activation of superoxide dismutase [6,7]. CBN can interact with DNA via hydrophobic interactions [8]. Another therapeutic benefit of CBG and CBN could be their combined application with CBD [9,10]. However, rationalised medicinal use is not possible without a thorough and detailed understanding of their biological and biochemical properties. Cannabinoids can also cause harmful side effects due to dysregulation of the epigenome. This includes changes in the DNA methylation pattern of schizophrenia patients [11], disturbances in DNA methylation and hydroxymethylation patterns in the sperm [12], and changes in histone acetylation and methylation patterns [13]. After chronic exposure to cannabis, epigenome shifts, such as methylation patterns in the nucleus accumbens, can be observed even in offspring [14].

Some of the above-mentioned effects can also be explained by interference with TET1 protein activity. The TET1 protein (ten-eleven translocation methylcytosine dioxygenase 1) is an Fe(II)- and α-ketoglutarate-dependent dioxygenase that converts 5-methylcytosine to 5-hydroxymethylcytosine. Due to its importance, investigations into the mechanisms that control TET1 activity can be a promising strategy for the development of new therapies or for explaining the biological and medicinal properties of pharmacologically active agents. However, only a small number of substances are known that can directly affect the activity of the TET1 protein. Thus, the potential of this approach is currently limited. Nevertheless, a few studies have suggested the application of iron chelators to inhibit TET activity. In the case of α-ketoglutarate-dependent dioxygenases, including TET1 protein, chelators can occupy the enzyme active site and complex Fe(II) ions [15,16]. For example, Sakurai et al. implied that inhibition of Jumonji histone demethylase by phenolic compounds could be based on chelation of Fe(II) ions in the active site of the enzyme [17]. This work also showed that a suitable structural motif for Fe(II) chelation should include phenyl groups. It is very probable that the potential chelation ability of cannabinoids could influence their biological effects.

According to the above, few high-impact studies have reported that the biological effect of CBD could be associated with the suppression of toxic iron effects, such as ROS production [18–21]. In the case of phenolic and polyphenolic compounds and their biological properties, the relationship between iron binding affinity and biological activity is an intensively discussed topic, but cannabinoids are rarely taken into consideration [22]. Nevertheless, Silva et al. reported that CBD in iron overdose rats reversed the decrease in methylcytosine and hydroxymethylcytosine levels in mitochondrial DNA [19]. The tested cannabinoids CBD, CBG and CBN contain one or two phenyl groups, and therefore, we can expect that some of their biological functions could be associated with their chelation ability. According to Petrovici et al., the antioxidant effect of cannabinoids (e.g., suppression of the Fenton reaction) could be explained, at least in part, by the chelation of Fe(II) ions [21]. Therefore, we studied the chelation ability of CBD, CBG and CBN for Fe(II) ions and their possible inhibitory effect on TET1 protein activity. The potential interactions with the TET protein were also verified by a molecular docking study using a TET1 homology model based on the TET2 protein structure.

2. Results

2.1. Chelation ability towards iron(II) ions

The structures of the cannabinoids CBD, CBG and CBN used are shown in Fig. 1.

The anticipated inhibition model of TET1 protein by the tested cannabinoids is based on the complexation of Fe(II) ions, thus the cannabinoid chelation ability for Fe(II) ions in water medium (water/:MeOH; 9:1, v/v) was investigated by UV–Vis spectroscopy (Figs. 2-4). In the presence of Fe(II) ions, a decrease in the absorbance of CBD and CBN absorption peaks was observed. In the case of CBG, we observed the opposite trend.

For the calculation of K and complex stoichiometry, we used the program Letagrop spefo 2005. The determined values of the binding constants and complex stoichiometry are shown in Table 1. The highest Fe(II) binding affinity was found for CBG. In the case of CBD and CBN, the K values were comparable. The higher CBG affinity for Fe(II) ions against CBN can be explained by the higher number of hydroxy groups in this case of CBG. CBD also has two hydroxy groups, such as CBG, but in this case, compared that in to CBG, the bulky cycloalkyl substituent in CBD can sterically block the second hydroxy group significantly more sterically.

This phenomenon could also explain the various spectroscopic behaviours of the observed iron complexes. CBN, which can interact with only one hydroxyl group, displayed a strong decrease in the absorbance of its spectral band. On the other hand, the CBG iron complex has a slightly higher absorbance maximum than that of CBG alone. CBD could also interact with Fe(II) ions by two hydroxyl groups, but the values of the interaction constants suggest that the primary hydroxy group is significantly more preferred. Therefore, the observed spectral behaviour (slightly decreased intensity in the absorbance maximum) lies between the properties of the studied cannabinoids.

