




RESEARCH ARTICLE

REVISED Quinazoline-2,4(1H,3H)-dione derivatives as new class of CB1 Agonists: A pharmacophore-based virtual screening workflow and drug discovery

[version 3; peer review: 3 approved]

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Abstract**Background**

The cannabinoid 1 (CB1) receptor is the primary target of Δ^9 -tetrahydrocannabinol (Δ^9 -THC), the psychoactive component of cannabis sativa (commonly known as "kif" in Morocco).

Methods


Here, we identified novel CB1 agonists using virtual screening approaches. First, we developed a pharmacophore model based on the known CB1 agonist AM11542 and screened a database of over three million compounds. Molecular docking using AutoDock Vina identified 61 hits with binding affinities of less than -9.00 Kcal/mol. Subsequent ADME-Tox (absorption, distribution, metabolism, excretion, and toxicity) analysis narrowed the selection to 18 promising candidates.

Results

Among these, three agonists exhibited strong characteristics, including a favorable inhibition constant (K_i) and key hydrogen-bond interactions with critical residues in the CB1 binding pocket:

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PUBChem157251136 ($K_i=2.09$ nM), ZINC64438485 ($K_i=0.262$ nM) and ZINC64438506 ($K_i=0.244$ nM). These agonists formed stable hydrogen bonds with CB1 binding pocket residues (Ser383, Ser173, His178 and Thr197). Molecular dynamics simulations (100 ns, GROMACS) demonstrated structural stability (RMSD < 1 nm) and low conformational flexibility (RMSF < 1 nm) for all complexes. MM-GBSA binding free energy calculations further confirmed the thermodynamic stability of all complexes, with interaction energies ranging from -30.59 to -49.98 kcal/mol. These comprehensive simulations confirm that all identified agonist complexes maintain stable binding conformations with optimal interaction profiles characteristic of CB1 receptor activation.

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article can be found at the end of the article.

Conclusion

These results could pave the way for researching and developing new quinazolin-2, 4(1H, 3H)-Dione derivatives as a new class of CB1 receptor agonists.

Keywords

CB1 agonists, pharmacophore based virtual screening, lead discovery, quinazoline-2, 4(1H, 3H)-dione derivatives



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REVISED Amendments from Version 2

Details of the manuscript update

Introduction section:

In the paragraph after Figure 1, line 2: "The AM11542CB1" was changed to "The AM11542-CB1".

In the paragraph after Figure 1, line 7: "is most likely do to the flexibility" was changed to "is most likely due to the flexibility".

In Table 2: "Kcal" was changed to "kcal" to correct the unit notation.

Any further responses from the reviewers can be found at the end of the article

I. Introduction

Cannabinoid receptors are classified as G protein-coupled receptors which belong to a family known as the endocannabinoid system, a master regulator of numerous physiological pathways throughout the human body.¹ There are two categories of cannabinoid receptors, known as CB1 and CB2. The CB1 receptor is found throughout multiple organs of the body; it is present in the digestive tract, liver, pancreas, and musculature, in addition to its primary site of localization being in the brain.² In fact, CB1 is the most highly expressed receptor in the brain relative to other receptors examined, possessing 7 transmembrane domains with G protein coupling. The CB1 receptor mediates cannabinoid-induced psychotropic effects. The CB2 receptor is more so located within immune cells, where it exerts its immunomodulatory role.

Cannabinoids are a class of chemical compounds which are championed globally for their psychoactive and physiologic whole body functions; for at least 5,000 years,^{3,4} mankind has been able to capitalize on the value of cannabinoids. One such natural source of cannabinoids is cannabis, known as "kif" in Moroccan culture, a natural product with a plethora of phytoconstituents including Δ^9 -tetrahydrocannabinol (THC). The phytochemical THC exerts its psychotropic effects by acting at the CB1 receptor, which also serves as the primary receptor for the endogenous endocannabinoids anandamide (AEA) and 2 arachidonoylglycerol (2-AG).⁵ Activation of the CB1 receptor upregulates potassium channel currents and calcium ion channel polarization, making receptor signaling dose dependent and responsive to treatment with pertussis toxin.⁶ In addition, CB1 can exist as a homodimer and/or form complexes as heterodimers or heterooligomers with other G Protein-Coupled Receptors (GPCRs). Lastly, the CB1 receptor is in a complex with GABAergic and glutamatergic cells, and therefore, CB1 receptor stimulation decreases release from GABAergic and glutamatergic cells.⁷

The structural complexes of the cannabinoid receptor CB1 with THC analogs are an important topic of research to help us understand the molecular interactions that underlie cannabinoid signaling. Thus far, the repertoire of known complexes of the CB1 receptor includes the structure of CB1, bound to AM8411,⁸ CP55940⁹ and AM11542¹⁰ (Figure 1). These structures have a resolution of 2.8-3.4 Å and provide a highly informative account of the binding modes and associated conformational changes that can accompany receptor activation. Several important interacting residues have been found to be conserved between agonist-bound structures, including hydrophobic interactions with LEU193^{3,29}, VAL196^{3,32}, PHE200^{3,36}, TYR275^{5,39}, LEU276^{5,40}, TRP279^{5,43}, TRP356^{6,48}, LEU359^{6,51} and MET363^{6,55}.¹¹ These observations further emphasize the importance of these residues in their role of stabilizing binding of agonists to the receptor, while also promoting receptor activation.

There were also three structures solved by means of cryo-electron microscopy (cryo-EM) that included full active states along with the Gi1-2 G protein subunit.¹² The AM11542-CB1 complex is a crystalline structure and had a fusion protein, flavodoxin, which contributes to stabilization of TM6 in an active confirmation.¹¹ All of the structural coordinates cover the full CB1 protein sequence, covering anywhere between approximately 58% and 62% of each residues in all active structures.⁸ The fact that the coordinates do not cover the full sequence is most likely due to the flexibility imparted by both the N and C-terminus and the long three intracellular loops (ICL3). These structural descriptions not only allow constructs for determining the molecular mechanisms for THC analog activation of the CB1 receptor but also create opportunities for future studies.

In recent years the harmonization of approaches have created several avenues to create CB1 receptor agonists with less adverse effects. This study will previously develop a CB1 agonist pharmacophore model that was used as a virtual screening tool for unique/novel class of CB1 agonists. Using the pharmacophore this was able to screen over 300 million hits, of compounds, parsed from 11 different databases and were able to identify many candidates that have not been

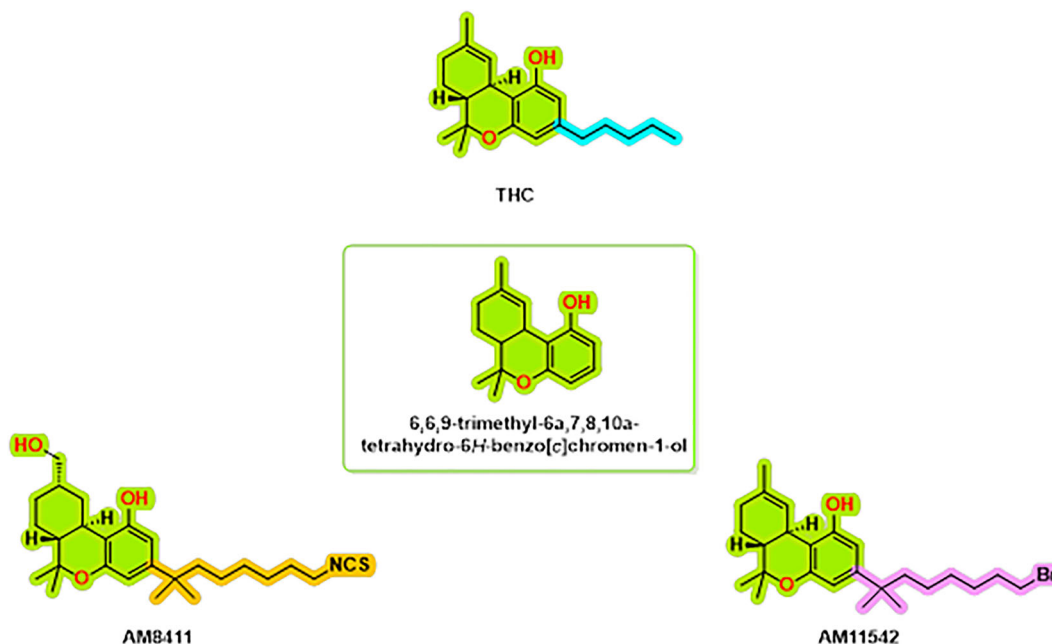


Figure 1. CB1 receptor agonist chemical structures.

screen for cannabinoid receptor binding assay. This highlights the usefulness of computational strategies to find novel therapeutics that have the potential to provide safer alternatives.

II. Materials and method

1. Pharmacophore modeling and virtual screening

Pharmacophore based virtual screening were performed using the Pharmit web interface (<http://pharmit.csb.pitt.edu/>), which has a number of built-in databases that provide access to comprehensive data; Molprot (4,742,020 molecules), ChEMBL34 (2,264,112 molecules), ZINC (13,127,550 molecules), ChemDiv (1,456,120 molecules), ChemSpace (50,181,678 molecules), Enamine (4,117,328 molecules), MCULE (39,843,637 molecule), MCULE-ULTIMATE (126,471,502 molecules), NCI Open Chemical Repository (52,237 molecules), LabNetwork (1,794,286 molecules), and PubChem (103,302,052 molecules). The pharmacophore model was constructed using selected PDB code 5XRA from the RCSB Protein Data Bank (<http://www.rcsb.org/structure/5xra>), with agonist AM11542. The model utilized a pharmacophore framework built on five features, adhering to the default parameters of the Pharmit server. The Pharmit filters were applied based on the Lipinski rule of five and Veber's rule to refine the screening process and identify the most selective CB1 agonist. **Figure 2** illustrates the multi-step virtual screening process used in this work.

