

Chapter 5

Computational Screening of Coumarin Derivatives as NLRP3 Inflammasome Inhibitors for Alzheimer's Disease

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5.1. Introduction

Neuroinflammation involves inflammatory responses in the brain mediated by the release of specific molecules like cytokines and neurotrophic factors, alongside the production of neurotoxic substances such as reactive oxygen species (ROS) and nitric oxide (NO). These mediators are secreted by microglia, astrocytes, endothelial cells, and immune cells from the periphery [238]. A key component of this process is the inflammasome, a complex protein structure activated by pathogens and danger signals. The nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) family, particularly the NLRP3 inflammasome, has been extensively studied in the context of neuroinflammation [239]. NLRP3 is composed of three primary domains: the N-terminal pyrin domain (PYD), the central NACHT domain, and the C-terminal leucine-rich repeat (LRR) domain. The NACHT domain further includes several subdomains essential for its function (Figure 5.1 (a) and (b)) [240].

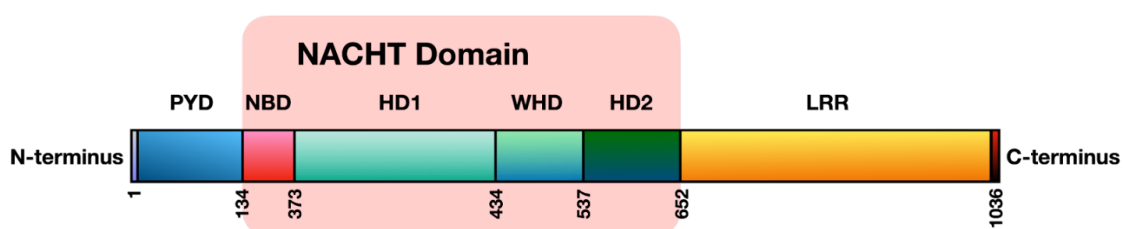


Figure 5.1 (a) Schematic representation of the structural domains of the NLRP3 inflammasome.

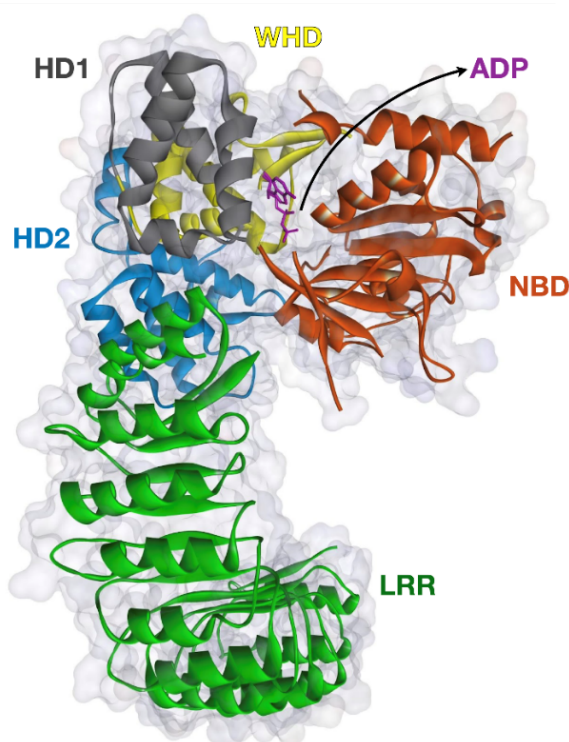


Figure 5.1 (b) 3D cartoon depiction of the NLRP3 inflammasome with bound ADP, omitting the pyrin domain. The domains are colour-coded as their labels and the protein surface outline is shown in light grey.

The NLRP3 inflammasome plays a significant role in the innate immune response and is closely linked to neuroinflammation and AD. Its activation can be triggered by various stimuli, including A β , NFTs, and inflammatory cytokines, leading to the release of pro-inflammatory cytokines like IL-1 β and IL-18, which contribute to neuroinflammation and CNS damage [241]. Increased activation of the NLRP3 inflammasome correlates with disease progression, suggesting that it may serve as a promising target for therapeutic intervention in AD. **MCC950**, a selective small-molecule inhibitor of the NLRP3 inflammasome, has been identified for its ability to inhibit both canonical and non-canonical activation pathways at nanomolar concentrations. Other notable inhibitors include IFM-2427, Inzomelid, and Somalix [242, 243].

Coumarins, known as 1,2-benzopyrones, are secondary metabolites prevalent in various plant species and can be classified into different categories based on their structural diversity [244]. Research into coumarin derivatives has highlighted their diverse biological activities, which include antioxidant, anticancer, anti-inflammatory, and neuroprotective effects [245-248]. Their antioxidant and anti-inflammatory properties may help mitigate oxidative stress and inflammation in the brain, both of which are implicated in the pathogenesis of AD.

Several studies have investigated the potential of coumarin derivatives as NLRP3 inhibitors. For instance, Scoparone (6,7-dimethoxycoumarin) has shown significant anti-inflammatory and neuroprotective effects, demonstrating the ability to inhibit NLRP3 inflammasome activation in an ovariectomy/D-galactose-induced rat model of AD [249]. Daphnetin (7,8-dihydroxycoumarin), derived from the traditional Chinese plant *Daphne giraldii* Nitsche, has been found to enhance cognitive function in AD mouse models [250]. Furthermore, exposure to fine particulate matter has been linked to increased neuronal damage through NLRP3 inflammasome activation in AD models [251]. Daphnetin effectively inhibits NLRP3-mediated pyroptosis in inflammation models, while Fraxin (8-(β -D-(β glucooxygenyl)-7-hydroxyl-6-methoxycoumarin), a compound from *Cortex Fraxini*, suppresses NLRP3 activation and the release of inflammatory cytokines induced by LPS [252]. Additionally, Nordalbergin from wood bark of *Dalbergia sissoo* has been shown to reduce inflammatory mediator release and ROS production by inhibiting NLRP3 activation in stimulated microglial cells [249]. Beyond their benefits in AD, coumarin derivatives have demonstrated inhibitory effects on NLRP3 inflammasomes in vascular dementia models, leading to decreased A β deposition and improved cognitive performance [253]. Isosibiricin has been reported to significantly

reduce levels of NLRP3, caspase-1, IL-1 β , and IL-18 in a concentration-dependent manner, indicating its potential to inhibit the NLRP3/caspase-1 pathway [254].

Targeting the NLRP3 inflammasome with coumarin derivatives presents a promising strategy for mitigating neuroinflammation and the associated degenerative changes in AD. In this study, the aim was to identify potential NLRP3 inhibitors that bind to its NACHT domain from a library of coumarin derivatives. Various computational techniques were employed, including molecular docking studies, *in silico* predictions of BBB permeability, and ADMET analyses, as well as MD simulations and binding free energy calculations, to evaluate the binding affinities of the ligands.

5.2. Materials and methods

5.2.1. Dataset preparation (library construction & filtering)

The library of coumarin derivatives was assembled by integrating subsets of molecules from five distinct databases: (i) MolPort, (ii) ChEMBL, (iii) Chem-Space, (iv) PubChem, (v) NPASS, and (vi) ZINC20 (**Figure 5.2**). To ensure uniqueness, duplicate entries represented by different SMILES strings for the same molecule were removed using the IUPAC Chemical Identifier (InChI) within the Open Babel GUI (version 3.1.1) [211].

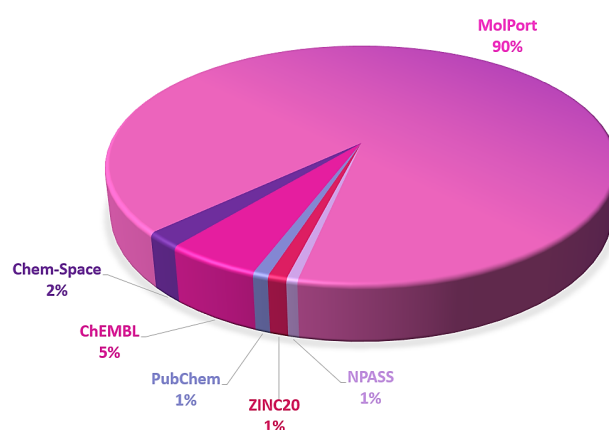


Figure 5.2 Distribution of the initial dataset of coumarin derivatives based on the source of the molecules

5.2.2. Drug-likeness and PAINS filtering

To assess various molecular properties such as molecular weight, TPSA, xLogP, the number of rotatable bonds, HBAs, HBDs, and heavy atom count, the open-source KNIME analytics platform (version 4.6.3) [255] was employed (**Figure 5.3**).