2.2. Inhibition of TET1 protein

It is well known that in complexes of phenolic and polyphenolic compounds, Fe(II) ions can interact with more hydroxyl groups (usually two to four). We can therefore expect that CBN can bind only one Fe(II) ion, but one Fe(II) ion can bind more CBN molecules. The observed complex stoichiometry for every tested cannabinoid was 1:4 (Fe(II):cannabinoid, Table 1). In the case of CBG and CBD, the mutual position of their hydroxyl groups enables an effective interaction with only one hydroxyl group at a time. Therefore, their behaviours are similar to those of CBN.

The inhibition of TET1 was determined by a fluorometric TET hydroxylase activity quantification kit. Unexpectedly, the application of CBG with the highest binding affinity for Fe(II) ions did not lead to the inhibition of TET1 protein activity, whereas CBD and CBN both displayed significant inhibitory effects. The IC_{50} values of CBD and CBN were comparable (approximately 5 and 6 μM, respectively) (Fig. 5 and Table 2).

Since Fe(II) ions are essential for the activity of the TET1 protein and the tested cannabinoids were found to be iron(II) chelators, they could suppress the enzymés activity. Although the corresponding concentration of tested chelators for IC_{50} reduced the enzymés activity to half, the maximal amount of chelated Fe(II) was significantly lower (approximately 5 and 6% for CBN and CBD in this case of hypothetical complex 1:1, respectively). In the case of complex stoichiometry (e.g., 1:4), the maximum amount of chelated iron was above 1% of the Fe(II) ion used (0.1 mM $FeSO_4$).

2.3. In silico docking of cannabinoids to the TET1 model

All three cannabinoids - docked to both the TET1 and TET2 protein models, albeit the binding scores were different (Table S2). These results

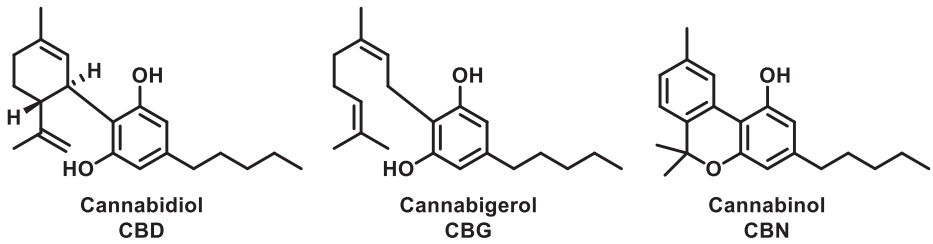

Fig. 1. Structure of cannabinoids used.

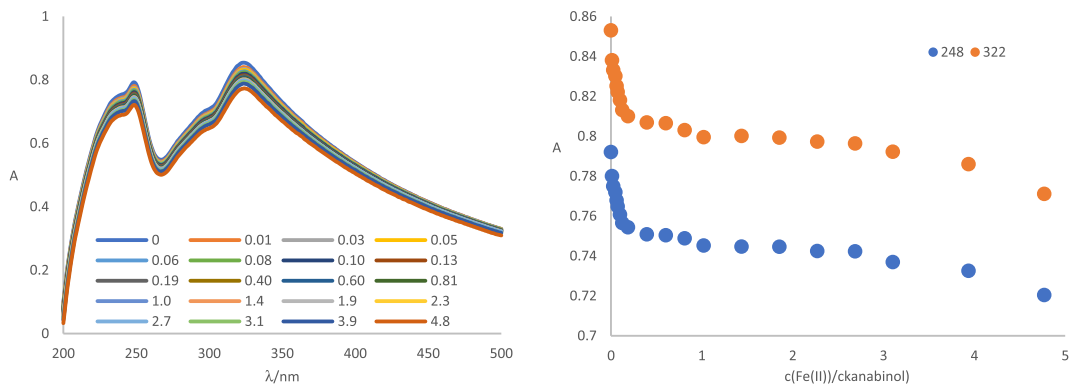

Fig. 2. Titration and titration curve of CBD (248 and 322 nm) (100 μM) with $Fe(ClO_4)_2$ in aqueous solution (water/MeOH, 9:1, v/v).