2. ADMET profiling

The ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) filter is one of the phases of the drug discovery and development process.^{13–15} This allows researchers to determine potentially druggable drug-like properties and toxicology early on to assess which compounds will be effective drugs with safety potential. Thus, relative to virtual screening, such a filter can detect adverse properties that would fail at much later stages, such as insufficient absorption, excessive toxicity, and/or suboptimal bioavailability. Two of the trusted websites relied upon to evaluate such properties based upon lipophilicity, logP, solubility, etc., are SwissADME (<http://www.swissadme.ch>) and pKCSM (<https://biosig.lab.uq.edu.au/pkcsml/prediction>). Therefore, when these are filtered out early on, time and costs will be effectively saved down the line.

3. Molecular docking studies

To estimate the binding affinities of the 433 highest-ranked compounds to CB1, molecular docking was carried out using AutoDock Vina integrated within the PyRx platform. The crystal structure of CB1 (PDB ID: 5XRA), with a resolution of 2.8 Å,^{8,16,17} was retrieved from the Protein Data Bank. Before docking, water molecules and the native ligand were removed from the protein, followed by the addition of polar hydrogen atoms and Kollman charges. The prepared protein structure was then energy-minimized using UCSF Chimera and saved in PDBQT format.

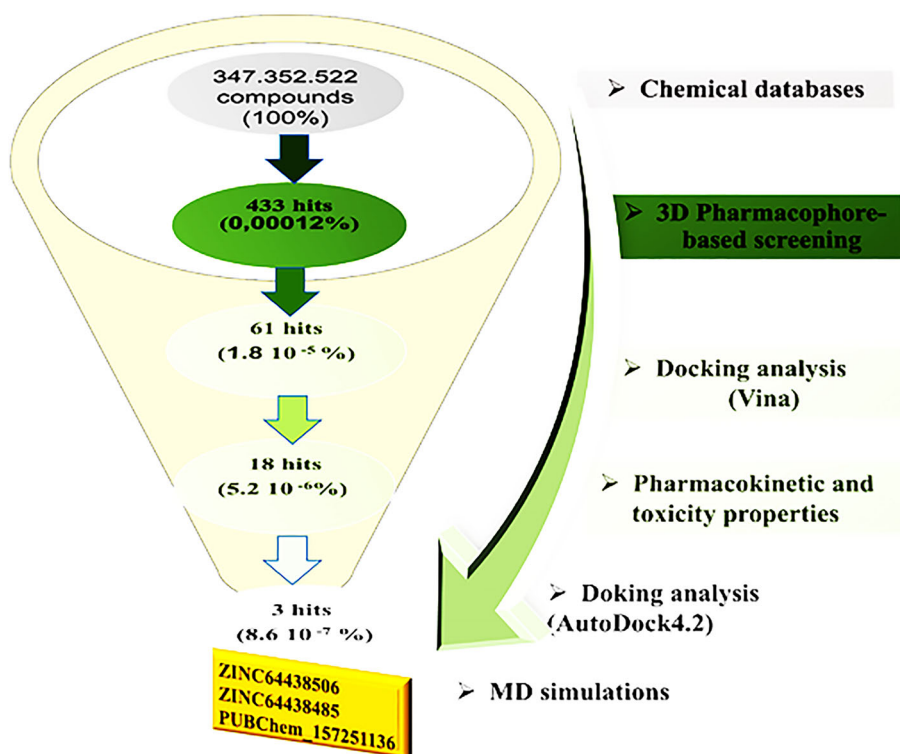


Figure 2. General workflow used in the present study for identifying new CB1 agonists.

Prior to docking, all 433 compounds underwent pre-optimization utilizing the Universal Force Field (UFF) in combination with the conjugate gradient algorithm.¹⁸ Optimization parameters were set to 2000 total steps, with updates occurring every step, and the process was programmed to terminate once the energy difference fell below 0.01 kcal/mol.¹⁹ After optimization, the compounds were converted to PDBQT format and docked at specific binding site coordinates ($x = -42.052$, $y = -164.338$, $z = 306.631$). Molecules demonstrating the lowest binding energies and root-mean-square deviation (RMSD) values below 2 Å were selected for subsequent analyses.

4. Molecular dynamics simulations

Molecular dynamics (MD) simulations were carried out on the top-ranked docking conformations using the GROMACS 2024.4 software package, employing the CHARMM27 all-atom force field.²⁰ The topology files for the CB1 receptor were generated using the pdb2gm tool,²¹ while the ligand topologies were prepared through the CHARMM General Force Field (CGenFF) using the Param-Chem server.^{22–24} Each receptor-ligand complex was embedded in a triclinic simulation box and solvated with TIP3P water molecules, maintaining a minimum distance of 1.0 nm from the box boundaries. To neutralize the systems, Na⁺ and Cl⁻ ions were added accordingly.

Prior to the production runs, energy minimization was performed using the steepest descent algorithm for 50,000 steps to eliminate steric clashes and ensure system stability. Subsequently, two equilibration phases were conducted: first under the NVT ensemble to stabilize temperature, followed by the NPT ensemble to equilibrate pressure and density. Input files for both equilibration and production stages were customized to adjust parameters such as trajectory-saving intervals, energy monitoring, and other essential simulation settings. Finally, each ligand-receptor system underwent 100 ns of production MD simulation at a constant temperature of 300 K, pressure of 1 bar, and a time step of 2 femtoseconds.

5. MM/GBSA method

The binding free energy was calculated via MM/GBSA (Molecular Mechanics Generalized Born Surface Area).²⁵ This approach utilizes force fields of molecular mechanics and an implicit solvent approach to conclude stability and binding tendencies of molecular complexes. It is particularly useful for classifying ligands and understanding overall energetic contributions of molecular recognition. The binding free energy ΔG_{bind} of a complex can be calculated as follows:

$$\Delta G_{\text{bind}} = \Delta E_{\text{MM}} + \Delta G_{\text{solv}} - \Delta \text{TAS} \quad (\text{Eq.1})$$

Where:

$$\Delta E_{MM} = E_{MM}^{complex} - (E_{MM}^{receptor} + E_{MM}^{ligand}) \quad (\text{Eq.2})$$

$$\Delta G_{solv} = G_{solv}^{complex} - (G_{solv}^{receptor} + G_{solv}^{ligand}) \quad (\text{Eq.3})$$

$$G_{solv} = G_{GB} + G_{SA}$$

Where $T\Delta S$, ΔE_{MM} and ΔG_{sol} are the conformational entropy upon binding, the changes of the gas phase molecular mechanism (MM) energy and the solvation free energy, respectively.

III. Results and discussion

1. Pharmacophore

The pharmacophore model of the CB1 receptor agonist was generated based on the AM11542 agonist. The results are shown in Figure 3. A five-point model with 1 aromatic, 2 hydrogen bond acceptors and 2 hydrophobic regions was generated using the Pharmit web server.²⁶

Subsequently, virtual screening was performed across 11 databases, identifying 433 compounds containing molecular groups that matched the pharmacophore mode.

2. Virtual screening

Pharmacophore-based virtual screening was performed using information from the previous pharmacophore model. Approximately 300 million compounds from the Pharmit database were filtered. The criteria for filtering the library were as follows: molecular weight limits were set at 250-500 Da, the maximum number of hydrogen bond donors was less than 4, the maximum number of hydrogen bond acceptors was less than 9, the maximum number of rotatable bonds was less than 9, Log P was between 2 and 5, and polar surface area was less than 140 Å. The results are computed and classified according to different criteria such as energy minimization. The 433 top-ranked compounds are presented in Table 1.

Next, molecular docking analysis was performed using AutoDock Vina, implemented in the PyRx software. Compounds with a binding affinity of less than -9.00 kcal/mol were selected for further analysis, while the others were excluded. Additionally, the predicted binding modes were required to have a root-mean-square deviation (RMSD) of less than 2Å when superimposed onto the native agonist (AM11542). The top 61 selected compounds are listed in Table 2, while the remaining candidates are provided in Table S1 (see supplementary data).

3. Toxicity filters

The ranked compounds from docking analysis were evaluated for potential toxicity, including an AMES toxicity test, an acute oral toxicity test in rats (LD_{50}), a skin sensitization test and a maximum tolerated dose analysis. Highly toxic compounds are not considered in further studies. The top selected compounds are presented in Table 3. The other are presented in the supplementary data (Table S2).

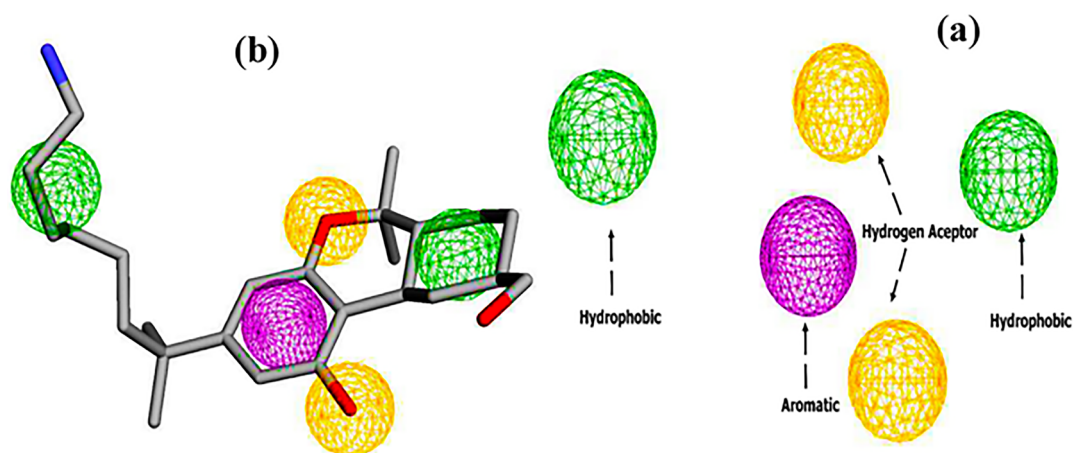


Figure 3. Pharmacophore features (a) two hydrogen bond acceptors (2HBA, yellow color), two hydrophobic groups (2HY, Green color), and one aromatic group (1AR, purple color), (b) Pharmacophore feature mapping of AM11542 agonist.