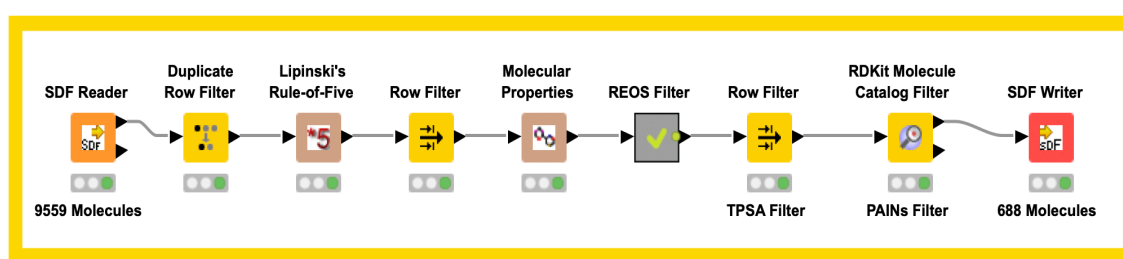


Figure 5.3 Knime workflow for drug-likeness and PAINS filtering

The distribution of the compounds was illustrated using histograms (**Figure S4**). Only the compounds fulfilling the criteria of “Lipinski’s Rule of Five” [256] and the “REOS” [257] were retained. Given that compounds with a TPSA below $60 \text{ \AA}^2$ were more likely to cross the BBB [258], those with TPSA values exceeding $60 \text{ \AA}^2$ were excluded from further analysis. Additionally, PAINS [259] were filtered out from the dataset (**Figure 5.3**). For redocking purposes, the co-crystal ligand corresponding to PDB ID 7ALV was incorporated into the library.

5.2.3. Protein structure selection and preparation

The X-ray crystallized structure of the NACHT domain of NLRP3 inflammasome in complex with 1-[4-chloranyl-2,6-di(propan-2-yl)phenyl]-3-[4-(2-oxidanylpropan-2-yl)furan-2-yl]sulfonyl-urea or RM5, an analogue of **MCC950** (a potent inhibitor of NLRP3), (PDB ID: 7ALV, Resolution: 2.83 Å) from the RCSB PDB database (<https://www.rcsb.org/>) was selected for protein preparation (**Figure 5.4** and **Figure 5.5**).

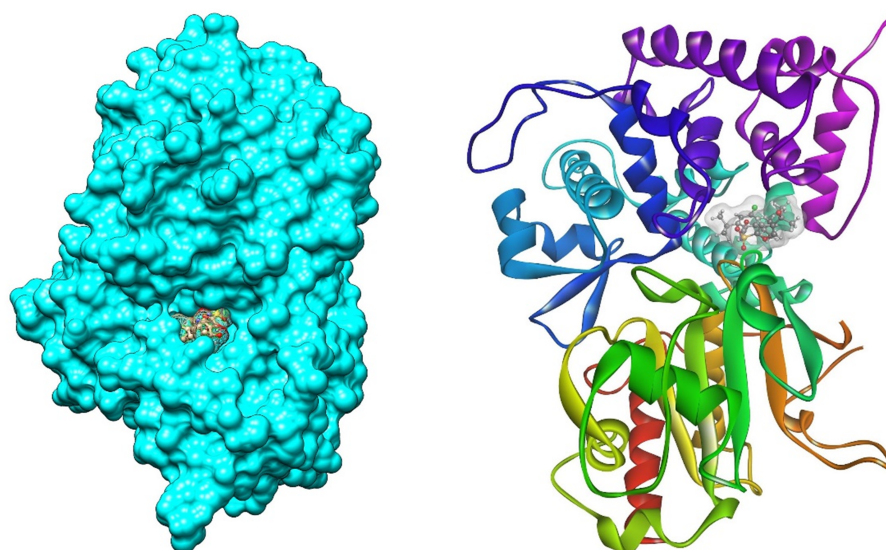


Figure 5.4 NLRP3 NACHT domain in complex with a small molecule inhibitor (PDB ID 7ALV) with (left) and without (right) visible protein surface

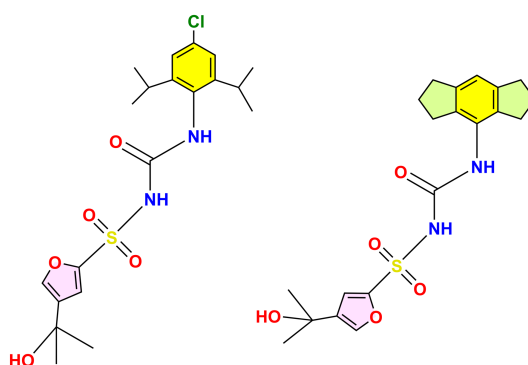


Figure 5.5 Chemical structure of compound RM5 (PDB ID 7ALV) (left) and compound **MCC950** (right)

The missing residues in the protein structure were incorporated using the SWISS-MODEL webserver (<https://swissmodel.expasy.org/>) with the ‘User Template’ option. The protein sequence was obtained from UniProtKB (<https://www.uniprot.org/>) (UniProt

ID Q96P20), and the minimised PDB structure (PDB ID: 7ALV) served as the template file [260]. The standard protonation states of the amino acids were assigned using PROPKA (PDB2PQR server: <https://server.poissonboltzmann.org/pdb2pqr>) at pH 7.4, while the N-terminus and C-terminus were neutralized using the PARSE force field [261]. Energy minimization of the protein structure was conducted using UCSF Chimera (version 1.15), involving 500 steps of the steepest descent algorithm (with a step size of 0.02 Å) followed by 50 steps of the conjugate gradient algorithm (also with a step size of 0.02 Å), employing the AMBER ff14SB force field [262].

5.2.4. Docking-based high-throughput virtual screening (HTVS)

The HTVS was conducted using PyRx (version 0.8) [263] to assess the binding modes and affinities of the selected coumarin derivatives to the NLRP3 receptor, aiming to identify compounds with optimal binding energies. The protein structure 7ALV was utilized as the macromolecule (receptor). Energy minimization of all ligands was carried out using UFF with the steepest descent algorithm, totalling 500 steps. The “number of steps to update” was set to 1, and minimization was halted when the energy difference fell below 0.1 kcal/mol. All minimized ligands were converted to AutoDock Ligand (PDBQT) format for HTVS. The energy-minimized ligands were docked into the binding sites of RM5.

The amino acid residues involved in non-covalent interactions were identified and visualized using BIOVIA Discovery Studio Visualizer [264] and LigPlot+ [265]. Discovery Studio generated detailed 2D interaction diagrams that illustrated a variety of interactions, including hydrogen bonds (H-bonds), hydrophobic contacts, and electrostatic interactions, offering a comprehensive analysis of the specific interactions between the protein and the co-crystal ligand. In contrast, LigPlot+ provided a simplified overview of these interactions, particularly highlighting H-bonds and hydrophobic

contacts, facilitating a quick interpretation of the key binding features. The identified residues served as references for developing the grid box in PyRx. The grid centre was placed at 18.5263 (X-axis), 33.9670 (Y-axis), 126.1612 (Z-axis) with grid dimensions of $26 \times 24 \times 21$ Å.

5.2.5. Molecular docking studies

Molecular docking studies of the compounds derived from HTVS were conducted using AutoDock (version 4.2) [266]. Protein preparation for docking was facilitated by AutoDock Tools (version 1.5.6), where water molecules and heteroatoms were removed, and polar hydrogens were added to enhance structural stability. Kollman united atom charges were assigned to the protein during energy minimization. All atoms were assigned AutoDock 4 atom types, and the prepared structure was saved in PDBQT format. A set of ligands exhibiting lower binding energies than the co-crystallized ligand RM5 were subjected to three consecutive stages of rigid molecular docking in AutoDock (version 4.2).

The grid boxes were designed accordingly and validated using BIOVIA Discovery Studio Visualizer. The grid was defined with dimensions of $71 \times 67 \times 55$ points along the X, Y, and Z axes, respectively, using a grid spacing of 0.4 Å. The grid centre was set at coordinates X: 19.201, Y: 32.899, and Z: 124.975. The grid validation involved redocking RM5 and calculating the RMSD value of the corresponding heavy atoms between the redocked pose and the experimental co-crystallized ligand structure retrieved from PDB ID 7ALV. Gasteiger charges were assigned to the ligands, which were also converted to PDBQT format using AutoDock (version 1.5.6).

