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Dream over life: Psychedelic terphenyl derivative induce hallucination via cannabinoid receptor 1

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Abstract. For ages, natural psychedelic resources have been used by ancient tribes for religious inspiration. In modern medicine, these compounds were prescribed to relieve severe distress and depression on cancer patients. Despite medical benefit, abuse of these compounds have become prevalent in our modern society. These compounds usually interacted with cannabinoid receptor 1 (CNR1) on neuron cell causing hallucination, and on other cell-types. In this study, chemically synthesized terphenyl derivative, 1,4-di(phenyl)benzene (13-BPB) interaction with human and its animal model were assessed. This derivative is an analogue found in fungi although their functional molecular mechanism is unknown. Terphenyl derivative known to have pharmacological activities - antifungal, anti-cancer, anti-coagulant. Our study designed includes in-vitro assessment and in-silico model of 13-BPB interaction to the molecular mechanism in human and its animal model, mice. Cytotoxicity assessment using MTT has shown that treatment of 13BPB on NIH-3T3 and RAW 264.7 have significant reduction in cell viability at 0.016mM and 0.08mM, respectively. Virtual database screening based on homologous compounds identified possible interaction with 15 different proteins from receptors, enzymes and transcription factors, in human and mice. Further docking analysis shows terphenyl derivatives binding affinities (pKd/pKi) are the highest with CNR1 and oestrogen receptors (ESRs).

1. Introduction

For ages, psychedelic plants and fungi were used by shamans of ancient tribe during religious ritual. They believe that these plants assist their ascension to the spiritual dimension [1]. Psychedelic plants have psilocybin that can alter the states of consciousness or hallucination. In modern medicine, psilocybin was prescribed by the cancer patients as it can relieve severe distress and depression [2].

Depression is synonym with cancer patients due to prolonged hospital admission and having a limited day of being alive. Psilocybin which can be found in *Psilocybe cubensis*, magic mushroom can alter the emotions and perceptions of the patients by causing hyperactivity of frontal regions of brain followed by other hallucinogenic effects such as euphoria. Other effect includes increase body temperature and blood pressure as well as having difficulty in thinking. This due to hypermetabolism



on right hemisphere of brain caused by the consumption of psilocybin [3,4]. At the molecular level, the hallucination is caused by psilocybin interaction with cannabinoid receptors (CNRs) of neuronal cells [5]. Hallucination also can be caused by terphenyl derivatives which mainly found in fungi.

In this study, pharmaceutical potential of a terphenyl analogue found in fungi, 1,4-di(phenyl)benzene (13-BPB) was assessed. Terphenyl derivative, 13-BPB is chemically synthesised which is more eco- friendly that mimic the characteristics of natural terphenyl. Our study designed included in-vitro assessment and in-silico model of 13-BPB interaction to the molecular mechanism in human.

2. Materials and method

2.1. Cell propagation assay

The 500 mL culture media Dulbecco's Modified Eagle's Medium (DMEM) from Nacalai Tescue and Roswell Park Memorial Institute (RPMI, Gibco) were used as the main culture media for the NIH 3T3 fibroblast cells and RAW 264.7 macrophage cells, respectively. Fetal bovine serum and penicillin/streptomycin (Gibco) were added into the media with the volume of 50 mL and 5 mL respectively. Cells were incubated at 36.5°C, 5%CO₂.

2.2. 1,4-di(phenyl)benzene

The 1,4-di(phenyl)benzene (13-BPB) is chemically synthesised terphenyl derivative based on derivative found in mushroom. The crystalized compound was dissolved with DMSO (as a vehicle) at 10mM stock concentration and stored in -20°C.

2.3. Cytotoxicity assessment

Tetrazolium salt, 3-4,5 dimethylthiazol-2,5 diphenyl tetrazolium bromide (MTT) assay was used to determine the cytotoxicity of 13-BPB. NIH 3T3 fibroblast cells and RAW 264.7 macrophage cells were treated for 24 hours' prior addition of 20 µL of 5 mg mL⁻¹ MTT. The incubated for three hours for formazon crystal formation. After that, MTT dye was removed from the wells carefully to avoid disturbance on the cell monolayer. The formed formazon that remained in the were dissolved with DMSO. Absorbance were measured using microplate reader at 570 nm wavelength.