Table 1. Pharmacophore-based virtual screening of compounds from 11 databases on the Pharmit server.

Pharmit database	Molecules	Hits
Molprot	4,742,020	58
ChEMBL34	2,264,112	134
ZINC	13,127,550	32
ChemDiv	1,456,120	76
ChemSpace	50,181,678	0
Enamine	4,117,328	5
MCULE	39,843,637	23
MCULE-ULTIMATE	126,471,502	0
NCI Open Chemical Repository	52,237	0
LabNetwork	1,794,286	63
PubChem	103,302,052	42
Total	347.352.522	433

Table 2. Top 61 compounds selected based on docking binding affinity (binding affinity < -9.00 Kcal/mol) and rmsd (rmsd < 2 Å) values.

Code	Binding affinity (kcal/mol)	rmsd	Code	Binding affinity (kcal/mol)	rmsd
ZINC35377792	-10.526	1.766	ZINC21525754	-9.349	1.086
ZINC17286185	-10.487	1.825	ZINC35377780	-9.311	1.265
ZINC45898833	-10.415	1.964	PubChem-16352732	-9.308	0.823
ZINC35562518	-10.410	1.055	PubChem-136659176	-9.294	1.082
ZINC35377809	-10.202	1.239	PubChem-135405892	-9.292	1.457
ZINC64438506	-10.189	1.395	PubChem-126853168	-9.271	1.275
PubChem-156469643	-10.074	1.867	PubChem-25220434	-9.254	1.924
PubChem-89734004	-10.054	1.291	ZINC20138980	-9.240	1.274
PubChem-50762870	-10.049	1.717	ZINC169	-9.236	1.227
PubChem-86747888	-10.035	1.776	ZINC96385691	-9.222	1.955
ZINC45899482	-10.006	1.796	ZINC65196494	-9.217	0.750
ZINC64438485	-9.944	1.964	MolPort-009-386-250	-9.208	1.702
PubChem-121231416	-9.858	1.197	PubChem-41119771	-9.192	1.542
ZINC21797190	-9.850	1.617	ZINC20113894	-9.173	0.794
ZINC45899726	-9.828	1.718	ZINC20113894	-9.173	0.794
ZINC45900106	-9.824	1.499	ZINC35377767	-9.130	1.670
PubChem-53794837	-9.820	1.352	PubChem-71600230	-9.127	1.653
ZINC45899547	-9.785	1.651	ZINC45899386	-9.127	1.416
CHEMBL1652254	-9.763	1.547	ZINC35377767	-9.121	1.351
ZINC2245716	-9.652	1.160	ZINC13365292	-9.100	1.219
ZINC45900103	-9.615	1.977	LN00379431	-9.098	1.083
ZINC35377763	-9.518	1.176	PubChem-3750748	-9.095	1.627
PUBChem 157251136	-9.492	1.362	ZINC4034881	-9.089	1.300
ZINC09598984	-9.489	1.654	ZINC65196500	-9.068	1.934
ZINC35377731	-9.485	1.417	ZINC100771598	-9.068	1.445

Table 2. *Continued*

Code	Binding affinity (kcal/mol)	rmsd	Code	Binding affinity (kcal/mol)	rmsd
ZINC 35377767	-9.465	1.474	PubChem-25352696	-9.067	1.172
ZINC 21723065	-9.463	1.471	PubChem-91428044	-9.045	1.614
PubChem-42810820	-9.454	1.742	ZINC35377731	-9.034	1.382
ChemDiv-C260-2692	-9.424	1.461	ZINC33057775	-9.027	1.058
PubChem-135869534	-9.413	1.985	ZINC96385592	-9.010	1.069
PubChem-91487881	-9.353	1.714			

As described in [Table 3](#), all selected compounds exhibited no Skin Sensitisation and no AMES toxicity. Additionally, the LD₅₀ values for oral rat toxicity, ranging from 1.439 to 3.354 mol/kg, suggest moderate acute toxicity levels, consistent with safety margins suitable for therapeutic use. Moreover, the maximum tolerated dose in humans' ranges from -1.12 to 1.059 Log mg/kg/day.

Table 3. *In silico* prediction of Skin Sensitisation. AMES toxicity, oral rat acute toxicity (LD₅₀), and Max Tolerated dose in humans.

Code	Skin Sensitisation	AMES toxicity	Oral Rat Acute Toxicity (LD ₅₀ (mol/kg))	Max. tolerated dose (human) (Log mg/kg/day)
ZINC35377792	No	No	2.785	0.412
ZINC17286185	No	No	2.474	0.222
ZINC45898833	No	No	2.617	0.328
ZINC35562518	No	No	2.441	0.215
ZINC64438506	No	No	2.744	0.685
PubChem-156469643	No	No	2.904	-1.12
PubChem-89734004	No	No	3.02	-0.825
PubChem-86747888	No	No	3.001	-0.913
ZINC64438485	No	No	2.816	0.381
PubChem-121231416	No	No	2.818	-0.595
ZINC21797190	No	No	3.085	0.61
ZINC45899726	No	No	2.748	0.647
PubChem-53794837	No	No	2.569	-0.725
CHEMBL1652254	No	No	3.004	0.428
ZINC2245716	No	No	2.469	0.586
PUBChem 157251136	No	No	2.812	-0.095
Zinc35377731	No	No	2.863	0.328
Zinc21723065	No	No	2.544	0.463
PubChem-42810820	No	No	2.73	0.562
ChemDiv-C260-2692	No	No	2.475	0.6
PubChem-135869534	No	No	3.077	0.604
PubChem-91487881	No	No	2.433	-0.088
ZINC21525754	No	No	2.753	-0.134
PubChem-16352732	No	No	2.218	0.971
PubChem-136659176	No	No	2.242	0.572
PubChem-135405892	No	No	3.075	0.607
PubChem-126853168	No	No	2.67	0.443

Table 3. *Continued*

Code	Skin Sensitisation	AMES toxicity	Oral Rat Acute Toxicity (LD ₅₀ (mol/kg))	Max. tolerated dose (human) (Log mg/kg/day)
ZINC65196494	No	No	3.354	-0.164
PubChem-41119771	No	No	2.38	0.016
ZINC20113894	No	No	2.574	0.429
ZINC20113894	No	No	2.574	0.429
PubChem-71600230	No	No	2.048	0.395
ZINC13365292	No	No	2.604	0.134
LN00379431	No	No	2.567	0.663
PubChem-3750748	No	No	3.038	-0.137
ZINC4034881	No	No	2.576	0.549
ZINC65196500	No	No	3.156	-0.141
ZINC100771598	No	No	3.008	0.471
PubChem-25352696	No	No	2.552	1.059
PubChem-91428044	No	No	1.439	0.563
ZINC35377731	No	No	2.863	0.328
ZINC33057775	No	No	2.503	0.769

4. Physicochemical properties and bioavailability

Physicochemical properties were evaluated using Lipinski's rule of five,²⁷ Ghose's rule,²⁸ Veber's rule,²⁹ Egan's rule³⁰ and Muegge's rule,³¹ with results detailed in Table 4. Based on these guidelines, it is suggested that for a compound to be effectively absorbed and administered orally, it must meet specific physicochemical parameters. These criteria serve as essential benchmarks for assessing the compound's potential for bioavailability and oral absorption. Compounds with two or more violations are not considered in the further analysis (see Table S3 in supplementary data).

Table 4. Physico-chemical properties based on the rules of Lipinski, Ghose, Veber, Egan and Muegge for the highest ranked compounds.

Code	Lipinski #violations	Ghose #violations	Veber #violations	Egan #violations	Muegge #violations
ZINC17286185	0	1	0	0	0
ZINC35562518	0	1	0	0	0
ZINC64438506	0	1	0	0	0
PubChem-156469643	0	0	0	0	1
PubChem-89734004	0	0	0	0	0
PubChem-86747888	0	0	0	0	1
ZINC64438485	0	1	0	0	0
PubChem-121231416	0	0	0	0	0
ZINC21797190	0	0	0	0	0
PubChem-53794837	0	0	0	0	1
CHEMBL1652254	0	0	0	0	1
PUBChem 157251136	0	0	0	0	0
PubChem-42810820	0	0	0	0	0
ChemDiv-C260-2692	1	1	0	0	0
PubChem-135869534	0	0	0	0	0
PubChem-91487881	0	0	0	0	0

Table 4. Continued

Code	Lipinski #violations	Ghose #violations	Veber #violations	Egan #violations	Muegge #violations
ZINC21525754	0	0	0	0	0
PubChem-16352732	0	0	0	0	0
PubChem-136659176	0	1	1	1	1
PubChem-135405892	0	0	0	0	0
PubChem-126853168	1	0	0	0	0
ZINC65196494	0	0	0	0	0
PubChem-41119771	1	1	0	0	1
ZINC20113894	0	0	0	0	0
PubChem-71600230	0	0	0	0	1
ZINC13365292	0	0	1	1	0
LN00379431	0	0	0	0	0
PubChem-3750748	1	1	0	0	1
ZINC4034881	0	0	0	0	0
ZINC65196500	0	0	0	0	0
ZINC100771598	0	0	0	0	0
PubChem-25352696	0	0	0	0	0
PubChem-91428044	0	0	0	0	0
ZINC33057775	0	0	0	0	0

5. Pharmacokinetic proprieties

To predict the pharmacokinetic properties of absorption, distribution, metabolism and excretion (ADME), pkCSM web server was used to calculate the following parameters: water solubility (log mol/L), Caco-2 cell permeability, human intestinal absorption (HIA), blood-brain barrier (BBB) permeability, central nervous system (CNS) permeability and total clearance.