Three sequential docking stages utilized the LGA 4.2, with an increasing number of GA runs set at 10, 50, and 100, respectively. After each docking stage, ligands that achieved better docking scores than **MCC950**, a highly specific NLRP3 inflammasome inhibitor,

were advanced to subsequent stages. Following the third docking stage, ligands demonstrating lower binding energies than **MCC950** were selected for further analysis. The comparison of docking scores among various coumarin derivatives, despite their diverse chemical groups and molecular sizes, primarily served as an initial screening method. This strategy facilitated the identification of compounds with favourable binding affinity to the NLRP3 neuroinflammasome binding site, akin to that of **MCC950**. Given that **MCC950** is a well-established specific inhibitor of the NLRP3 inflammasome, any compound exhibiting equal or superior binding affinity to the receptor may also possess inhibitory potential.

5.2.6. *In silico* BBB permeability and ADMET prediction

Ligands that exhibited lower binding affinities than **MCC950** in the final docking stage were analysed using SwissADME (<http://www.swissadme.ch/>), eMolTox (<https://xundrug.cn/moltox>), and PreADMET (<https://preadmet.webservice.bmdrc.org/>) web servers. These tools were employed to predict the compounds' potential to permeate the BBB, assess ADMET properties, and evaluate neurotoxicity, enabling the identification of molecules with the highest likelihood of brain penetration without eliciting toxicity.

5.2.7. MD simulations

MD simulations of the protein complexed with the selected compounds and the standard inhibitor (**MCC950**) derived from docking studies were conducted using the GPU version of the Amber 20 software package [267]. Utilizing **MCC950** as a control facilitated a direct comparison of the binding affinities and dynamic behaviour of the compounds relative to **MCC950** within the NLRP3 binding site.

Topologies and coordinates for the protein and protein–ligand complexes were generated using the *tleap* module of Amber 20. The refined system was solvated in a cubic box

filled with TIP3P water molecules, ensuring a cut-off distance of 12 Å between the protein and the box edge. Counter ions (Na^+ or Cl^-) were added using the ‘Monte Carlo’ method to neutralize any residual charges [268]. A non-bonded cut-off was set at 8 Å, and PME was employed to address long-range electrostatic interactions. The solvated MD systems then underwent energy minimization via the steepest descent and conjugate gradient algorithms, with the convergence criterion for the energy gradient established at 1.0×10^{-4} Kcal/mol-Å.

Equilibration was achieved in three phases. The constant volume ensemble (NVT) was run for 200 ps to gradually heat the system to 310.15 K, utilizing a Langevin thermostat with a collision frequency of $\gamma = 5 \text{ ps}^{-1}$. With a time step of 1 fs, SHAKE constraints were applied to all bonds. Following the temperature stabilization, a 200 ps MD run was conducted in the constant pressure (NPT) ensemble at 310.15 K and 1 bar pressure, maintained using a Berendsen barostat. The final 200 ns of the MD production phase was executed at 310.15 K in the NPT ensemble [228]. After the MD simulation concluded, the trajectories and coordinates generated were analysed using the *cptraj* module in Amber 20 [269]. Calculations for RMSD, RMSF, H-bonding, Rg, and solvent-accessible surface area (SASA) for both the screened compounds and **MCC950** within the NLRP3 binding site were performed to evaluate the strength of the protein–ligand interactions. These analyses provided a comprehensive comparison of their binding affinities and highlighted the potential advantages of the screened compounds over **MCC950**.

5.2.8. Binding free energy calculations

The molecular mechanics–Generalised Born surface area (MM-GBSA) and molecular mechanics–Poisson-Boltzmann surface area (MM-PBSA) methods are commonly employed to estimate the free energy associated with the binding of small ligands to biological macromolecules. These methods typically utilize MD simulations of the

receptor–ligand complex, offering a balance between accuracy and computational cost when compared to empirical scoring and more rigorous alchemical perturbation techniques [270]. The MM-GBSA and MM-PBSA calculations for the docked ligand–protein complexes were carried out using the MMPBSA.py tool from Amber 20 [271]. This tool extracts snapshots of the protein, ligand, and the protein–ligand complex from the trajectory of the solvated complex. The calculations were conducted at 10 ns intervals over the final 50 ns of the simulation [269].

5.3. Results and discussion

5.3.1. HTVS and molecular docking studies

The analysis of protein–ligand interactions facilitated the identification of amino acid residues for generating grid boxes used in HTVS and molecular docking studies (**Figure 5.6**).

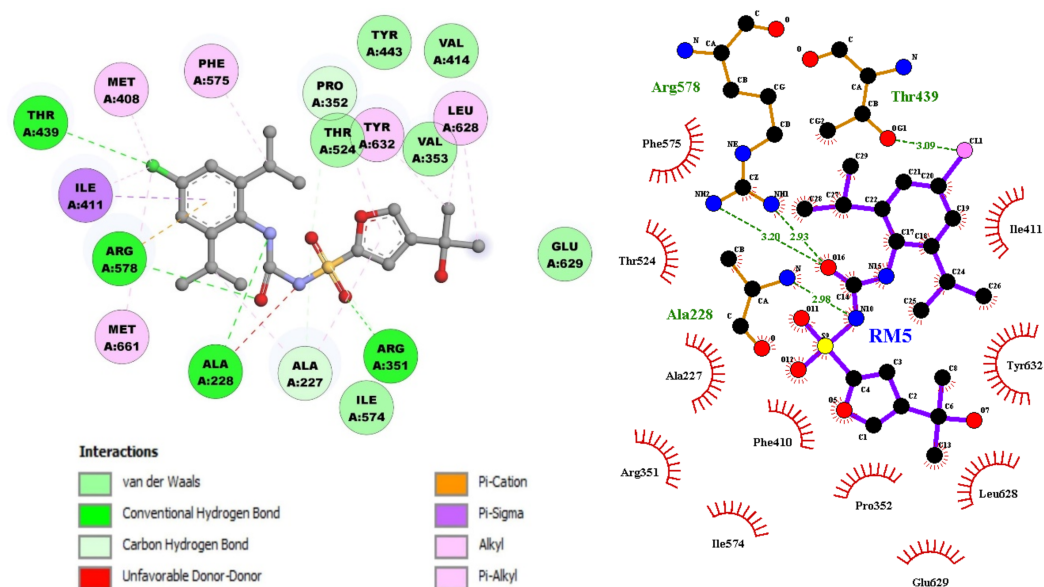


Figure 5.6 2D protein–ligand interaction diagram visualized by BIOVIA Discovery Studio Visualizer (left) and LigPlot+ (right)

Based on the presence of a common coumarin moiety, a total of 9,559 unique compounds were sourced from the specified websites, of which 688 drug-like molecules were selected for HTVS using PyRx software. These compounds were then ranked according

to their estimated free binding energies. The binding energy for RM5 was measured at -9 kcal/mol. Out of this, 175 molecules exhibiting binding energies lower than -9 kcal/mol were chosen for docking studies. Molecular docking was performed utilizing AutoDock (version 4.2). The docking protocol was validated by calculating the RMSD between the redocked and crystallographic poses of RM5, yielding a value of 0.4413 Å, which demonstrates the reliability of the docking procedure (**Figure 5.7**).

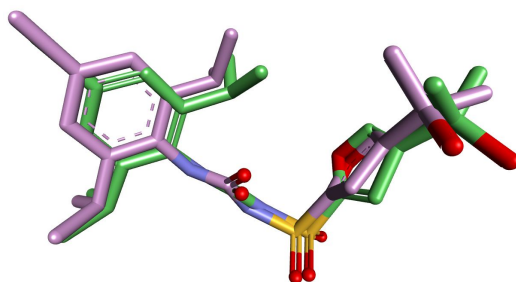


Figure 5.7 Superimposition of co-crystallised (green) and redocked RM5 (lavender)

The co-crystallized ligand aligned well with its redocked conformation. Additionally, the RMSD between the co-crystal protein structure and the homology-modelled protein structure was assessed using Maestro and found to be 0.686 Å (**Figure 5.8** and **Figure S5**).