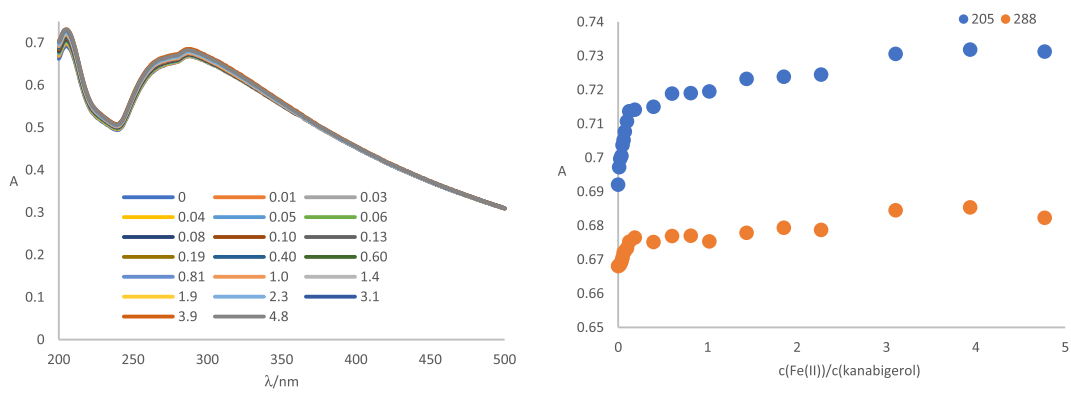

Fig. 3. Titration and titration curve of CBG (205 and 288 nm) (100 μM) with $Fe(ClO_4)_2$ in aqueous solution (water/MeOH, 9:1, v/v).

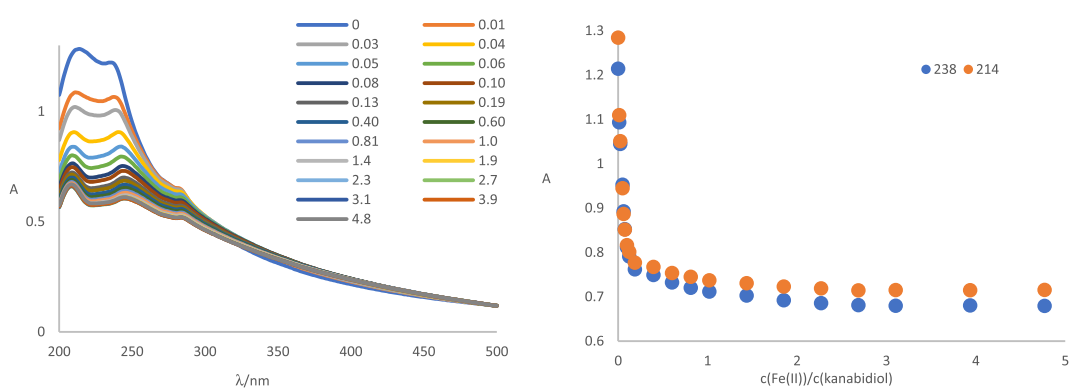

Fig. 4. Titration and titration curves of CBN (214 and 238 nm) (100 μM) with $Fe(ClO_4)_2$ in aqueous solution (water/MeOH, 9:1, v/v).

imply that the experimental inhibitory effect of CBD and CBN does not rely solely on free Fe(II) ion chelation but also on other mechanisms. Therefore, it cannot be speculated that chelating or any other interactions with the metallic centre suffice for the observed inhibition. On the other hand, the similarity between the IC_{50} values of CBD and CBN suggests that CBD's interaction with the enzyme's Fe(II) ion would

Table 1
Conditional binding constants and complex stoichiometry of cannabinoid complexes with Fe(II) ions.

Cannabinoids	Log (K)	St[a]
CBD	17.5	1:4
CBG	18.9	1:4
CBN	17.1	1:4

[a] Stoichiometry Fe(II): cannabinoid.

be possible via the hydroxyl group of the resorcinyl ring present in all three compounds.

Putatively, the presence of the second ring, a cyclohexenyl or an aromatic C-ring of CBD and CBN, respectively, is necessary for interaction with the enzyme, or the aliphatic chain of CBG may cause steric hindrance to this interaction. Structure-activity relationship studies using other types of cannabinoids s are necessary to confirm this hypothesis that two rings are a prerequisite for activity, but most of these compounds occur only as minor constituents in the *Cannabis* herb. However, for in-depth studies, including *in silico* modelling, the details of the regulation of TET1 isoform protein activity are needed. Currently, only limited data on ligand interactions with catalytic and noncatalytic domain regulation are available [23]. In the present study, we used a homology TET1 model based on available TET2 information (Fig. S1) [24].