2.4. Virtual screening of potential protein target

Identification of possible proteins targeted by 13-BPB was screened using online database, SwissTargetPrediction (<http://www.swisstargetprediction.ch>). The prediction is based on similarity in 3-dimensional structure of ligands that are known to be interacted with the protein [6]. From the list, top three human proteins from the prediction were selected for docking analysis.

2.5. Molecular docking

The crystal structure of selected proteins was obtained from RSCB protein database. Type of interaction and binding affinities of 13-BPB with the selected proteins were assessed using online SystemsDock application (<http://systemsdock.unit.oist.jp>).

3. Result and discussion

3.1 Cytotoxicity assessment of 13-BPB to NIH 3T3 fibroblasts and RAW264.7 macrophages

The cytotoxicity of 13-BPB was determined by treating NIH 3T3 murine embryonic fibroblast (MEF) and RAW264.7 macrophage cell line for 24 hours at various concentration. Cytotoxic level of vehicle (DMSO) has to be determined to avoid false positive. Both cell-type were treated with vehicle at equivalent concentration to the 13-BPB concentration ratio. The data revealed that the vehicle caused the reduction in cell viability measurement at 0.4mM (4% v/v) and 1mM (1% v/v) for NIH 3T3 MEF and RAW264.7 macrophage cell lines, respectively (figure 1a). 24 hours' treatment with 13-BPB on the NIH 3T3 and RAW 264.7 at gradient concentration, shows significant reduction (two-tailed T-test, $p_{\text{value}} < 0.01$) in cell viability at 3.2 μ M for both cell-type, lower than the vehicle. This indicates that the 13-BPB can induce cell death at very low concentration, less than an order of magnitude micromolar (figure 1b). Previous study revealed that other terphenyl derivatives treatment caused significant reduction in cell viability at concentration from 0.87 μ M up to 0.91 μ M when tested on human cancerous cell lines, lung and hepatocellular carcinoma cells however cytotoxicity was not observed when tested on normal human hepatocytes cell lines [7,8].

3.2 Database screening of protein targeted by 13-BPB

Screening of proteins being targeted by 13-BPB using online database shows 15 potential proteins in human (figure 2a) and mouse (figure 2b). Ranging from transcription factors, enzymes and membrane receptors. The potential interaction was predicted based on similarity in the three-dimensional structure of compound that known to interact with the listed proteins [6]. Almost the same proteins in human and in mice are being targeted by 13-BPB especially in the top five. The top four proteins are membrane receptors that are presence in majority of cell-type including neuronal cells. These receptors not just responsible for neuronal signalling but also will influence activity of cyclin dependent kinases, hence affecting the fate of cell in cell cycle phases and cell death [9,10].

3.3 Molecular docking analysis

A total of five proteins were selected for docking analysis to determine binding affinities and to characterise their interaction structure. The selection is based on the highest probability scores and availability of crystallised structure in the RSCB protein database. Results from 13-BPB docking were compared with the native ligand of selected proteins (figure 2c, d). The model predicted that 13-BPB highest bind affinity is with the CNR1 at 5.622 pKd/pKi, even stronger than the native ligands (Taranabant) which is at 3.4 pKd/pKi. Taranabant is a cannabinoid-1 receptor reverse agonist that used as anti-obesity where it can induce the feeling of satiety [11]. Structural inspection on the docking site shows that 13-BPB docks on the same site as Taranabant (figure 3). A total of nine amino acid residues hydrophobically interacted with 13-BPB compared to 12 residues with Taranabant (table 1). Both share eight amino acid residues thus indicate potential competitor or could cause very similar physiological outcome. Higher binding affinity (pKd/pKi) of 13-BPB is owing to its structure is more stable and has higher surface area than Taranabant, which has open structure thus unstable to allow optimal hydrophobic interaction.