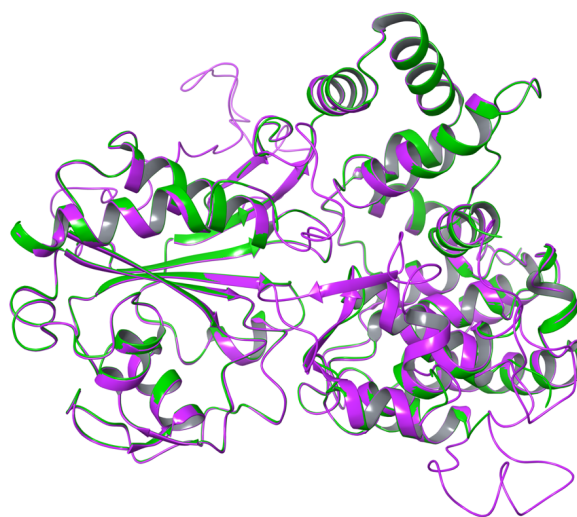


Figure 5.8 Alignment of co-crystal protein 7ALV (green) to homology modelled protein

Subsequently, the 175 molecules derived from HTVS underwent sequential docking (**Table 5.1**). The outcomes for 36 compounds demonstrating comparable or superior binding affinity to **MCC950** during the third stage of docking are presented in **Table 5.2**.

Table 5.1 Molecules subjected to HTVS and molecular docking

Study	Platform	Number of compounds
HTVS	PyRx 0.8	688
Docking stage 1	AutoDock 4.2	175
Docking stage 2	AutoDock 4.2	45
Docking stage 3	AutoDock 4.2	36

Table 5.2 Results of the third stage of docking displaying mean binding energy, binding efficiency, and cluster size percentage

Sl No.	Compound ID	Mean binding energy (kcal/mol)	Ligand binding efficiency	Cluster size percentage
1	MolPort-050-872-358	-11.08	-0.38	95
2	MolPort-050-923-053	-11.03	-0.38	36
3	MolPort-051-403-360	-10.90	-0.40	47
4	MolPort-050-916-394	-10.88	-0.39	59
5	MolPort-050-897-577	-10.86	-0.39	66
6	MolPort-050-923-051	-10.75	-0.38	100
7	MolPort-050-697-151	-10.71	-0.41	45
8	MolPort-050-866-640	-10.64	-0.35	90
9	MolPort-050-897-585	-10.60	-0.38	60
10	MolPort-050-884-068	-10.56	-0.39	88
11	MolPort-051-022-786	-10.54	-0.38	100
12	MolPort-051-396-435	-10.48	-0.42	48
13	MolPort-050-848-682	-10.43	-0.37	91
14	MolPort-051-321-471	-10.42	-0.42	42
15	MolPort-051-327-862	-10.42	-0.42	33
16	MolPort-051-405-230	-10.37	-0.37	73
17	MolPort-050-873-234	-10.29	-0.38	76
18	MolPort-050-921-741	-10.29	-0.38	62
19	MolPort-050-910-683	-10.17	-0.41	84
20	MolPort-050-927-595	-10.13	-0.36	75
21	MolPort-050-778-875	-10.04	-0.37	49
22	MolPort-050-872-704	-10.03	-0.36	98
23	MolPort-050-840-916	-10.01	-0.37	95
24	MolPort-050-884-445	-10.00	-0.36	71
25	MolPort-050-860-181	-9.99	-0.40	90
26	MolPort-051-401-400	-9.94	-0.40	51
27	MolPort-050-894-075	-9.93	-0.35	88
28	MolPort-051-402-595	-9.91	-0.38	62
29	MolPort-050-810-558	-9.86	-0.38	64
30	MolPort-050-935-260	-9.76	-0.31	58
31	MolPort-050-241-114	-9.75	-0.41	70
32	MolPort-050-825-444	-9.74	-0.37	86
33	MolPort-050-677-457	-9.72	-0.40	61
34	MolPort-050-830-570	-9.71	-0.37	55
35	MolPort-050-911-405	-9.69	-0.35	99
36	MolPort-051-135-630	-9.59	-0.34	52
37	MCC950	-9.59	-0.34	30

5.3.2. *In silico* BBB permeability and ADMET prediction

The compounds were evaluated for *in silico* BBB permeability and ADMET properties using the SwissADME [272], eMolTox [273], and PreADMET [274] web servers. All 36 molecules exhibited BBB permeability with high gastrointestinal absorption and a bioavailability score of 0.55 according to SwissADME predictions. Following the removal of potential P-gp substrates, an additional 12 molecules identified by eMolTox as having potential neurotoxicity were excluded. Ultimately, the PreADMET server indicated that three compounds (**MolPort-050-872-358**, **MolPort-050-884-068**, and **MolPort-051-135-630**) were non-mutagenic in the Ames test and non-carcinogenic in *in silico* rat and mouse models. A summary of various parameters for the selected compounds predicted by SwissADME is provided in **Table 5.3**, while toxicity predictions are detailed in **Table 5.4**. The BOILED-Egg plots for both sets of compounds are displayed in **Figure 5.9**.

Table 5.3 SwissADME results of the selected compounds

Compound ID	GI absorption	BBB permeability	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
MolPort-050-872-358	High	Yes	No	Yes	Yes	Yes	Yes
MolPort-050-884-068	High	Yes	No	Yes	Yes	Yes	Yes
MolPort-051-135-630	High	Yes	No	Yes	Yes	No	Yes

Table 5.4 PreADMET results of the selected compounds

Compound ID	MolPort-050-872-358	MolPort-050-884-068	MolPort-051-135-630
algae_at	0.025752	0.033579	0.017331
Ames_test	non-mutagen	non-mutagen	non-mutagen
Carcino_Mouse	negative	negative	negative
Carcino_Rat	negative	negative	negative
daphnia_at	0.02616	0.054266	0.016304
hERG_inhibition	medium_risk	medium_risk	medium_risk
medaka_at	0.00146	0.005812	0.000618
minnow_at	0.007463	0.028486	0.003884
TA100_10RLI	negative	negative	negative
TA100_NA	negative	negative	negative
TA1535_10RLI	negative	negative	negative
TA1535_NA	negative	negative	negative

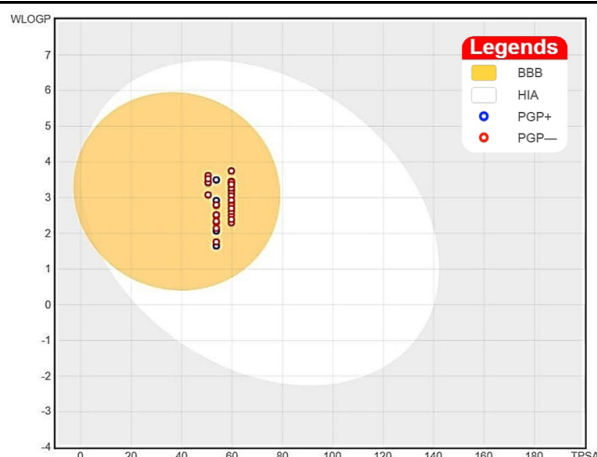


Figure 5.9 BOILED-Egg plots of compounds analyzed by SwissADME

5.3.3. Docking-based protein-ligand interaction studies

Three selected compounds demonstrated significant interactions with NLRP3, forming robust H-bonds at various lengths (Table 5.5). Compound **MolPort-050-872-358** exhibited the highest binding affinity (−11.08 kcal/mol), characterized by excellent hydrophobic interactions within the receptor binding site, in addition to hydrogen bonding with amino acid residues ALA228, ARG351, PRO352, and TYR443, along with electrostatic interactions with ARG578 and GLU629. **MolPort-050-884-068** also showed good binding affinity (−10.56 kcal/mol) with multiple hydrophobic interactions and hydrogen bonding with ARG578 and ALA227. **MolPort-051-135-630** had a comparatively lower binding affinity (−9.59 kcal/mol), forming hydrogen bonds with ALA228 and ARG578, as well as exhibiting alkyl and electrostatic interactions. The reference compound, **MCC950** (−9.59 kcal/mol), displayed several hydrophobic interactions and hydrogen bonding with ALA228, ARG351, ARG578, and GLU629. **MCC950** also engaged in electrostatic interactions with ARG578 and GLU629, similar to **MolPort-050-872-358** and **MolPort-051-135-630**. The 2D and 3D interactions of the inflammasome with the three ligands and the reference compound are illustrated in **Figure 5.10**, visualized using BIOVIA Discovery Studio Visualizer.