Additionally, docking to the TET2 protein was performed to compare the docking poses of all 3 compounds with docking to the TET1 protein. TET2 protein was obtained from the PDB database (PDB code: 4NM6). The binding site residues of the TET2 protein, Arg1261, Asp1384, Tyr1902 and His1904, correspond to the following residues in the case of the TET1 protein: Arg1551, Asp1675, Tyr2049 and His2051 (Supplementary Figures S1-S3, Supplementary Tables S1, S2).

Similar to TET1, CBD also interacts with all 4 of the residues in the binding site of the TET2 protein and forms hydrogen bonds with Arg1261 and His1904 as well as -π-interactions with Asp1384 and Tyr1902. CBN interacts with Arg1261 (hydrogen bond) and His1904 (alkyl interaction) residues in the binding site. Conversely, CBG does not form any hydrogen bonds with any of the 4 main residues from the binding site but only π-interaction with ASP1384 and Tyr1902 and alkyl interaction with His1904.

Comparison of the interactions of the redocked ligand from TET2 (Fig. S2) showed that CBD interacts with all 4 residues, which are also observed in the case of the *N*-oxalylglycine ligand, and creates a conventional hydrogen bond with Arg1551, carbon hydrogen bond with Asp1674, π interaction with His2051 and π-sigma Tyr2049. Additionally, CBN interacts with all 4 residues and creates conventional hydrogen bonds with Arg1551 and Tyr2049 and an additional carbon-hydrogen bond His2051 and π-interaction with Asp1674. In the case of CBG, conventional hydrogen bonds with His2051 and π interactions with Arg1551 and Tyr2029 are present. Thus, the docking revealed that CBD/CBN exhibit the ability to bind with certain amino acid residues (Fig. 6, Fig. S3). The Arg1551 residue interacted by hydrogen bonds with CBD/CBN, while with CBG, only an π alkyl interaction was possible.

The aliphatic part of the molecule has a higher degree of freedom than in the cyclic structures of CBD/CBN; thus, whether stable binding was impeded remains unresolved in the present study. Furthermore, CBD was a stronger in vitro inhibitor and showed more complex apparent interactions with more residues *in silico* compared to those of CBN, and this would at least partially explain the experimental results.

3. Discussion

Until an experimental confirmation of each residue's role in ligand binding is obtained, these findings remain hypothetical. In addition, a complex *in silico* TET1 model must also consider a coordination of iron ions; however, this is not available with the current state-of-the art technology. The potential roles of each residue in the catalytic centre of TET2 are available based on mutant studies [25], but these data are not conclusive in the context of our results.

At the present time, it is difficult to determine which physiological consequences may result from the inhibition of TET1 activity by cannabinoids. It is well known that TET1 protein is expressed in the nucleus accumbens, which controls neural and behavioural plasticity after a drug is added [26]. It cannot be excluded that this phenomenon is associated with a change in the activity or the expression of TET1. For example, Feng et al. suggested that the increase in drug sensitivity after repeated cocaine application in mice was associated with a significant downregulation of TET1 protein in the nucleus accumbens [27]. Similarly, Zhang et al. observed that mice with TET1 protein deficits displayed impaired hippocampal neurogenesis associated with poor learning and memory [28]. This disturbance strongly correlates with hypermethylation of CpG islands and slight reduction of their hydroxymethylation [29]. Accordingly, chronic cannabinoid exposure is associated with a change in methylation pattern [30] or neurocognitive impairments [31].

Another intensively studied effect of chronic cannabinoid applications is its association with lung cancer [32]. Numerous studies have demonstrated that reduced expression/activity of TET1 protein and

Table 2
Influence of cannabinoids on the activity of TET1 protein.

Cannabinoids	IC_{50} [μM]	Maximal Amount of Chelated Fe(II)[a] [%]
CBD	4.8	1.2
CBG	–	
CBN	6.27	1.7

[a] Expected complex stoichiometry is 1:4 (Fe(II):cannabinoid), and all cannabinoids participate in the complexation.