13-BPB also interacted with receptors for steroid hormones such as androgen receptor (AR), estrogen receptor 1 (ESR1) and estrogen receptor 2 (ESR2) which affecting the cell cycle. This interaction would affect MAPK signalling pathway that involved in cell proliferation and cell survival [9]. For example, 13-BPB also interacted with ESR2, the second highest protein after CNR1. However, the binding affinity of 13-BPB with ESR2 at 5.037 pKd/pKi is lower compared to its native ligand, Genistein at 5.6 pKd/pKi. Genistein is an isoflavone that had been used in cancer therapy together with protein tyrosine kinase where it activates MAPK pathway thus inhibits the inflammatory action of cancer cells [12]. Structural inspection on the docking site shows that 13-BPB docks on the same site as Genistein (figure 4). A total of eleven amino acid residues form hydrophobic interaction with 13-BPB compared to eight residues with Genistein (table 2) and both share five amino acid residues. Higher binding affinity of Genistein than 13-BPB is due to the formation of hydrogen bond of oxygen atom of Genistein with the arginine-346 and Histidine-475. While 13-BPB only interacted hydrophobically with the residues which is weaker compared to the hydrogen bond.

Interaction of 13-BPB with CNR1 and receptors for steroids that directly connected to the MAPK signalling pathway would have significantly affect homeostasis of this pathway and the downstream physiological components. Notably affecting the homeostasis of cyclin-dependent kinases of cell cycle hence may causes the cell-death as observed in cytotoxicity assessment (figure 1).