Water solubility (LogS) indicates the solubility of a compound in water at 25°C. Generally, water-soluble drugs are more readily absorbed than lipid-soluble ones. *in vitro* Caco-2 cell permeability is a crucial measure of drug absorption, with a compound considered to have high Caco-2 permeability if its value surpasses 8×10^{-6} cm/s. Within the pkCSM model, high Caco-2 permeability aligns with predicted values exceeding 0.90. The intestine typically serves as the primary site for drug absorption from orally administered solutions. A compound with absorbance below 30% is deemed poorly absorbed. An established gauge of blood-brain barrier (BBB) penetration is the log BB ratio, which reflects drug molecule concentrations in the brain and blood. Compounds with log BB > 0.3 exhibit high BBB permeability, while those with log BB < 1.0 show limited BBB distribution. Furthermore, central nervous system (CNS) permeability is a vital parameter for assessing the blood-brain permeability of a drug candidate, expressed as LogPS. Compounds with LogPS < -3 are considered incapable of penetrating the CNS.

Based on these ADME properties, 18 compounds were selected for further analysis. The results for these compounds are detailed in Table 5, while the other parameters are shown in Table S4 in the supplementary data.

6. Docking Validation

Docking validation was performed using AutoDock 4.2. The results are presented in Table 6.

To identify the top CB1 agonist several criteria were taken into account including energy values (e.g. binding energy or Ki values), interactions with key amino acids in the CB1 binding site, number of hydrogen bonds and distances between hydrogen bonds. These interactions are essential for designing effective and selective CB1 agonists.¹⁰ For instance, π - π interactions with aromatic residues (Phe296, Phe170, Phe174, Trp 279) and hydrogen bonds with Ser383 or Thr197 stabilize the agonist-receptor complex, while residues such as Phe379 and Asp366 play a key role in receptor activation through electrostatic interactions.¹⁰ Based on these criteria three compounds were identified as CB1 receptor agonists: ZINC64438506, ZINC64438485 and PUBChem157251136.

Table 5. Some ADME parameters of the top-ranked compounds.

Code	Water solubility	Caco2 permeability	Intestinal absorption (human)	BBB permeability	CNS permeability	Total Clearance
ZINC17286185	-5.117	0.818	83.062	-0.367	-2.784	0.727
ZINC35562518	-4.994	0.819	82.23	-0.389	-2.876	0.732
ZINC64438506	-5.467	1.165	96.008	-0.435	-2.363	0.804
ZINC64438485	-5.241	1.163	97.137	-0.98	-2.573	0.505
PubChem-121231416	-4.14	0.979	94.665	-0.031	-2.006	0.608
ZINC21797190	-4.273	1.083	96.539	-0.669	-2.597	0.623
PUBChem 157251136	-4.222	1.106	94.303	-0.893	-2.705	1.167
PubChem-42810820	-5.266	1.294	94.667	-0.394	-1.924	0.316
ChemDiv-C260-2692	-5.941	1.284	97.247	-0.422	-2.392	0.819
PubChem-91487881	-2.946	1.374	90.351	-0.966	-2.226	0.369
PubChem-126853168	-4.351	1.428	96.515	-0.399	-2.234	0.514
ZINC65196494	-4.278	1.131	98.641	-0.725	-2.5	0.947
PubChem-41119771	-4.601	1.251	95.633	0.709	-1.937	0.039
PubChem-3750748	-5.732	1.102	91.195	0.215	-1.931	-0.313
ZINC4034881	-4.991	1.18	97.628	-0.693	-2.477	0.972
PubChem-25352696	-2.773	1.444	98.767	-0.866	-2.82	0.534
PubChem-91428044	-5.217	1.3	94.768	0.399	-2.503	0.758
ZINC33057775	-4.528	1.202	93.212	-0.615	-2.241	-0.075

Table 6. Binding energy (B.E), Intermolecular Energy (I.M.E), Internal Energy (I.E), Torsional Energy (T.E) and constant of inhibition (K.I).

Code	B.E (Kcal/mol)	I.M.E (Kcal/mol)	I.E (Kcal/mol)	T.E (Kcal/mol)	KI (nM)
ChemDiv-C260-2692	-12.43	-14.82	-2.39	2.39	0.768
PubChem3750748	-12.3	-14.69	-2.05	2.39	0.958
PubChem25352696	-10.58	-12.63	-1.06	1.79	17.63
PubChem41119771	-11.23	-13.02	-1.22	1.79	5.91
PubChem42810820	-11.6	-12.79	-0.77	1.19	3.13
PubChem91428044	-10.2	-12.88	-1.1	2.68	33.36
PubChem91487881	-10.71	-13.09	-1.13	2.39	14.15
PubChem121231416	-11.63	-14.32	-0.86	2.68	2.98
PubChem126853168	-12.26	-13.75	-0.93	1.49	1.04
PUBChem157251136	-11.84	-14.23	-1.61	2.39	2.09
ZINC4034881	-11.51	-13.89	-2.1	2.39	3.67
ZINC17286185	-11.41	-14.39	-1.84	2.98	4.36
ZINC21797190	-12.37	-14.45	-1.22	2.09	0.862
ZINC33057775	-12.25	-14.04	-1.64	1.79	1.06
ZINC35562518	-12.22	-14.9	-1.74	2.68	1.11
ZINC64438485	-13.07	-15.76	-1.37	2.68	0.262
ZINC64438506	-13.11	-15.8	-1.37	2.68	0.244
ZINC65196494	-11.7	-13.49	-1.28	1.79	2.65

Table 7. Ligand-receptor interactions of the three highest-ranked agonists.

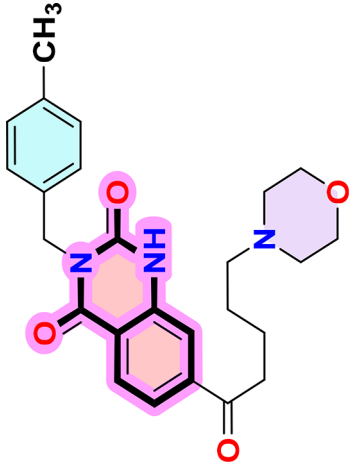
Hydrophobic interactions		Hydrogen Bonds		π-Stacking		Halogen Bonds						
Residue	AA	Distance	Residue	AA	Distance H-A	Distance D-A	Residue	AA	Distance (Å)	Residue	AA	Distance
												
PUBChem 157251136												
174A	PHE	3.83	173A	SER	3.23	3.72	268A	PHE	4.71			
174A	PHE	3.70	178A	HIS	1.94	2.93	279A	TRP	3.94			
177A	PHE	3.26	383A	SER	2.04	2.81						
193A	LEU	3.37										
197A	THR	3.13										
268A	PHE	3.48										
276A	LEU	3.98										
279A	TRP	3.60										
379A	PHE	3.49										

Table 7. Continued

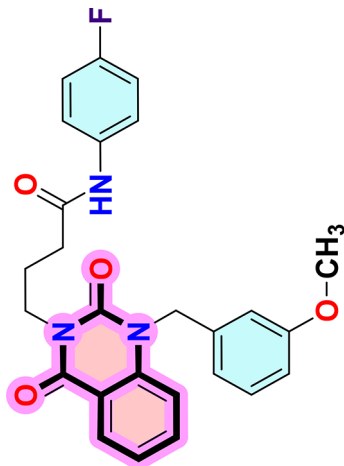
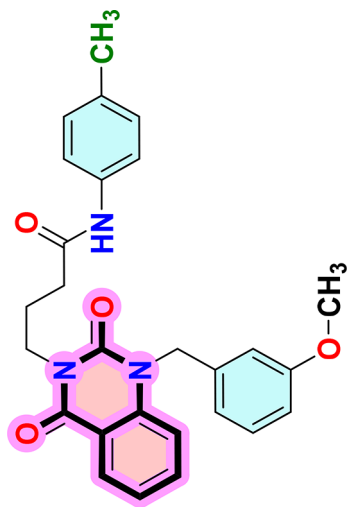
Hydrophobic interactions		Hydrogen Bonds			π -Stacking			Halogen Bonds				
Residue	AA	Distance	Residue	AA	Distance H-A	Distance D-A	Residue	AA	Distance (Å)	Residue	AA	Distance
												
ZINC64438485												
174A	PHE	3.87	197A	THR	1.94	2.93	174A	PHE	5.09			
177A	PHE	3.68	383A	SER	3.08	3.74	178A	HIS	4.62			
177A	PHE	3.37					268A	PHE	4.77			
177A	PHE	3.88					279A	TRP	4.93			
189A	PHE	3.58										
193A	LEU	3.42										
193A	LEU	3.81										
196A	VAL	3.19										
271A	ILE	3.91										
276A	LEU	3.40										
279A	TRP	3.65										
279A	TRP	3.77										
379A	PHE	3.95										
379A	PHE	3.40										
380A	ALA	3.34										

Table 7. Continued

Hydrophobic interactions		Hydrogen Bonds			π -Stacking			Halogen Bonds				
Residue	AA	Distance	Residue	AA	Distance H-A	Distance D-A	Residue	AA	Distance (Å)	Residue	AA	Distance
108A	PHE	3.77	173A	SER	3.34	3.82	268A	PHE	4.77			
177A	PHE	3.82	383A	SER	1.79	2.67						
177A	PHE	3.22										
177A	PHE	3.47										
189A	PHE	3.22										
193A	LEU	3.47										
197A	THR	3.59										
268A	PHE	3.63										
275A	TYR	3.13										
276A	LEU	3.26										
279A	TRP	3.40										
279A	TRP	3.71										
279A	TRP	3.25										
279A	TRP	3.56										
379A	PHE	3.76										

ZINC64438506



ZINC64438506: The docking analysis of CB1 receptor and ZINC64438506 selected agonist is shown in [Table 7](#) and [Figure 4](#). The ZINC64438506 agonist was fixed in the CB1 binding pocket (cavity size = 2963 Å) through various type of interactions, including hydrogen bonds with key amino-acid residues SER A: 173 and SER A: 383, hydrophobic interactions with residues PHE A: 108, PHE A: 177, PHE A: 189, LEU A: 193, THR A: 197, PHE A: 268, TYR A: 275, LEU A: 276, TRP A: 279 and PHE A: 379 and π - π interaction with PHE A: 268. These interactions likely contribute to the compound's low binding affinity (-13.11 Kcal/mol) and low inhibition constant ($K_i = 0.244$ nM) ([Table 6](#)). The strong binding affinity may be attributed to the presence of short hydrogen bonds with SER A: 173 (3.34 Å) and SER A: 383 (51.79 Å).