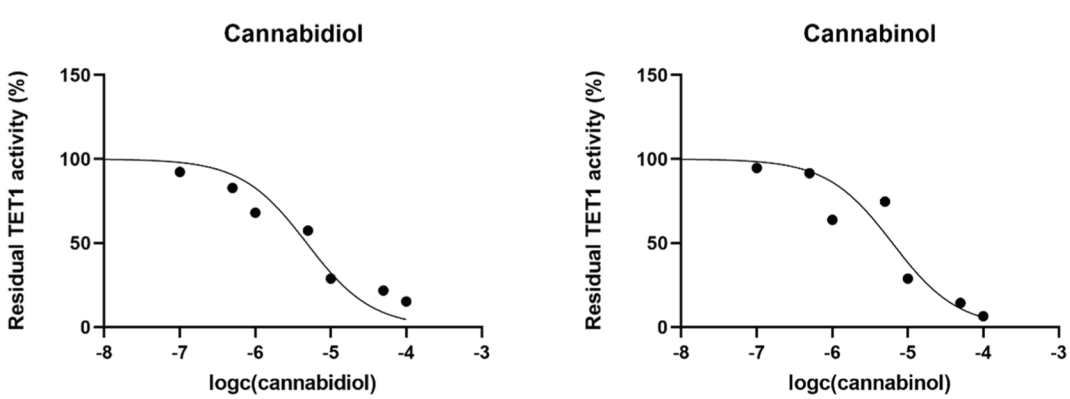

Fig. 5. Influence of CBD and CBN on the activity of TET1 protein.