Table 5.5 Hydrophobic, hydrogen, cation- π , and π - π stacking interactions and amino acid residues for the compounds with the target protein

Compound ID	H-bond with bond length (Å)			Hydrophobic		
	Conventional H-Bond	Carbon H-bond	π -Donor H-Bond	Alkyl	π -alkyl	π -cation π -anion
MolPort-050-872-358	ALA228 (2.21),	ARG351 (2.04),	TYR443	ALA227, ILE411,	ALA227, PRO352,	
	ALA228 (1.96),	PRO352 (2.62)	(2.78)	MET408, MET661	VAL353, ILE411, LEU413, VAL414	ARG578 GLU629
	ARG351 (2.44)					
MolPort-050-884-068	ARG578 (2.05)		ALA227 (2.88)	ALA227, MET661,	ILE230, PHE410, ILE411, TYR632, MET661	
MolPort-051-135-630	ALA228 (2.25), ARG578 (2.31)	TYR632 (3.68)		LEU628, ALA227, ALA228, ILE411		ARG578, ARG578 GLU629
MCC950	ALA228 (2.33), ARG351 (2.47), ARG578 (1.87), ARG578 (2.69), GLU629 (1.83)	ALA227 (2.30), PRO352 (2.10)		ALA228, ILE411, LEU413, MET661	TYR632, ILE411	ARG578, ARG578 GLU629

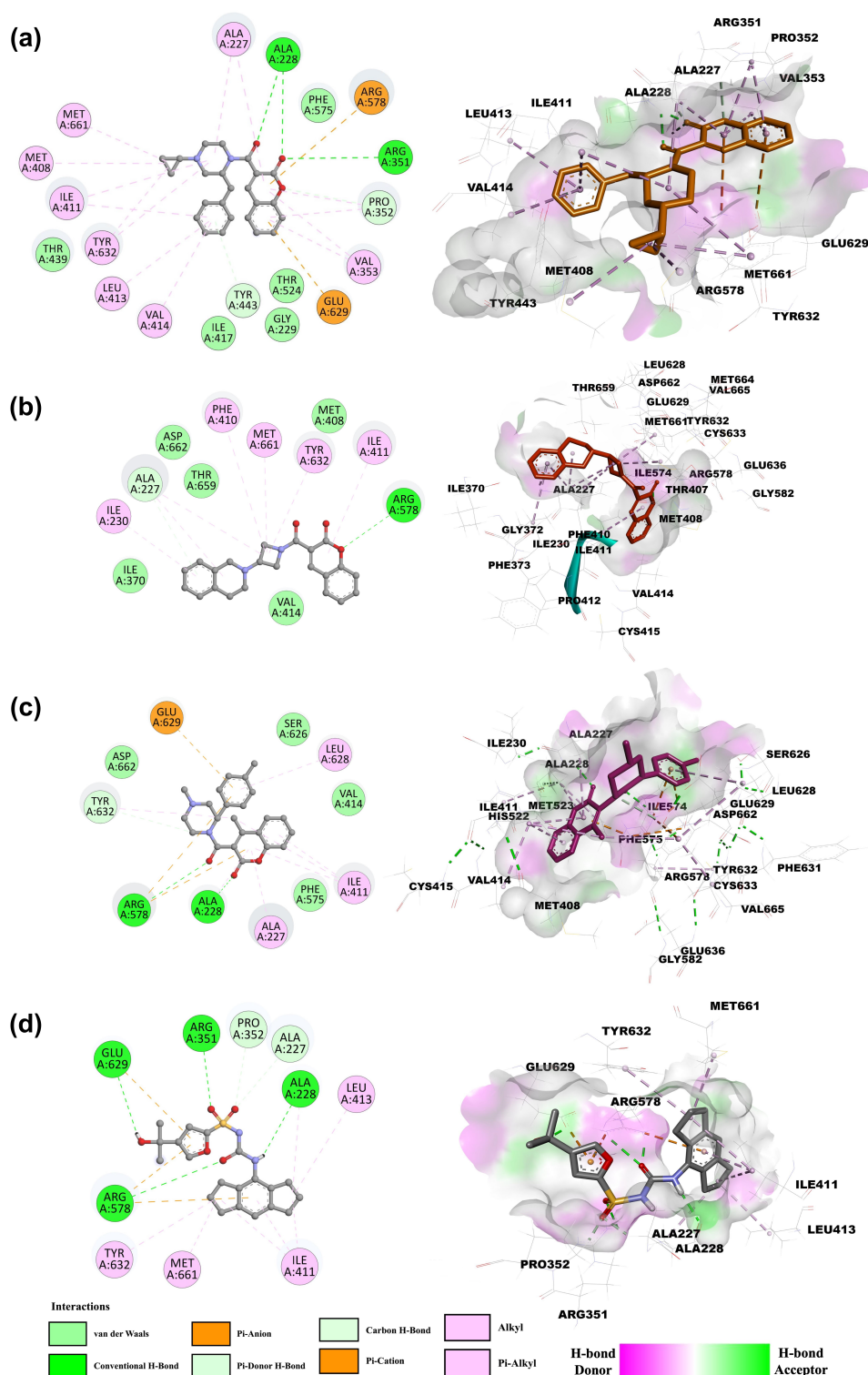


Figure 5.10 2D and 3D protein-ligand interaction diagrams of (a) MolPort-050-872-358, (b) MolPort-050-884-068, (c) MolPort-051-135-630, and (d) protein-MCC950 complexes

5.3.4. MD simulation study

Snapshots from the MD simulation trajectories taken at 40 ns intervals for all selected ligands, including the reference ligand, are presented in **Figure S6**. These conformations illustrate the structural dynamics and notable conformational changes within the ligand-protein complex, offering a detailed understanding of its dynamic behaviour throughout the simulation.

5.3.4.1. RMSD analysis

RMSD values of 1–3 Å are well accepted for small, globular proteins. The mean protein C α RMSD observed for the protein–**MolPort-050-872-358**, protein–**MolPort-050-884-068**, protein–**MolPort-051-135-630**, and protein–**MCC950** complexes were 0.87, 0.85, 0.86, and 0.88 Å, respectively (**Figure 5.11 (a)**). The ligand–protein interactions underwent conformational changes within the initial 40 ns of the simulation. The RMSD trajectories displayed an upward trend during the initial frames until stabilization around 40 ns, followed by trajectories that remained close to their average values for the subsequent 160 ns. The protein C α RMSD values were similar across all four complexes, indicating that, on average, the protein structures experienced a comparable degree of deviation from their initial conformations during the MD simulation. The relatively low values suggest that the protein–ligand complexes maintained overall structural stability and did not deviate significantly from their initial conformations throughout the 200 ns simulation.

During the MD simulation, ligands shifted from their initial docking pose and glided deeper into the receptor pocket. If a ligand exhibits a considerably greater RMSD than the protein, it indicates potential diffusion from its initial binding site. The average RMSD values for ligands **MolPort-050-872-358**, **MolPort-050-884-068**, **MolPort-051-135-630**, and **MCC950** were 0.89, 0.30, 0.50, and 0.59 Å, respectively, indicating stability

with respect to the complexed protein's binding pocket (**Figure 5.11 (b)**). The maximum RMSD values for the ligands were 1.25, 0.74, 1.24, and 1.16 Å at around 76, 18, 41, and 195 ns, respectively. All three protein–ligand complexes remained stable throughout the simulation, with the ligands maintaining good proximity to the protein up to 200 ns. The ligand RMSD of **MolPort-051-135-630** was similar to that of the reference molecule **MCC950** during the MD simulation, while **MolPort-050-872-358** displayed slightly higher and **MolPort-050-884-068** slightly lower RMSD values than **MCC950**. The RMSD of **MolPort-050-884-068** was nearly stable from the beginning, while **MolPort-050-872-358**, **MolPort-051-135-630**, and **MCC950** stabilised at around 25, 110, and 129 ns, respectively. This indicates that all three selected ligands attained a stable binding conformation faster than the reference ligand.