ZINC64438485: The docking analysis of CB1 receptor and ZINC64438485 selected agonist is shown in [Table 7](#) and [Figure 5](#). The ZINC64438485 agonist was fixed in the CB1 binding pocket (cavity size = 2963 Å) through various type of interactions, including hydrogen bonds with key amino-acid residues THR A: 197 and SER A: 383, hydrophobic interactions with residues PHE A: 174, PHE A: 177, PHE A: 189, LEU A: 193, VAL A: 196 and ILE A: 271 and π - π interaction with PHE A: 174, HIS A: 178, PHE A: 268 and TRP A: 279. These interactions likely contribute to the compound's low binding affinity (-13.07 Kcal/mol) and low inhibition constant ($K_i = 0.262$ nM) ([Table 6](#)). The strong binding affinity may be attributed to the presence of short hydrogen bonds with THR A: 197 (1.94 Å) and SER A: 383 (3.08 Å).

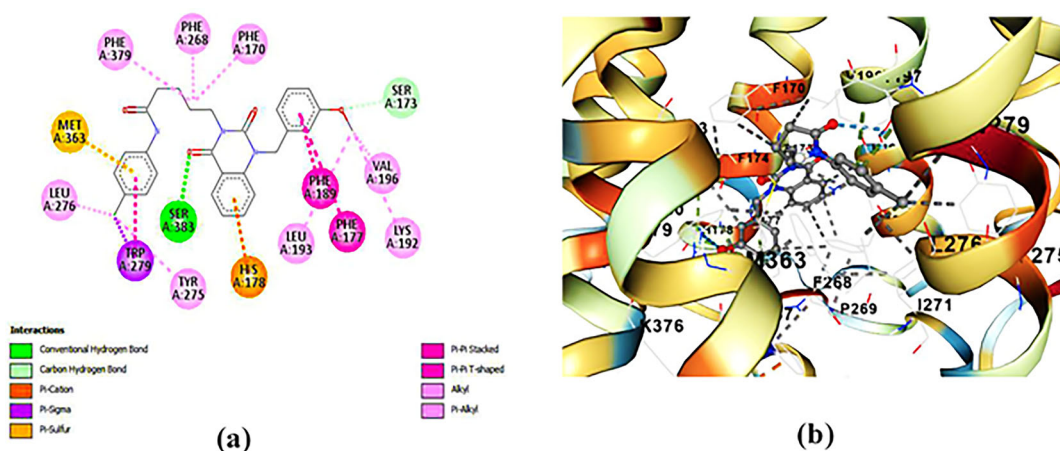


Figure 4. Docking analysis of ZINC64438506 agonist with CB1 receptor. (a) 2D view of binding site interactions (b) 3D view of binding conformation.

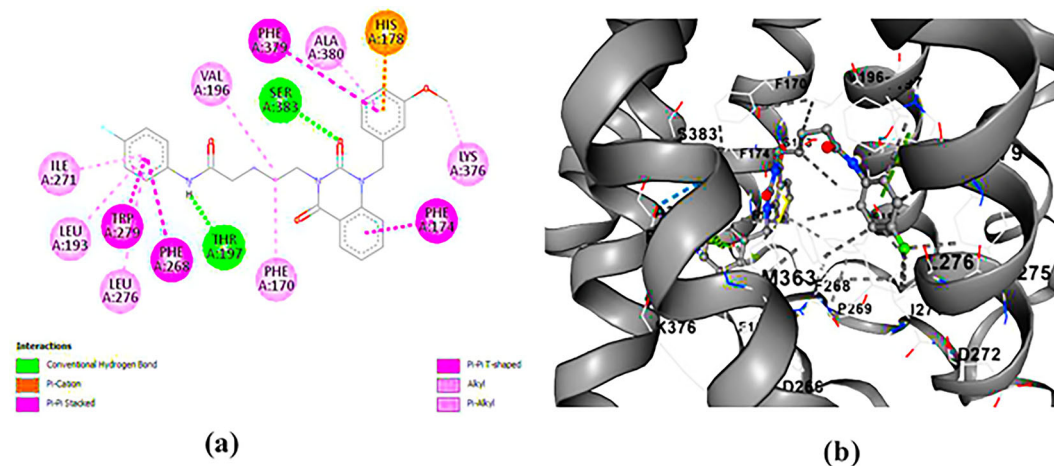


Figure 5. Docking analysis of ZINC64438485 agonist with CB1 receptor. (a) 2D view of binding site interactions (b) 3D view of binding conformation.

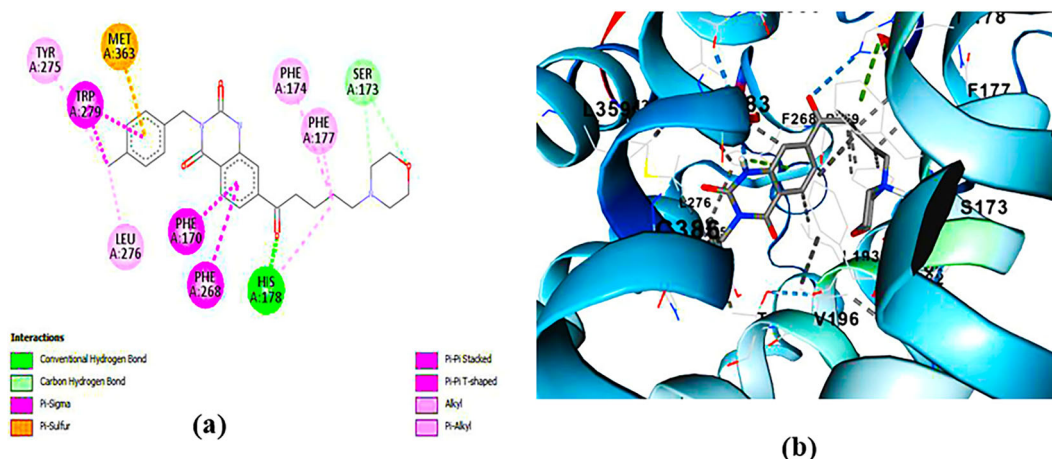


Figure 6. Docking analysis of PUBChem_157251136 agonist with CB1 receptor. (a) 2D view of binding site interactions (b) 3D view of binding conformation.

PUBChem-157251136: The docking analysis of CB1 receptor and PUBChem-157251136 selected agonist is shown in Table 7 and Figure 6. The PUBChem157251136 agonist was fixed in the CB1 binding pocket (cavity size = 2963 Å) through various type of interactions, including hydrogen bonds with key amino-acid residues SER A: 173, HIS A: 178 and SER A: 383, hydrophobic interactions with residues PHE A: 174, PHE A: 177, LEU A: 193, THR A: 197, PHE A: 268, LEU A: 276, TRP A: 279 and PHE A: 379 and π - π interaction with PHE A: 268 and TRP A: 279. These molecular interactions likely contribute to the compound's low binding affinity (-11.84 Kcal/mol) and low inhibition constant ($K_i = 2.09$ nM) (Table 6). The strong binding affinity may be attributed to the presence of three hydrogen bonds with SER A: 173 (3.23 Å), HIS A: 178 (1.94 Å) and SER A: 383 (2.04Å).

7. MD simulations

While molecular docking analyses are enough to understand possible bindings based on ligand positioning and receptor-ligand interactions, it should be noted that such methodologies evaluate the flexibility of the ligand only while keeping the protein in a rigid form. Thus, in order to evaluate the best-docked candidates for binding pose stability and dynamics of protein conformation, molecular dynamics (MD) simulations were performed over 100 ns. The results of the MD simulations, including root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), and Ligand-Receptor Interaction Plot analyses, can be found in Figures 7–10. These studies provide insights into the dynamic behavior and stability of the protein–ligand complexes over time.

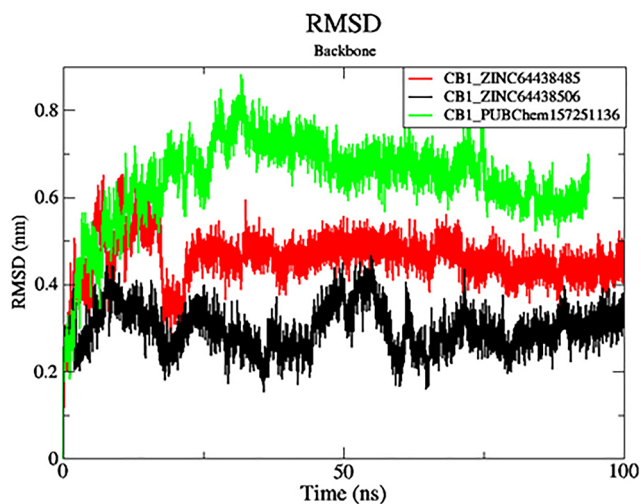


Figure 7. Plots of RMSD over the 100 ns MD simulation. The black color was the CB1_ZINC64438506 complex, the red was the CB1_ZINC64438485 complex and the green was the CB1_PUBChem157251136 complex.