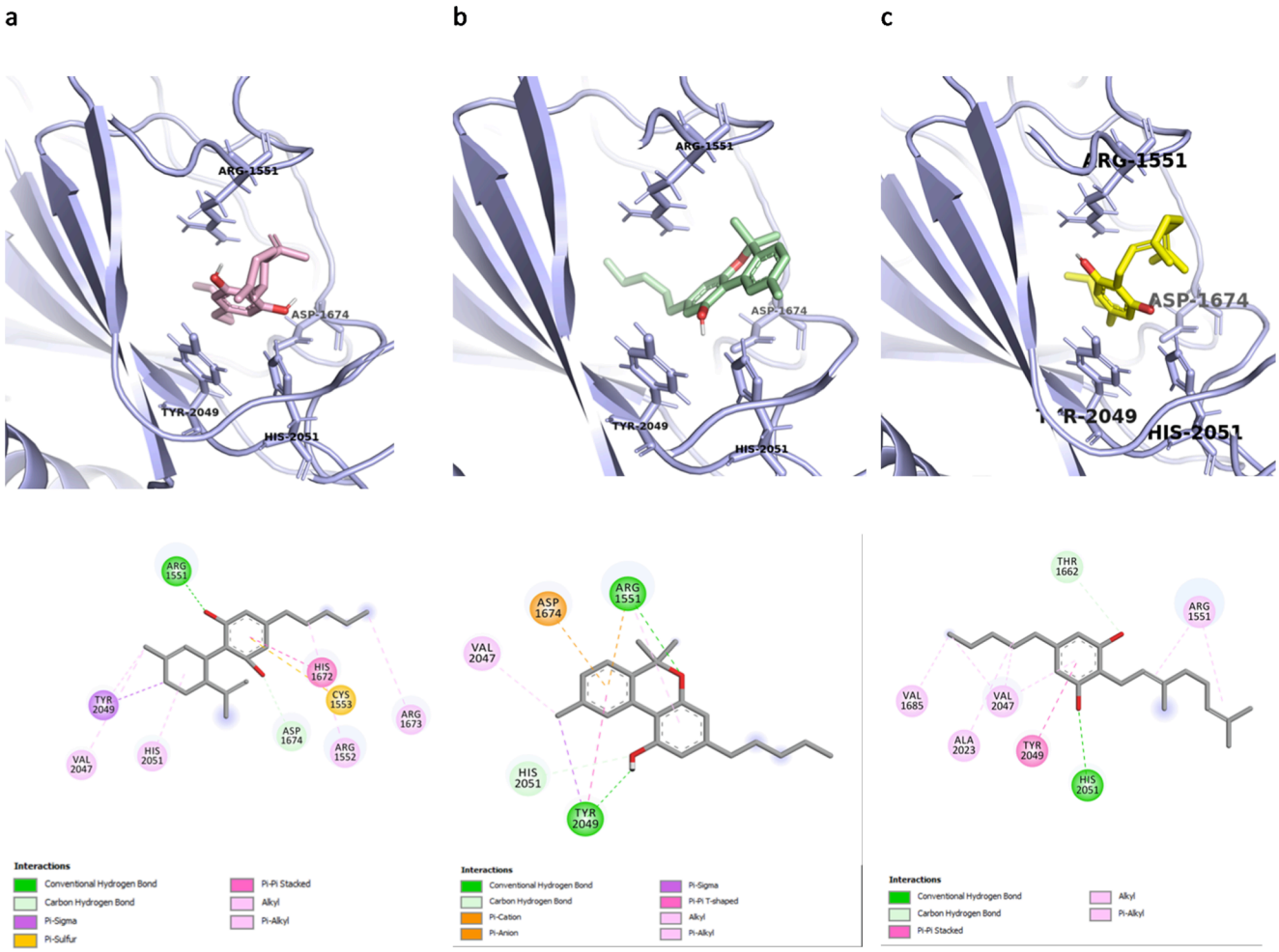

Fig. 6. a) Interaction of CBD b) interaction of CBN c) interaction of CBG with the residues of homology model of TET1.

hydroxymethylcytosine levels are strongly associated with the development of oncological diseases, including lung cancer [28,33].

This suggests that the chronic effects of exposure to cannabinoids could result from the inhibition of TET1 activity and thereby the DNA methylation pattern. However, there are unquestionable limitations of this hypothesis, as listed below.

1) The decrease in TET1 protein activity may have a different phenotypic effect than the suppression of gene expression. For example, it was observed that mutant TET1 protein - without enzymatic activity could modulate gene expression, similar to native proteins [34].
2) In some pathological states, such as mitochondrial iron overloading, the application of CBD may cause an increase in hydroxymethylcytosine levels in mitochondrial DNA, most likely by restoration the activity of succinate dehydrogenase [19].
3) The TET2 and TET3 proteins can also catalyse hydroxymethylation of the methylcytosine group, and their possible effect on gene expression should be considered. TET2 proteins play important roles in the haematopoietic system, and mutation of these proteins is associated with numerous oncological diseases [35]. Unlike the TET1 and TET3 proteins, the single TET2 isoform completely lacks a CXXC domain for the recognition of CpGs and therefore requires the assistance of other proteins containing DNA-binding domains. TET3 protein is highly expressed in oocytes, zygotes and neuronal cells [36]. Changes in hydroxymethylcytosine levels in DNA are associated with neurodegenerative diseases and brain tumours [37]. Nevertheless, it was observed that the neuronal form of the TET3 protein [38,39] mostly prefers CpGs that are modified by 5CaC and binds to unmethylated promoters [40]. Hahn et al. reported that in mammalian neuronal cells, TET3 protein expression was not associated with demethylation in CpGs, but an increase in intragenic 5hmC was found [39]. This may suggest that TET1 may play a prominent role in 5hmC formation at the gene promotor and stimulation of gene expression [39,41].
4) Physiological concentrations of cannabinoids s cannot suffice to significantly inhibit TET proteins. In clinical trials and animal studies, the maximal serum level of CBD with observed clinical effects varied from 36 to 1248 ng/ml [42–44]. Nevertheless, CBD levels that were much smaller than 1 μmol/l were sufficient to achieve a clinical effect. Marijuana and CBD users had concentrations of CBD from 1.8 ng to 3202.3 (10.2 μM) ng/ml in their urine [45]. This may suggest that chronic exposure could be associated with changes in the activity of TET proteins. Another point could be made by analysis of in vitro studies. In the case of cannabinoids anticancer effects, their effective concentrations vary from 10 to 100 μM [46–48]. For the anti-inflammatory effects in lung cells and macrophages, the lowest effective CBD concentration was 3 mg/ml (9.5 μM) [49].
5) The effects of cannabinoids on the activity of TET proteins were not validated by using an in vitro or *in vivo* model. However, the tested cannabinoids s are potent iron chelators, and their effect on iron homeostasis can be neglected. Depending on the type of cells, external conditions and methods used, the intracellular level of free iron ions varies in the interval from submicromal to micromal