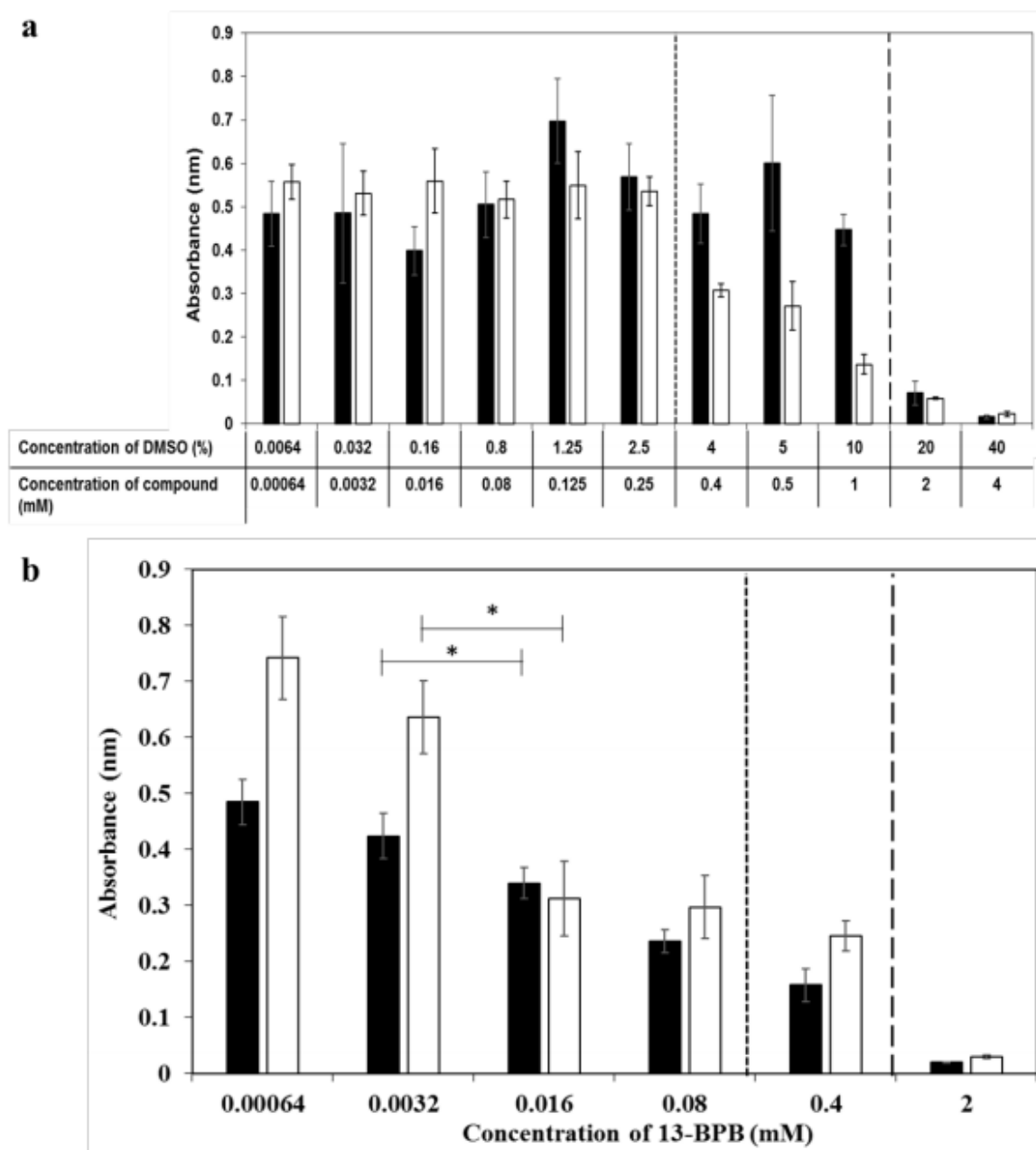


Figure 1. MTT assay for assessment of 13-BPB cytotoxicity. Treatment of NIH-3T3 murine embryonic fibroblast cell line (□) and RAW264.7 macrophage cell line (■) with DMSO (a) and 1,4-di(phenyl)benzene (13-BPB) (b). Dotted line and dashed line indicate assay limit threshold for NIH3T3 fibroblast and RAW264.7 macrophage, respectively. Both cell density; 1×10^4 cell/well in 96 wellplate. Error bars mean \pm SE; $n=8$, * indicate $p_{\text{value}} < 0.01$ for two-tailed Student's T-test.