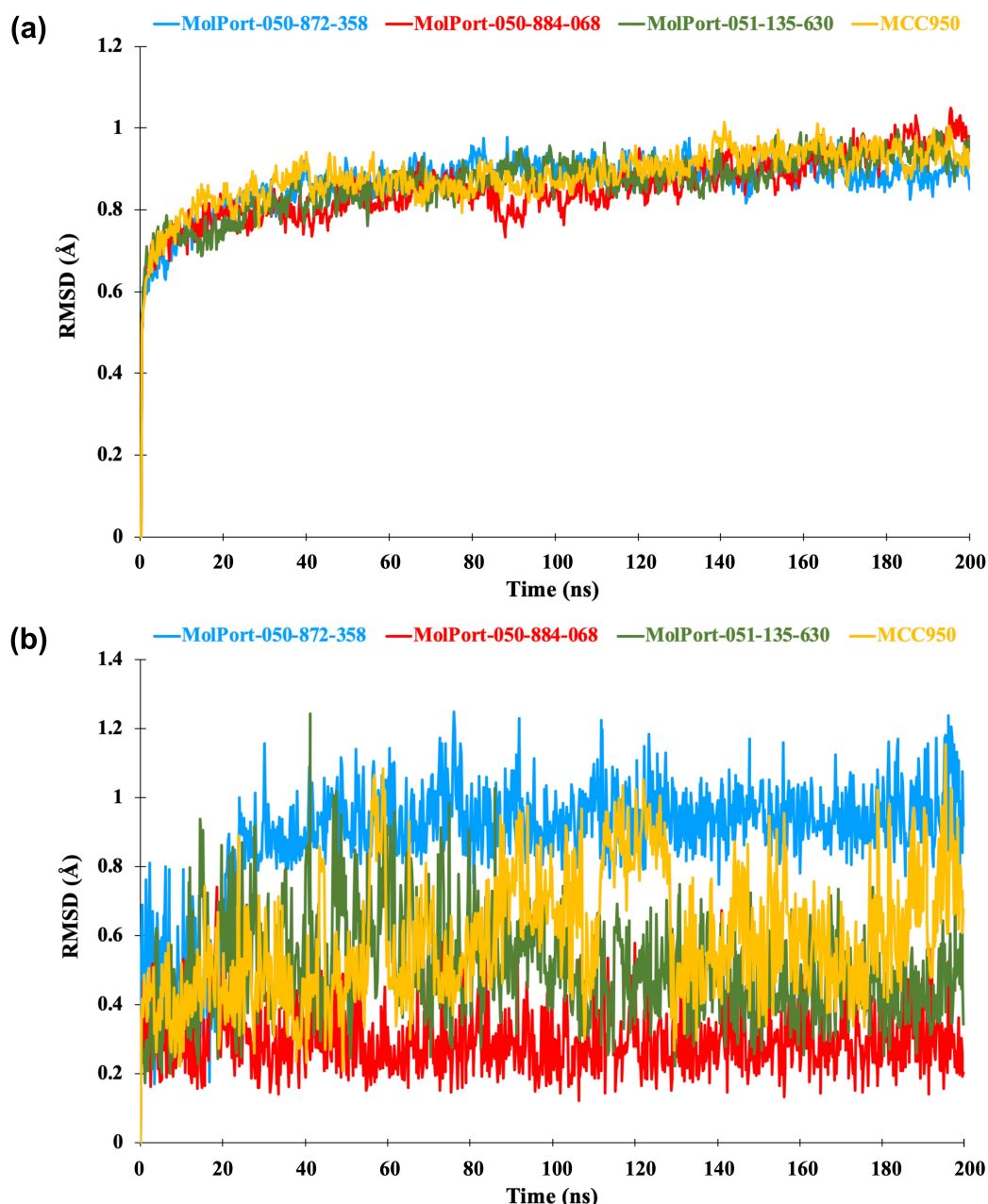


Figure 5.11 (a) RMSD of the C α of the protein–MolPort-050-872-358 (blue), protein–MolPort-050-884-068 (red), protein–MolPort-051-135-630 (green), and protein–MCC950 (yellow) complexes. (b) RMSD of heavy atoms of the ligands MolPort-050-872-358 (blue), MolPort-050-884-068 (red), MolPort-051-135-630 (green), and MCC950 (yellow)

5.3.4.2. RMSF

The average RMSF values for the C α of the protein were found to be 0.59, 0.62, 0.60, and 0.60 Å for the protein–MolPort-050-872-358, protein–MolPort-050-884-068, protein–MolPort-051-135-630, and protein–MCC950 complexes, respectively (Figure

5.12). These values indicate relatively low fluctuation and stability in the protein structure at the C α atom level for each complex. The highest fluctuation observed was 2.00 Å for the protein–**MolPort-051-135-630** complex. Besides the two terminals, consistent fluctuations were noted at around 25, 55-62, 80, 295, 325, 409, and 487 amino acid residues across all systems. The RMSF values of the protein in complex with the ligands were similar to those with the reference ligand (**MCC950**), suggesting that the ligands had a comparable stabilizing or perturbing effect on the NLRP3 structure. Overall, the protein exhibited a consistent level of stability and flexibility in the presence of the ligands, similar to that of **MCC950**.

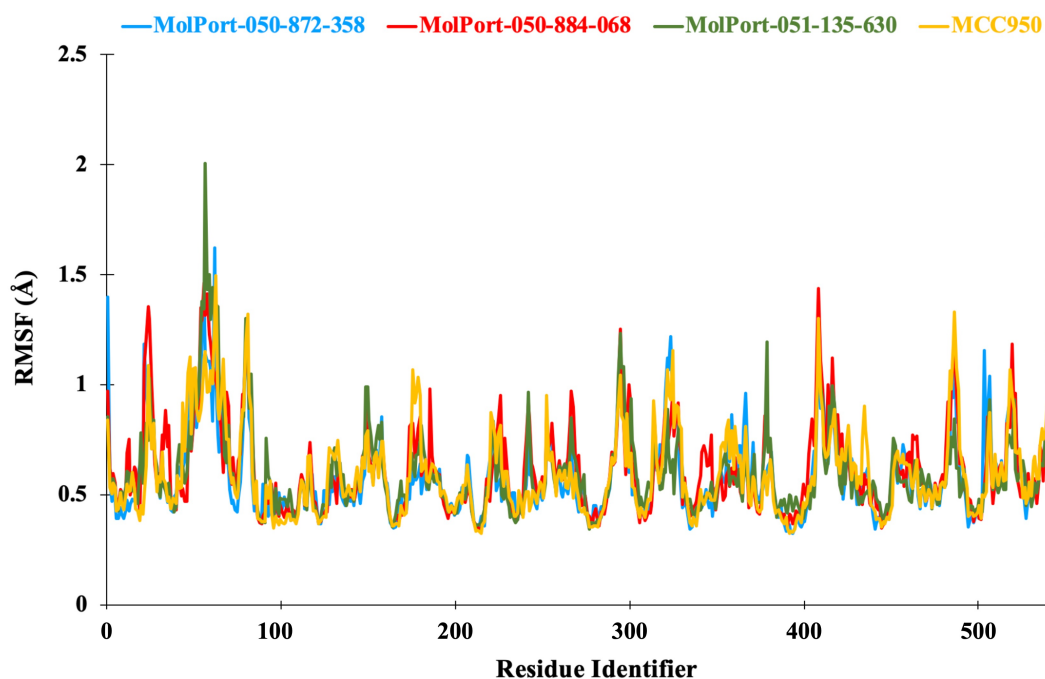


Figure 5.12 Residue-wise protein RMSF of the C α of the protein–**MolPort-050-872-358** (blue), protein–**MolPort-050-884-068** (red), protein–**MolPort-051-135-630** (green), and protein–**MCC950** (yellow) complexes

The ligand-RMSF characterises the positional alterations of ligand atoms. The average ligand-RMSF values for the protein–**MolPort-050-872-358**, protein–**MolPort-050-884-068**, protein–**MolPort-051-135-630**, and protein–**MCC950** complexes were 0.96, 0.65, 1.50, and 0.83 Å, respectively (**Figure 5.13**).