concentrations [50]. We suggest that their application at effective inhibition concentrations represented by the determined IC_{50}s will significantly influence cellular iron homeostasis and that the activity of Fe(II)-dependent enzymes such as TET proteins will be significantly repressed. For example, Badal et al. reported that the application of iron chelators to HEK293 cells led to increased global DNA methylation associated with a decrease in TET1 activity [51]. Distinguishing the direct inhibition of TET proteins from decreased activity caused a lower level of free iron ions. This phenomenon could be least partially limited by iron supplements. Our results suggest that a higher level of Fe(II) ions (0.1 mmol/l) did not significantly suppress the CBD and CBN effects on the activity of TET1 proteins. Nevertheless, in the rat model, iron applications lead to the repression of succinate dehydrogenase and thereby decrease the mitochondrial level of hydroxymethylcytosine, and CBD application represses iron-induced effects and thereby restores hydroxymethylation of mitochondrial DNA [19]. Higher succinate levels inhibit TET protein activity [52]. The above results imply that in biological systems, the effect of cannabinoids s on DNA methylation/hydroxymethylation will be dependent on other factors, such as iron levels, and its possible prediction is strongly limited.

Despite these objections, the role of TET proteins in the pleiotropic effects of cannabinoids s on the organism level should not be ruled out. However, for either confirmation or rejection of this hypothesis, further studies involving *in vivo* models are urgently needed.

4. Conclusion

In summary, we demonstrated that some biological properties of cannabinoids can be explained by their affinities for Fe(II) ions. In this study, we observed that the CBD and CBN that exhibit affinity for Fe(II) ions displayed an inhibitory effect on the TET1 protein. A molecular docking study using the TET1 homology model based on TET2 data showed different binding between all three compounds and suggested a higher potential of CBD and CBN to interact with amino acid residues in the active centre; however, the results did not explain the lack of CBG activity. Therefore, even if the observed iron chelating can partially contribute to the inhibitory properties, the molecular interactions with the catalytic cavity are essential for the inhibition. The determined IC_{50} values imply that under chronic exposure, some of the non-psychoactive cannabinoids could cause a reduction in TET1 protein activity. However, the structural attributes necessary for such activity remain elusive and warrant further studies.

5. Experimental procedures

5.1. Materials and methods

All chemicals and solvents were purchased from Sigma–Aldrich (Czech Republic) and were used without further purification. The recombinant TET1 protein and TET hydroxylase activity fluorometric quantification kit were obtained from Active Motif (USA) and Abcam (UK), respectively.

The UV–Vis absorption spectra were recorded using a Varian Cary 400 SCAN UV–Vis spectrophotometer (Varian, USA), in which the reference spectrum of the plain solvent was subtracted from all sample spectra.

5.2. Determination of conditional binding constants and complex stoichiometry of CBD, CBG and CBN with Fe(II) ions

The association of CBD, CBG and CBN with Fe(II) ions was studied using UV–Vis spectroscopy in aqueous solution (water/MeOH, 9:1, v/v) in the same way that was used to study the interactions of the organic hosts with metal ions in aqueous medium [53–56]. Because the solvent always significantly affects the binding constants, all titrations were performed in the same environment, and the ratio of MeOH to water was held constant. Conditional constants (Ks) were calculated from the absorbance changes ΔA of CBD, CBG and CBN at their spectral maxima and spectral maxima of their complexes with Fe(II) by nonlinear regression analysis using the Letagrop Spefo 2005 software.

The concentrations of CBD, CBG and CBN were 100 μM. The concentrations of Fe(II) ions varied in the range of 0–0.5 mM. UV–Vis spectra were measured from 200 to 600 nm, with 1-nm data spacing in a 1-cm quartz cell at a scan rate of 600 nm/min.

5.3. IC_{50} determination of CBD, CBG and CBN for the TET1 protein

The activity of TET1 protein was determined using the Abcam TET Hydroxylase Activity Quantification Kit (Fluorometric). A 96-well microtiter plate was activated according to the manual (application of binding solution and TET substrate). Accurately weighed amounts of CBD, CBG or CBN were dissolved in MeOH to obtain a concentration of 0.01 M in a 1 mL volumetric flask. A total of 25 μg of TET1 protein was diluted in TET1 assay buffer in a 5 mL volumetric flask. The concentrations of ascorbic acid, α-ketoglutarate and ferric sulfate in diluted TET assay buffer were 2, 1 and 0.1 mM, respectively. MeOH solutions of CBD, CBG or CBN were subsequently diluted with TET assay buffer to concentrations of 10^{-6} 5×10^{-6}, 10^{-5}, 5×10^{-5}, 10^{-4}, 5×10^{-4}, and 10^{-3} with 10% MeOH in a 1 mL volumetric flask. Subsequently, 50 μL of the final TET assay buffer was applied, with the addition of TET1 protein and 5 mL of CBD, CBG or CBN solutions, into the prepared microarrays. In the case of control experiments (TET1 alone without cannabinoids), 5 μL of final TET assay buffer with 10% MeOH was used. The tested concentrations of CBD, CBG or CBN were 10^{-7} 5×10^{-7}, 10^{-6}, 5×10^{-6}, 10^{-5}, 5×10^{-5}, 10^{-4}, and 0 M. The subsequent steps of the kit were carried out according to the manual. In the next step, wells were washed with wash buffer, and a capture antibody was applied. In the subsequent step, wells were washed with wash buffer, a detection antibody was added, wells were washed again, and an enhancer solution was added. After the application of the fluorescence development solution, fluorescence was measured at 590 nm (λex = 530 nm) and was used to calculate the residual activity of TET1. We determined IC_{50} values for CBD, CBG and CBN.