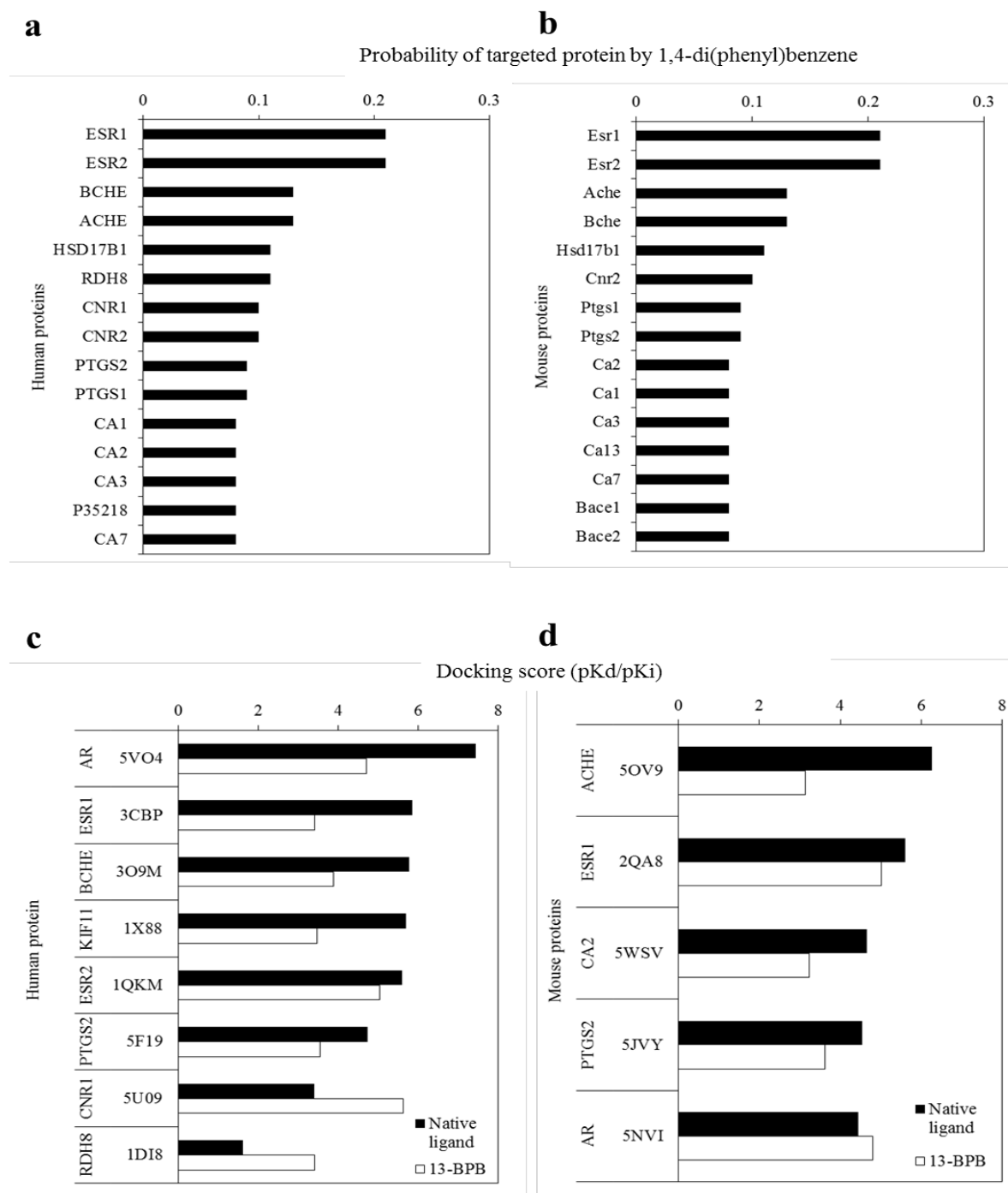


Figure 2. List of potential proteins to be targeted by synthesized 1,4-di(phenyl)benzene in human (a) and mouse (b). The prediction is based on similarity in 3-dimensional structure of known ligands available in www.swisstargetprediction.ch. The protein abbreviation is based on GeneCard ID or UniProt ID. Binding strength of 1,4-di(phenyl)benzene interaction with human (c) and mouse (d) proteins in comparison with their native ligands. The binding strength was determined based on docking score. Unlisted proteins indicate the database could not determine type of interaction with the predicted proteins in fig. 2. ACHE is acetylcholinesterase; AR is androgen receptor; BCHE is cholinesterase; CA2 is carbonic anhydrase; CNR1 is cannabinoid receptor 1; ESR1 is estrogen receptor; ESR2 is estrogen receptor beta; KIF11 is kinesin-like protein KIF11; PTGS2 is prostaglandin G/H synthase 2; RDH8 is retinol dehydrogenase 8. The protein ID is based on GeneCard ID and PDB ID.