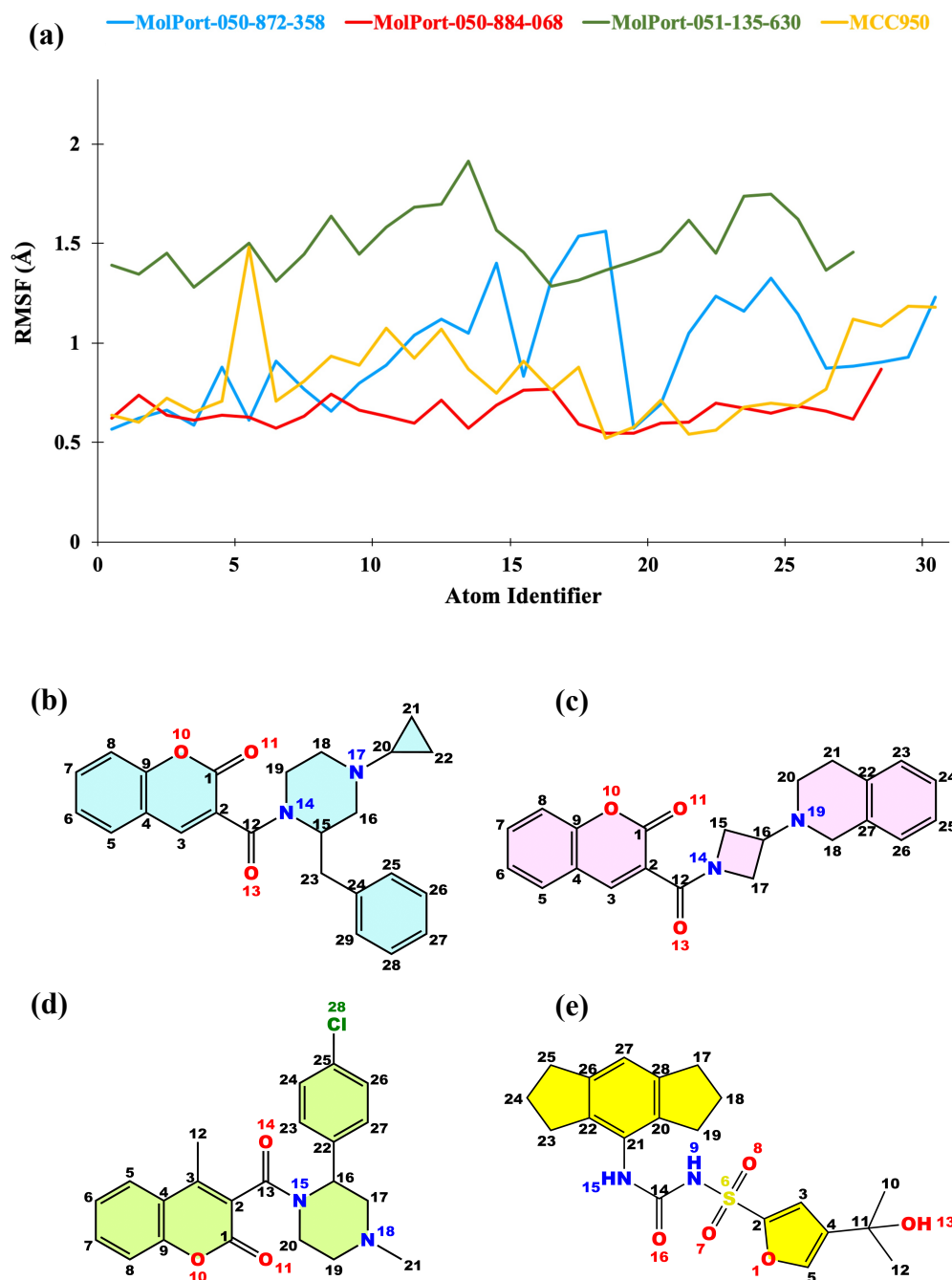


Figure 5.13 (a) Residue-wise ligand RMSF of the molecules **MolPort-050-872-358** (blue), **MolPort-050-884-068** (red), **MolPort-051-135-630** (green), and **MCC950** (yellow). Atom identifier numbers are shown for the molecular structures of (b) **MolPort-050-872-358**, (c) **MolPort-050-884-068**, (d) **MolPort-051-135-630**, and (e) **MCC950**

5.3.4.3. H-bond analysis

The H-bonds were detected using a distance cut-off of 3.5 Å and an angle cut-off of 140°.

Figure 5.14 shows the number of H-bonds formed between the molecules and the protein structure during the simulation runs. Plotting the number of H-bonds as a function of time provides a dynamic view of how these interactions evolve throughout the simulation. The peaks in the plots indicate instances of H-bond formation between the ligand and receptor.

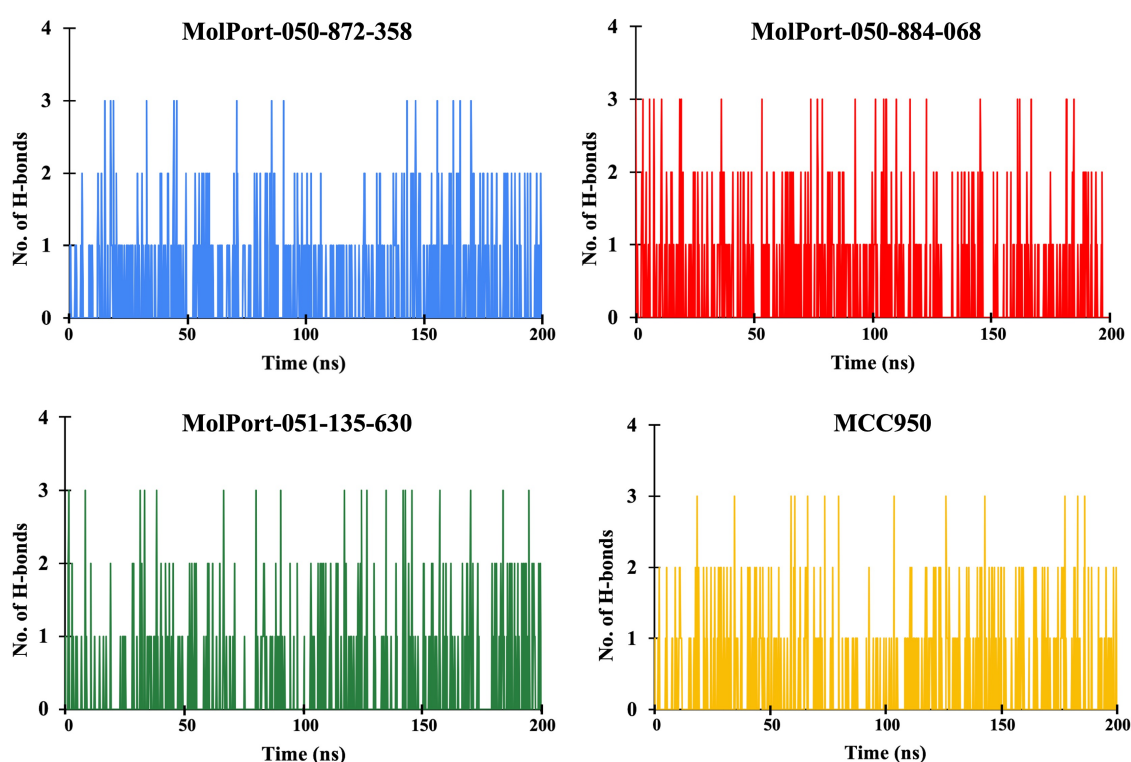


Figure 5.14 The number of intermolecular H-bonds formed between ligand and protein during MD simulation for **MolPort-050-872-358** (blue), **MolPort-050-884-068** (red), **MolPort-051-135-630** (green), and **MCC950** (yellow)

5.3.4.4. Rg analysis

The average Rg values for the protein–**MolPort-050-872-358**, protein–**MolPort-050-884-068**, protein–**MolPort-051-135-630**, and protein–**MCC950** complexes were 23.72 Å, 23.73 Å, 23.98 Å, and 23.98 Å, respectively. These results indicated that all the protein–ligand complexes, including the protein–standard complex, exhibited similar Rg values during the simulation run (**Figure 5.15**). This suggests that the selected compounds fit well within the binding site of NLRP3, interacting with the protein in a manner comparable to the standard compound **MCC950**. The interactions contributing to the compactness of the protein–ligand complexes are likely comparable in strength and orientation for all compounds. Notably, the Rg values for complexes with ligands **MolPort-050-872-358** and **MolPort-050-884-068**, as well as those with **MolPort-051-135-630** and **MCC950**, exhibited similar trends throughout the 200 ns simulation.