7.1 Root Mean Square Deviation (RMSD)

RMSD analysis was carried out for the protein backbone to have an idea of each protein-ligand complex's structural stability during the simulation.^{32,33} The results are shown in Figure 7. As shown in Figure 7, mean RMSD values for the CB1_ZINC64438506, CB1_ZINC64438485 and CB1_PUBChem157251136 complex are 0.29 nm, 0.45 nm and

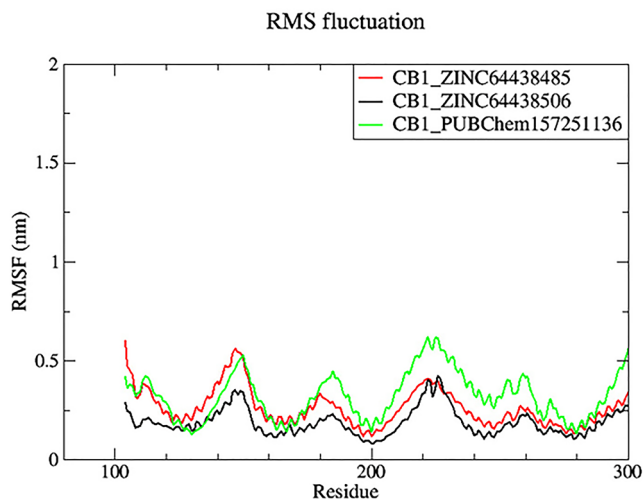


Figure 8. Plots of RMSF over the 100 ns MD simulation. The black color was the CB1_ZINC64438506 complex, the red was the CB1_ZINC64438485 complex and the green was the CB1_PUBChem157251136 complex.

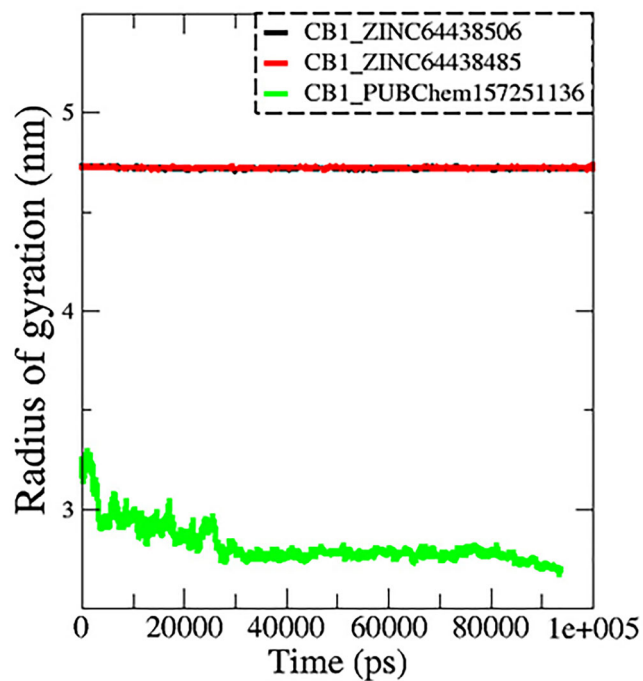


Figure 9. Plots of Rg during the 100 ns of MD simulation. The black color represents the CB1_ZINC64438506 complex, the red color represents the CB1_ZINC64438485 complex and the green color represents the CB1_PUBChem157251136 complex.

0.64 nm, respectively. The global RMSD values for CB1_ZINC64438506, CB1_ZINC64438485 are small which indicates that these ligands remain stable during the simulation of 100 ns and remained in the binding pocket of the CB1 receptor. The stability of the ligands can be attributed to several strong hydrogen bonds being mediated between these agonists and some of the key amino acids positioned within the binding pocket of the CB1 receptor.

7.2 Root mean square fluctuation (RMSF)

Root mean square fluctuation (RMSF) was used to determine the rigid and flexible regions of the CB1 receptor over the 100 ns of MD simulations.¹⁴ RMSF has been term definition, instead of just RMSD values, so that the maximum range of motion of a bound ligand can be seen. RMSF is defined as a standard measure of deviation of a molecule from its initial position.³⁴ Molecules and residues should not present a high value, which indicates a flexibility, and those that appears low value has a greater rigidity. The RMSF plot for all complexes (Figure 8) indicates that most residues located in the CB1 receptor has a low RMSF value, indicating they were rigid and retained stability over the entire 100 ns MD simulation.

7.3 Radius of gyration (Rg)

The radius of gyration (Rg) is another important metric measured during MD simulations to determine spatial characteristics of a protein-ligand complex. The radius of gyration is the root mean square distance of all atoms making

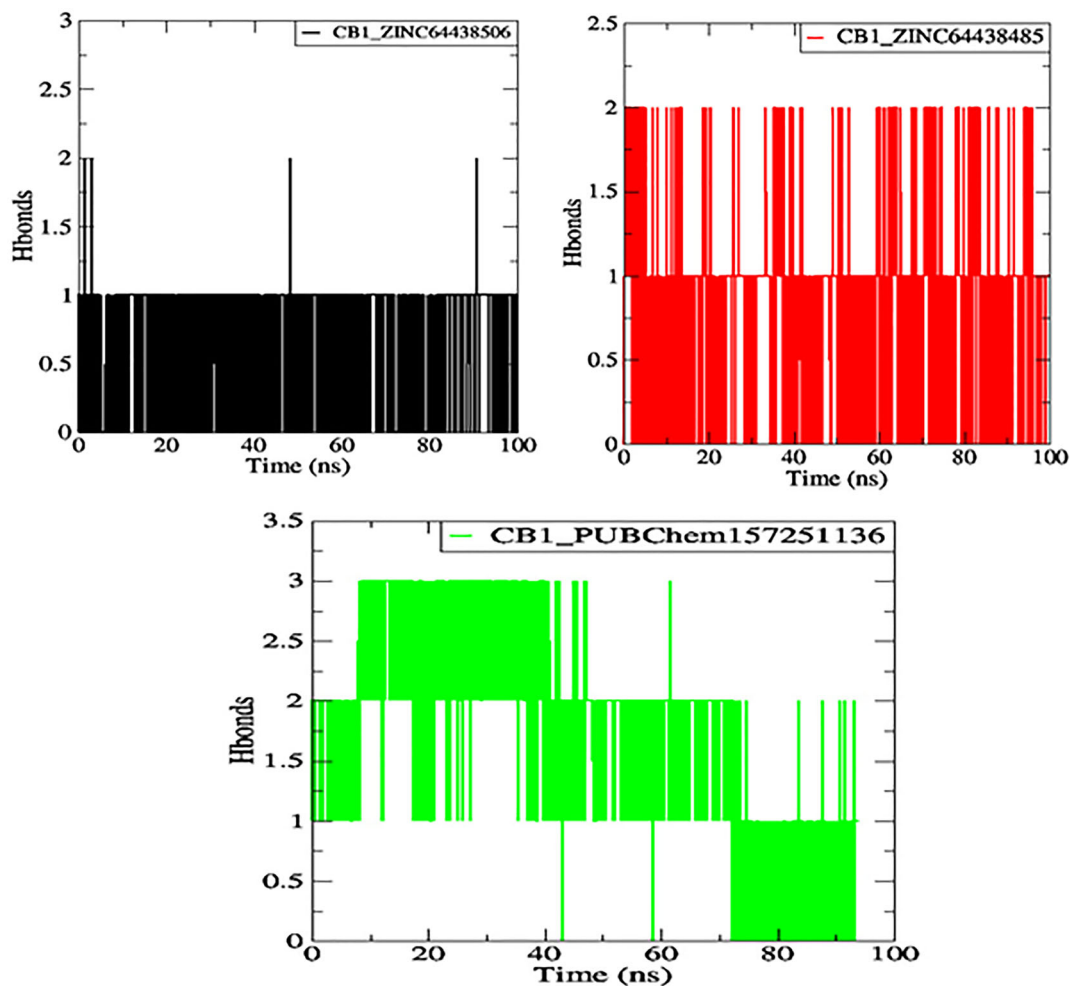


Figure 10. Plot of H-bonds during the 100 ns of MD simulation. The black color represents the CB1_ZINC64438506 complex, the red color represents the CB1_ZINC64438485 complex and the green color represents the CB1_PUBChem157251136 complex.

Table 8. MM-GBSA calculations for the top three ranked compounds.

Complex	ΔG_{GAS} (Kcal/mol)	ΔG_{SOLV} (Kcal/mol)	ΔG_{bind} (Kcal/mol)
CB1_ZINC64438506	-80.76	30.99	-49.77
CB1_PUBChem157251136	-107.10	76.51	-30.59
CB1_ZINC64438485	-82.29	32.31	-49.98

up a given structure from a relative center common point (the center of mass) and signifies extension versus folding of the given structure. Therefore, a lower Rg would imply stability and a more tightly folded structure, while a higher Rg would imply extension and flexibility of possible structures. In this work, the Rg profiles remained stable over the course of 100 ns of MD simulation for the complexes CB1_ZINC64438506 (mean = 4.70 nm; [Figure 9](#)) and CB1_ZINC64438485 (mean = 4.72 nm), while the complex CB1_PUBChem157251136 decreased in Rg (mean = 2.81 nm).