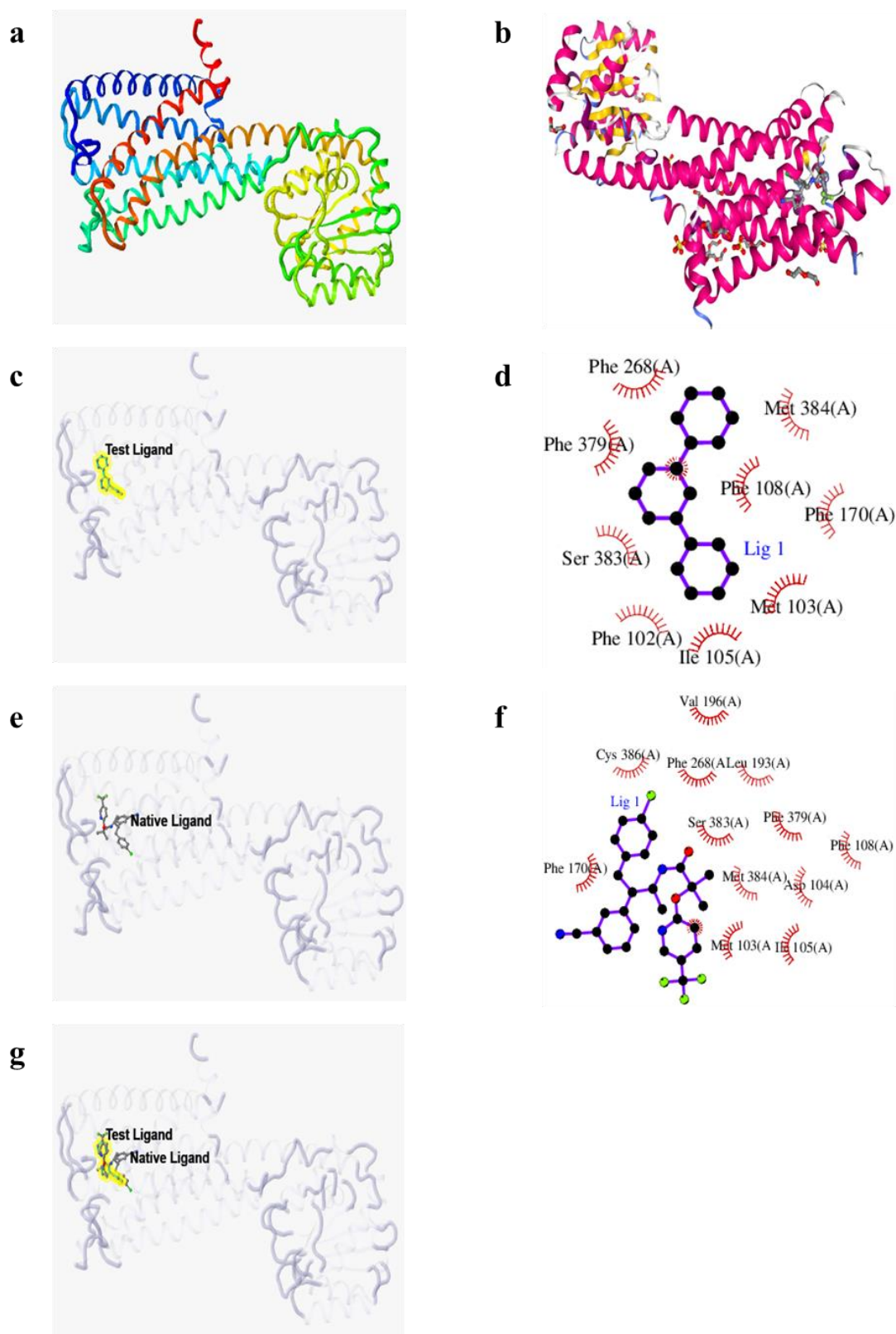


Figure 3. Docking simulation between cannabinoid receptor 1 (CNR1; PDB ID: 5U09) and 1,4-di(phenyl)benzene. (a) 1,4-di(phenyl)benzene (c) shows the synthesized compound dock at the same binding Taranabant (b and e), the native compound (e). 2D views of CNR1 docking result for native ligand (F) and 1,4-di(phenyl)benzene (d). (g) Merge 3D view of synthesized compound and Taranabant at CNR1 docking site.