5.4. In silico modelling – Molecular docking

For docking studies, a homology model of TET1 was created. The homology model of TET1 is based on the alignment to a solved crystal structure of human TET2 enzyme, which is bound to DNA (PDB code: 4NM6) [25], and the structure provided in the supplementary materials published by Chua *et al.* [24] The section between amino acid numbers 1752 and 1988 was omitted because there was no reliable alignment and because the binding site was not located in this region of the protein (Fig. S1) [24,25].

For the insertion within the alignment as well as for the replacement of some parts of the alignment with no matching prolines, a database of loop conformations SuperLooper2 (SL2) was used [57]. This server uses the conformational space of real loops within crystal structures taken from the Protein Data Bank (PDB) [58]. The quality of the fitting of the loops is rated according to the fitting of the stem atoms, and the alignment of the sequence attaches great importance to the matching of prolines. The final energy minimisation was performed using the DeepRefiner webserver [59]. In the optimisation process, the deep learning model DeepCNF was used, and the 'Adventurous' refinement mode. Fig. S1 presents the visualisation of protein TET2 (PDB code: 4NM6) and the obtained homology model of protein TET1 created with PyMOL, Version 2.3.5 software [60].

Molecular docking was performed with the CB-Dock web server [61], which is based on AutoDock Vina docking software [62]. CB-Dock is a blind docking server that predicts protein binding sites.

First *N*-oxalylglycine, which is the co-crystallised ligand of TET2, was redocked to the TET2 structure (PDB code: 4NM6). Five different cavities were detected, and the cavity with the top docking score (-5.7 kcal/mol) is shown below (Fig. S2a). Visualisation of the protein–ligand complex was created with PyMOL software [60]. All ligand poses from this cavity with the top docking score were visually inspected with BIOVIA Discovery Studio [63], and interactions of the best ligand pose with the protein residues are shown in Fig. S2. Redocking of the co-crystalised ligand showed that the cavity with the top docking score was the same as the binding site of TET2 described in previous works [24,25], and conventional hydrogen bonds with Arg1261, Asp1384, His1904, and Tyr1902 residues were observed (Fig. S2b).

The numbers of residues in the homology model of TET1 are based on the full sequence of the enzyme, and they correspond to the residue numbers of TET2 and are presented in Table S1.

The same docking procedure as in the case of the co-crystalised ligand was repeated in the case of docking of CBN, CBD and CBG ligands into the homology model of the TET1 protein. Vina docking scores are presented in the supplementary materials (Table S2).

All docking poses from this cavity were visually inspected with BIOVIA Discovery Studio Visualiser [63] and 2D interaction diagrams were generated for the best pose and presented. 3D visualisations of the protein–ligand complex were created with PyMOL software [60].

6. Author contributions.

Conceptualisation, project administration and funding were achieved by Milan Jakubek, Michal Masařík, Adam Matkowski and Robert Preissner. Original draft preparation and supervision of the manuscript were performed by Milan Jakubek, Zdeněk Kejík, Adam Matkowski and Andrean Goede. Veronika Antonyová, Tereza Brogyanyi, Petr Novotný and Jakub Gburek performed the TET1 protein measurements and participated in the writing of the manuscript. Zdeněk Kejík, Robert Kaplánek, Kateřina Veselá and Tomáš Ocelka performed the analytical studies and participated in the writing of the manuscript. Nikita Abramenko, Renata Abel, Andrean Goede and Robert Preissner performed *in silico* modelling and molecular docking and participated in manuscript writing.

All authors have read and agreed to the published version of the manuscript.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2022.105793.

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