7.4 Hydrogen bonds

One of the main factors influencing the affinity of a molecule for the protein binding pocket is its ability to form and maintain hydrogen bonds with the binding site residues. The stability of the selected agonist was assessed by analyzing the hydrogen bonds between the ligand and the protein. The results are presented in [Figure 10](#).

Compounds ZINC64438506, ZINC64438485, and PUBChem157251136 form two, two and three hydrogen bonds with the CB1 binding site, respectively, indicating strong and specific interactions with the protein. These results are consistent with the docking results.

8. MM-GBSA calculation for the top three ranked compounds

The binding free energy of all complexes was calculated to revalidate the binding affinity obtained from molecular docking analysis. The results are presented in [Table 8](#). A ΔG_{bind} value below -7 Kcal/mol indicates strong binding, a value between -5 and -7 kcal/mol suggests moderate binding, and a value between -2 and -5 kcal/mol corresponds to weak binding.³⁵ The results show that all the agonists exhibit exceptionally low binding free energies, indicating a remarkable affinity for the target protein.

The results also highlight the crucial role of Van der Waals and electrostatic interactions in mediating protein-ligand binding throughout the molecular dynamics simulations ([Figure 11](#)).

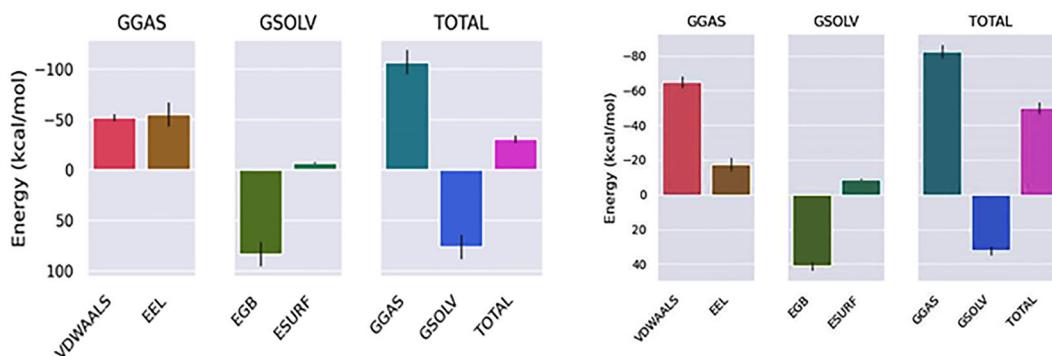
9. Quinazoline-2,4(1H,3H)-dione derivatives

Quinazoline-2,4(1H,3H)-dione derivatives represent a highly promising class of heterocyclic compounds with broad therapeutic potential, particularly as anticancer,³⁶⁻³⁸ antibacterial,^{39,40} antihypertensive,⁴¹ phosphodiesterase (PDE) 4 inhibition,⁴² 5-HT3A receptor antagonist,⁴³ anti-inflammatory,⁴⁴ and an an up-and-coming antiviral agents.⁴⁵ Their fused benzopyrimidinedione scaffold provides an excellent pharmacophore for targeting key biological pathways ([Figure 12](#)). According to the article's findings, quinazoline-2,4(1H,3H)-dione derivatives may be the first of a new class of CB1 agonists.

10. Different synthesis routes of substituted quinazoline-2,4-dione scaffold

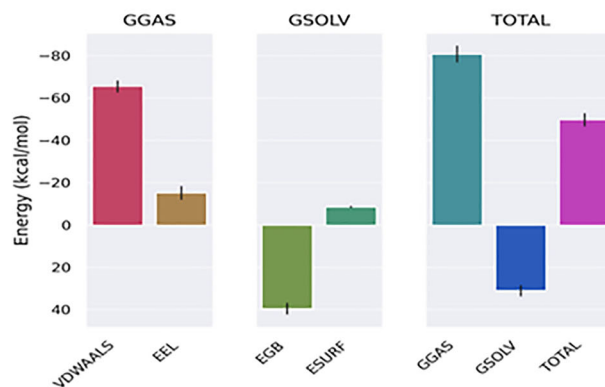
To synthesise the substituted quinazoline-2,4-dione and the highest-ranking agonists identified by virtual screening, namely ZINC64438506, PUBChem157251136 and ZINC64438485, several plausible synthesis strategies were developed based on established methodologies.⁴³ These routes use various precursors such as 2-aminobenzoic acid,⁴⁴ methyl 2-nitrobenzoate, 2-iodobenzamides⁴⁶ or 2H-benzo [d][1,3]oxazine-2,4(1H)-dione⁴⁷ ([Figure 13](#)). The choice of starting material is guided by both its availability and synthetic feasibility. Depending on the substrate, the desired compounds can be obtained by catalytic transformations, particularly transition metal-catalysed couplings, or by intramolecular cyclisation reactions that form the characteristic quinazoline skeleton. The synthetic flexibility offered by these precursors allows the reaction conditions to be adjusted to optimise yield and purity, making them suitable candidates for further pharmacological evaluation and development.

To synthesise substituted quinazoline-2,4-dione and the top-ranked agonists identified by virtual screening - namely ZINC64438506, PUBChem157251136 and ZINC64438485 - several plausible synthetic strategies have been devised, based on established methodologies.⁴⁶ These routes use various precursors such as 2-aminobenzoic acid,⁴⁷ methyl 2-nitrobenzoate⁴⁸ which undergoes catalytic hydrogenation under mild and green conditions (1 atm of H₂ in ethanol at



CBI_PUBChem157251136

CBI_ZINC64438485



CBI_ZINC64438506

Figure 11. Binding free energy plot of the three highest ranked agonists.

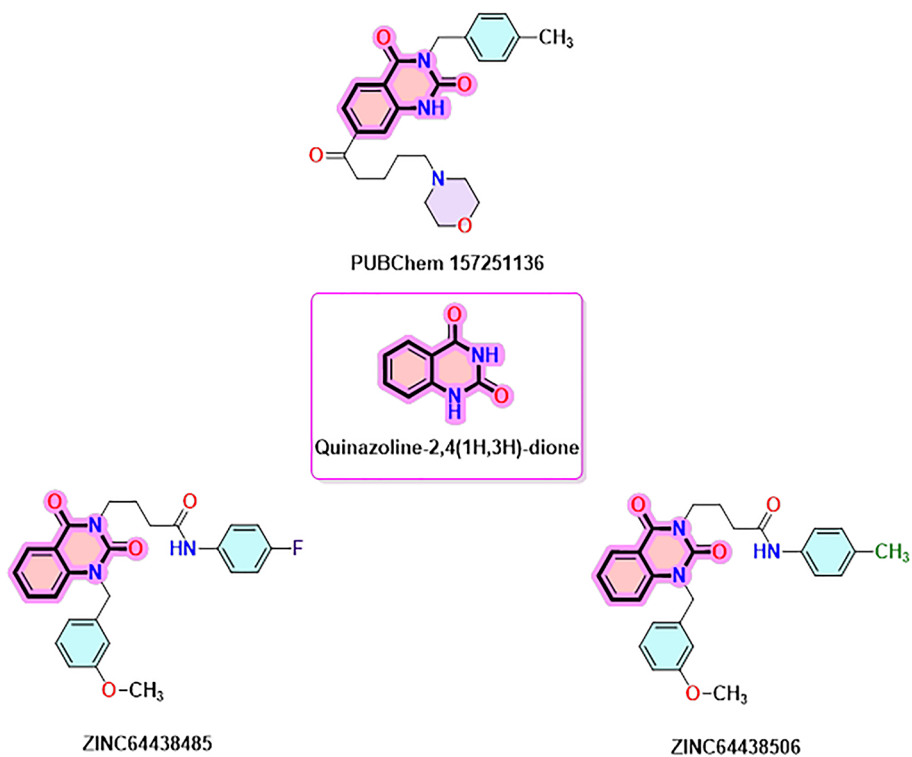


Figure 12. Quinazoline-1, 4(1H, 3H)-dione scaffold and the three highest-ranked agonists (ZINC64438506, PUBChem157251136 and ZINC64438485).

This project contains the following extended data:

- **Supplementary Table 1.** (Binding free energies of the 433 top-ranked compounds from pharmacophore-based virtual screening)
- **Supplementary Table 2.** (Computationally predicted toxicity profiles of top 61 compounds)
- **Supplementary Table 3.** (Physicochemical properties of studied compounds)
- **Supplementary Table 4.** (Some ADME parameters of the top-ranked compounds)

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Version 3

Reviewer Report 19 March 2026

<https://doi.org/10.5256/f1000research.197019.r466648>

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Bouchra Rossafi

Institution: Hassan II University of Casablanca, Morocco, Morocco, Morocco

Samir Chtita 

Hassan II University of Casablanca, Morocco, Morocco

The authors have addressed all of my recommendations, and I recommend the indexing of the article

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular modeling

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 2

Reviewer Report 03 February 2026

<https://doi.org/10.5256/f1000research.194643.r449401>

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Samir Chtita 

Hassan II University of Casablanca, Morocco, Morocco

Bouchra Rossafi

Institution: Hassan II University of Casablanca, Morocco, Morocco, Morocco

The manuscript titled "**Quinazoline-2,4(1H,3H)-dione derivatives as new class of CB1 Agonists: A pharmacophore-based virtual screening workflow and drug discovery**", present an in silico investigation aimed at identifying new CB1 agonists derived from quinazoline-2,4(1H,3H)-dione scaffolds, employing a pharmacophore-based virtual screening strategy followed by molecular docking, MD simulations and MMGBSA calculations. While the study provides interesting results, some aspects require improvement, including:

1. The authors should more clearly demonstrate the novelty and originality of the present study in the Introduction section, particularly in comparison with previously reported CB1 agonists and related computational studies.
2. After the pharmacophore-based virtual screening, the authors proceeded with molecular docking. However, the rationale behind the selection of 61 compounds with a docking score threshold of -9 kcal/mol as top molecules is not clearly justified. The authors should explain why this specific cutoff was chosen.
3. Although a reference agonist (AM11542) was used for the generation of the pharmacophore model, it is not clear whether this compound was subsequently used as a reference drug in the docking, ADMET and MD analyses.
4. In the context of the targeted pathology and the central role of the CB1 receptor, the analysis of BBB permeability and central nervous system CNS parameters is highly relevant. These aspects should be more thoroughly analyzed and discussed.
5. The Introduction identifies several key residues involved in CB1 receptor activation. However, it is not clearly demonstrated whether the identified compounds effectively interact with these key residues.
6. Comparing MM-GBSA results with docking scores or reference compounds could strengthen the interpretation.
7. The quality and clarity of the docking figures should be improved.
8. **In the table 2, the unit for binding energy should be written as "kcal" instead of "Kcal" to follow standard scientific notation.**
9. The manuscript would benefit from a dedicated section discussing the limitations of the study.
10. Experimental validation of the identified compounds perspectives should be included in the Conclusion.
11. Information included on the different modeling techniques in material and methods section is quite trivial and should be more detailed. To enhance the introduction of the computational section, it is recommended to provide a detailed discussion and comparison of different methods as reported in the literature. In order to facilitate a better understanding for readers, the following previous studies employing docking and molecular dynamic in this research area can be cited: Refer to reference 1, 2, 3, & 4

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Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular modeling

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Author Response 15 Feb 2026

ABDELLAH EL AISSOUQ

reponse to Q1: your remarks have been taken into account in the revised manuscript

response to Q2: this threshold was chosen based on the reference molecule (see sup data)

response to Q3: the agonist (AM11542) was selected as the reference compound for the whole study

response to Q4-9: your remarks have been taken into account in the revised manuscript

response to Q10: all selected compounds are known drugs

Competing Interests: No competing interests were disclosed.

Version 1

Reviewer Report 30 December 2025

<https://doi.org/10.5256/f1000research.189041.r439371>

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Mourad Fawzi

Faculty of Sciences Semlalia, Cadi Ayyad University, Department of Chemistry, Laboratory of Molecular Chemistry, Marrakech, Morocco

The study entitled "**Quinazoline-2,4(1H,3H)-dione derivatives as new class of CB1 Agonists: A pharmacophore-based virtual screening workflow and Lead discovery**" presents a pharmacophore-based virtual screening of a large database aimed at developing new CB1 agonists. I believe this work is suitable for publication in *F1000 Research* before the minor revisions below:

Comments for the authors:

1. Page 17: "form two, two and three hydrogen bonds with the CB1 binding site, respectively, ..." – The word "two" is repeated; please remove one.
2. Some abbreviations are not clearly defined in the manuscript, such as **GPCR** and **ICL3**. Please ensure all abbreviations are spelled out upon first use.
3. Page 19: The sentence "Translated with DeepL.com (free version)" should be removed.
4. More details about the advantage of work in conclusion are required
5. Grammatical errors and typos are present throughout the manuscript. A thorough proofreading is recommended to improve clarity and language quality.
6. Regarding **Table 6**: The reported K_i values indicate that the screened compounds exhibit promising drug-like properties. Therefore, I suggest modifying the title to better reflect this finding. Proposed revision:

" **Quinazoline-2,4(1H,3H)-dione derivatives as new class of CB1 Agonists: A pharmacophore-based virtual screening workflow and Lead discovery** "

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

No source data required

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Bioinformatics, Organic Chemistry, Medicinal Chemistry

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 01 Jan 2026

ABDELLAH EL AISSOUQ

Reviewer #2:

The study entitled "Quinazoline-2,4(1H,3H)-dione derivatives as new class of CB1 Agonists: A pharmacophore-based virtual screening workflow and drug discovery" presents a pharmacophore-based virtual screening of a large database aimed at developing new CB1 agonists. I believe this work is suitable for publication in F1000 Research before the minor revisions below:

Comments for the authors:

1. Page 17: "form two, two and three hydrogen bonds with the CB1 binding site, respectively, ..." – The word "two" is repeated; please remove one.
2. Some abbreviations are not clearly defined in the manuscript, such as GPCR and ICL3. Please ensure all abbreviations are spelled out upon first use.
3. Page 19: The sentence "Translated with DeepL.com (free version)" should be removed.
4. More details about the advantage of work in conclusion are required
5. Grammatical errors and typos are present throughout the manuscript. A thorough proofreading is recommended to improve clarity and language quality.
6. Regarding Table 6: The reported K_i values indicate that the screened compounds exhibit promising drug-like properties. Therefore, I suggest modifying the title to better reflect this finding. Proposed revision:

Response to Questions 1-6: We thank the reviewer for their insightful comments. All remarks have been addressed in the revised manuscript."

Competing Interests: No competing interests were disclosed.

Reviewer Report 29 December 2025

<https://doi.org/10.5256/f1000research.189041.r437306>

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Fatima En-nahli

University of Moulay Ismail, Meknes, Morocco

Dear Editor,

I would like to thank you for the opportunity to review the manuscript entitled "*Quinazoline-2,4(1H,3H)-dione derivatives as a new class of CB1 Agonists: A pharmacophore-based virtual screening workflow and Lead discovery.*" The study addresses a relevant topic and provides valuable insights into CB1 agonist identification through an in silico workflow. Overall, the manuscript is promising; however, I believe that **minor revisions** are required to improve clarity and scientific rigor.

Please find below my main remarks for the authors:

1. The current title would benefit from being reformulated to make it more concise and more attractive to readers. A clearer title highlighting the novelty and the integrated workflow is recommended.
2. The authors mention the use of molecular dynamics (MD) simulations in the Materials and Methods section, but MD is not mentioned in the Abstract. For coherence and completeness, this important component of the workflow should be briefly stated in the Abstract.
3. In the graphical abstract, the authors indicate two docking steps (first using AutoDock Vina, then AutoDock4). It would be helpful to briefly clarify the rationale behind this double-docking strategy and how each tool contributes to the screening workflow.
4. The authors are encouraged to integrate a comparative discussion between the pharmacophore hits, docking scores, and the stability of key amino acids involved in ligand recognition, supported by MD fluctuation analysis (e.g., RMSF). This would reinforce the consistency and predictive power of the multi-step in silico approach.
5. Since none of the proposed candidates were synthesized or experimentally validated, the authors should clearly state the value of the in silico-only approach and discuss its limitations. It is also recommended to suggest potential collaboration with experimental researchers for future synthesis and biological evaluation.
6. Given that the identified compounds appear to be promising drug candidates, it would be appropriate to add a short section in the conclusion outlining perspectives for future work.

Overall, the manuscript represents an interesting contribution, and after addressing these minor points, it will be suitable for publication.

Thank you again for entrusting me with the review of this manuscript.

Kind regards,

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: chimie informatique

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 01 Jan 2026

ABDELLAH EL AISSOUQ

Reviewer #1:

Dear Editor, I would like to thank you for the opportunity to review the manuscript entitled "*Quinazoline-2,4(1H,3H)-dione derivatives as a new class of CB1 Agonists: A pharmacophore-based virtual screening workflow and Lead discovery.*" The study addresses a relevant topic and provides valuable insights into CB1 agonist identification through an in silico workflow. Overall, the manuscript is promising; however, I believe that **minor revisions** are required to improve clarity and scientific rigor. Please find below my main remarks for the authors:

1. The current title would benefit from being reformulated to make it more concise and more attractive to readers. A clearer title highlighting the novelty and the integrated workflow is recommended.

Response to Q1: the title has been changed to "*Quinazoline-2,4(1H,3H)-dione derivatives as new class of CB1 Agonists: A pharmacophore-based virtual screening workflow and drug discovery*"

2. The authors mention the use of molecular dynamics (MD) simulations in the Materials and Methods section, but MD is not mentioned in the Abstract. For coherence and completeness, this important component of the workflow should be briefly stated in the Abstract.

Response to Q2: The molecular dynamics (MD) simulations are mentioned in the abstract, as shown in the following excerpt from the abstract section "*Molecular dynamics simulations (100 ns, GROMACS) demonstrated structural stability (RMSD < 1 nm) and low conformational flexibility (RMSF < 1 nm) for all complexes. MM-GBSA binding free energy calculations further confirmed the thermodynamic stability of all complexes, with interaction energies ranging from -30.59 to -49.98 kcal/mol.*"

3. In the graphical abstract, the authors indicate two docking steps (first using AutoDock Vina, then AutoDock4). It would be helpful to briefly clarify the rationale behind this double-docking strategy and how each tool contributes to the screening workflow.

Response to Q3: We thank the reviewer for raising this important point regarding our docking strategy. The double-docking approach was implemented to enhance the

robustness and reliability of our virtual screening workflow.

4. The authors are encouraged to integrate a comparative discussion between the pharmacophore hits, docking scores, and the stability of key amino acids involved in ligand recognition, supported by MD fluctuation analysis (e.g., RMSF). This would reinforce the consistency and predictive power of the multi-step in silico approach.

5. Since none of the proposed candidates were synthesized or experimentally validated, the authors should clearly state the value of the in silico-only approach and discuss its limitations. It is also recommended to suggest potential collaboration with experimental researchers for future synthesis and biological evaluation.

6. Given that the identified compounds appear to be promising drug candidates, it would be appropriate to add a short section in the conclusion outlining perspectives for future work.

Response to Q4, 4 and 6 : The identified compounds are promising drug candidates

Competing Interests: Not applicable. The authors declare no competing interests.

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