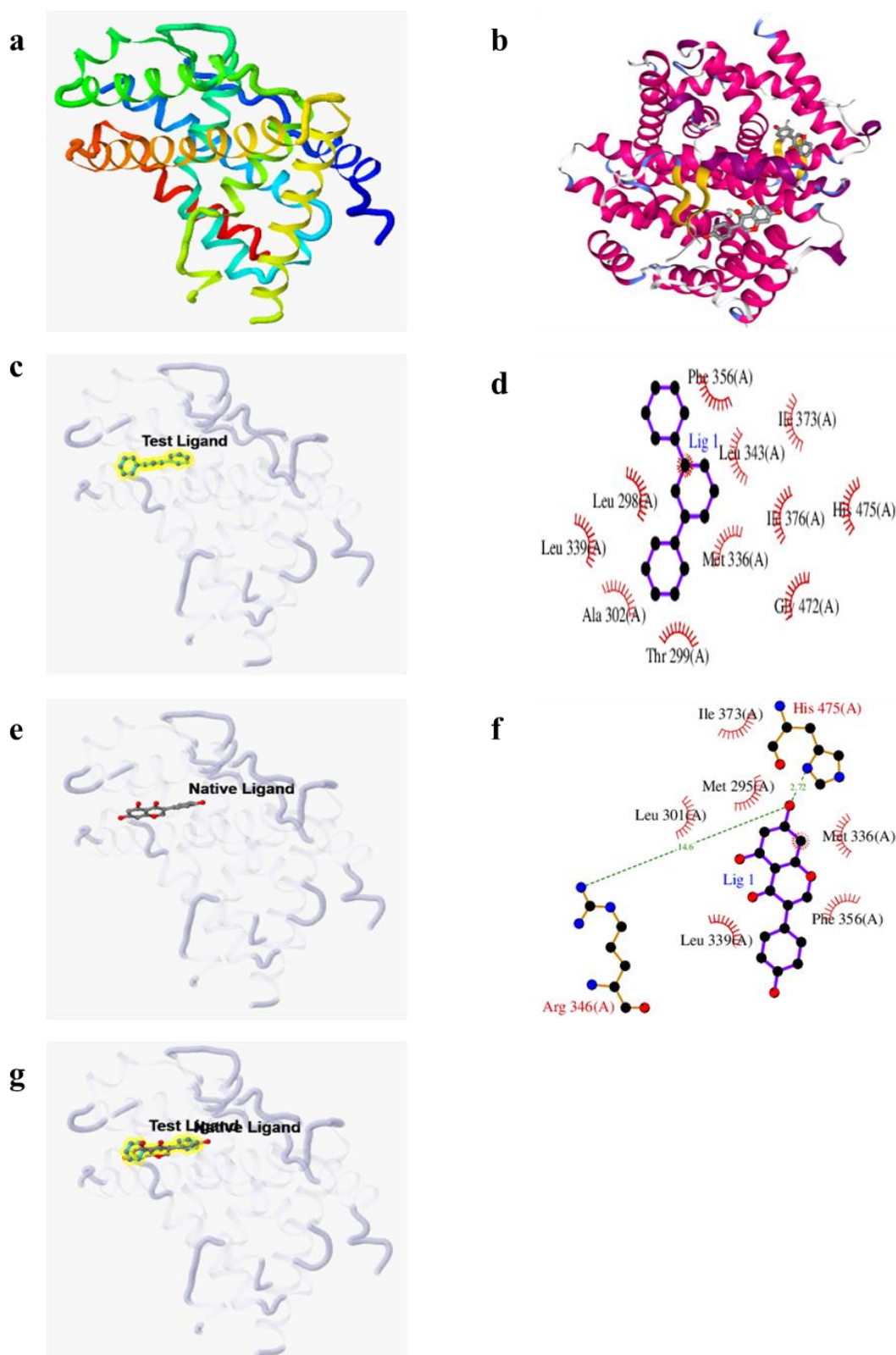


Figure 4: Docking simulation between estrogen receptor beta (ESR2), PDB ID: 1QKM (a) 1,4-di(phenyl)benzene (c) shows the synthesized compound dock at the same binding site as Genistein (b and e), the native compound (e). 2D views of ESR2 docking result for native ligand (f) and 1,4-di(phenyl)benzene (d). (g) Merge 3D view of synthesized compound and Genistein at ESR2 docking site.

Table 1: A total of 9 amino acid residues form an interaction with 1,4- di(phenyl)benzene. In comparison, taranabant (native ligand) interacted with 12 residues at the CNR1 docking site, although only 8 out of 12 amino acid residues are listed in this table.

1,4-di(phenyl)benzene	Taranabant
MET ₁₀₃	MET ₁₀₃
ILE ₁₀₅	ILE ₁₀₅
PHE ₁₇₀	PHE ₁₇₀
PHE ₂₆₈	PHE ₂₆₈
PHE ₃₇₉	PHE ₃₇₉
SER ₃₈₃	SER ₃₈₃
PHE ₁₀₈	PHE ₁₀₈
MET ₃₈₄	MET ₃₈₄
PHE ₁₀₂	-

Table 2: A total of 11 amino acid residues form an interaction with 1,4-di(phenyl)benzene. In comparison, genistein (native ligand) interacted with 8 residues at the ESR2 docking site, although only 5 out of 8 amino acid residues are listed in this table.

1,4-di(phenyl)benzene	Genistein
HIS ₄₇₅	HIS ₄₇₅
LEU ₃₃₉	LEU ₃₃₉
MET ₃₃₆	MET ₃₃₆
PHE ₃₅₆	PHE ₃₅₆
ILE ₃₇₃	ILE ₃₇₃
ILE ₂₉₈	-
THR ₂₉₉	-
ILE ₃₇₆	-
GLY ₄₇₂	-
ALA ₃₀₂	-
LEU ₃₄₃	-

4. Conclusion

Our study shows potential hallucinogen based on terphenyl derivatives found in mushrooms due to interaction with the receptor associated with hallucination. The compound, 13-BPB form a strong hydrophobic interaction with cannabinoid receptor 1 (CNR1) at the same docking site of known agonist, Taranabant with higher binding affinity. The interaction almost similar to Taranabant but with higher affinities indicate more potent inverse agonist for CNR1, potentially could cause similar side effect but worse. The compound also interacted strongly with other receptors such as ESRs family. Both CNR1 and ESRs are connected with cyclin-dependent kinases through MAPK signalling pathway thus may affecting the cell cycle and cell death. Cytotoxicity assessment reveals high toxicity of 13BPB at micromolar range.

5. Reference

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