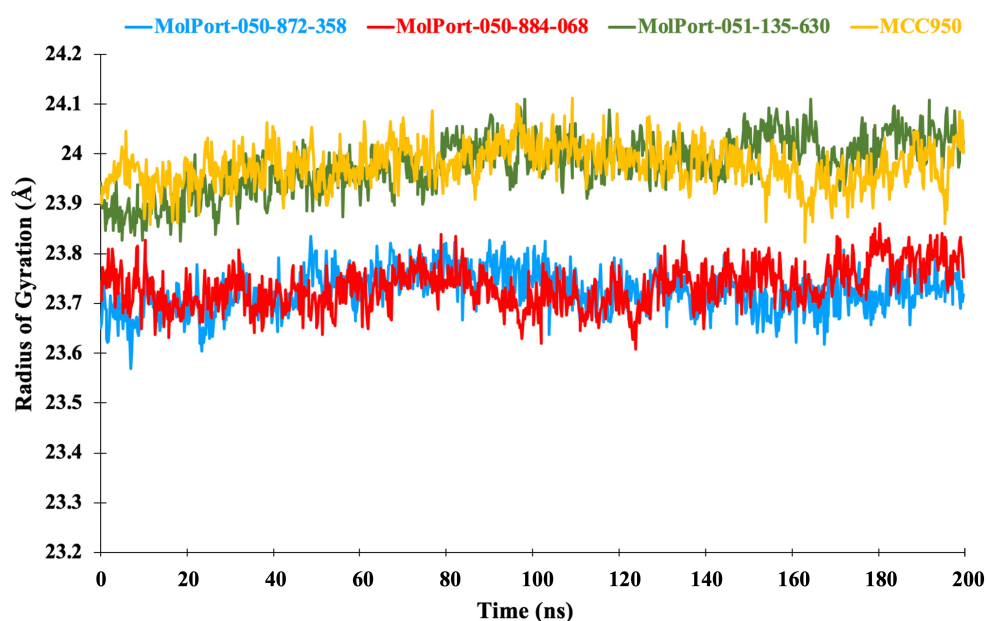


Figure 5.15 Rg of the ligands **MolPort-050-872-358** (blue), **MolPort-050-884-068** (red), and **MolPort-051-135-630** (green), and **MCC950** (yellow)

5.3.4.5. SASA analysis

The SASA of a protein-ligand complex represents the surface area of the combined structure (protein and ligand) that is accessible to solvent molecules, typically water. It is an important parameter for understanding the interactions and behaviour of the protein-ligand complex in a biological environment. SASA is typically computed by considering the combined van der Waals surfaces of the ligand and protein, calculating the area accessible to solvent molecules while accounting for the shape and size of water or relevant solvent molecules.

In this study, SASA was calculated using a probe with Van der Waals radii of 1.4 Å in the surf module of Amber 20. A large SASA value indicates that a significant portion of the protein–ligand binding interface is exposed to the solvent, suggesting more stable and specific binding interactions. The accessibility of these regions is crucial for ligands to exert their functional effects on the protein. A complex with higher SASA values may also be more flexible and dynamic, allowing the ligand to explore multiple binding orientations and conformational changes.

The average SASA values for the complete MD simulation were 17072.89 Å², 16799.76 Å², 17992.25 Å², and 16137.81 Å² for protein–**MolPort-050-872-358**, protein–**MolPort-050-884-068**, protein–**MolPort-051-135-630**, and protein–**MCC950** complexes, respectively (**Figure 5.16**). These values suggest accessibility and potential for extensive interactions at the binding interface.

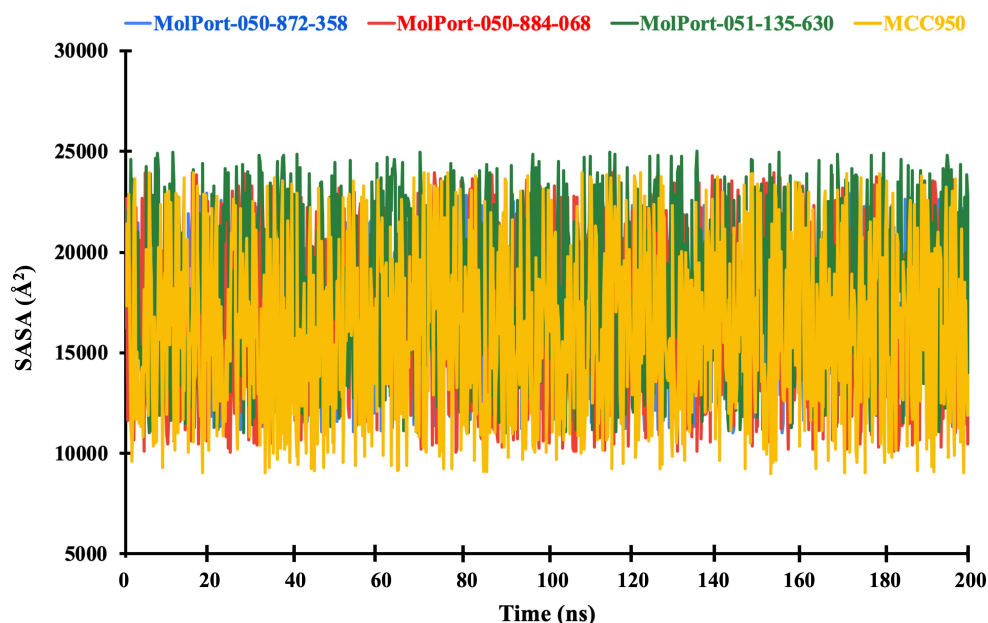


Figure 5.16 Graph of solvent accessible surface areas (SASAs) of protein–**MolPort-050-872-358** (blue), protein–**MolPort-050-884-068** (red), protein–**MolPort-051-135-630** (green), and protein–**MCC950** (yellow) complexes

5.3.4.6. Binding free energy calculations

A direct comparison of docking scores among chemically diverse molecules has limitations due to their unique structural and physicochemical properties. The primary goal of comparing docking scores was to perform an initial screening to identify molecules with more favourable docking at the binding site of **MCC950**, the reference drug. To enhance the accuracy of the ligand selection process and gain a more comprehensive understanding of receptor–ligand interactions, binding free energy values were calculated for all protein–ligand complexes. This provided deeper insight into binding affinities and facilitated the selection of potential ligands for further investigation. The combination of free binding energy calculations with docking scores created a robust framework for the comprehensive evaluation of ligands.

In MM-GBSA and MM-PBSA computations, molecular mechanics (MM) energies and solvation energies are considered for calculating binding free energy. The polar solvation

energy calculation depends on the implicit solvent model (GB or PB), while the non-polar solvation energy calculation relies on SASA. The binding free energies of the protein–ligand complexes, along with various components of binding free energy (van der Waals: ΔE_{vdw} , electrostatic: ΔE_{eel} , polar solvation free energy: $\Delta G_{\text{GB/PB}}$, nonpolar solvation free energy: $\Delta G_{\text{Nonpolar}}$, and total binding free energy: $\Delta G_{\text{MMGBSA/MMPBSA}}$), are summarized in **Table 5.6** and **Table 5.7**. Both MM-GBSA and MM-PBSA revealed low net binding energies, indicating high complex stability. The van der Waals contributions (ΔE_{vdw}) were significantly high, suggesting that they play a major role in stabilizing the ligands at the protein's binding site. The contribution of electrostatic energies (ΔE_{eel}) was also high, except in the case of MolPort-050-884-068 (7.44 ± 1.45 kcal/mol). In both the GB and PB solvation models, all selected ligands exhibited better binding free energy than the standard, indicating that all identified hits mediate promising interactions with the protein NLRP3.

Table 5.6 Energy contributions of protein-ligand complexes in MM-GBSA assay

Compound code	$\Delta E_{vdw}(\text{kcal/mol})$	$\Delta E_{ele}(\text{kcal/mol})$	$\Delta G_{PB}(\text{kcal/mol})$	$\Delta G_{Nonpolar}(\text{kcal/mol})$	$\Delta G_{MMGBSA}(\text{kcal/mol})$
MolPort-050-872-358	-65.29 ± 1.6084	-18.21 ± 1.89	34.50 ± 2.40	-6.50 ± 0.12	-55.51 ± 2.60
MolPort-050-884-068	-56.90 ± 1.47	7.44 ± 1.45	10.09 ± 2.08	-6.44 ± 0.06	-45.81 ± 1.86
MolPort-051-135-630	-49.88 ± 1.64	-12.95 ± 10.58	38.10 ± 10.01	-5.49 ± 0.09	-30.22 ± 3.08
MCC950	-46.21 ± 2.68	-51.16 ± 12.64	80.67 ± 11.39	-5.94 ± 0.14	-22.64 ± 4.68

Data expressed in mean $\pm$ S.D. (n = 6).

Table 5.7 Energy contributions of protein-ligand complexes in MM-PBSA assay

Compound code	$\Delta E_{vdw}(\text{kcal/mol})$	$\Delta E_{ele}(\text{kcal/mol})$	$\Delta G_{PB}(\text{kcal/mol})$	$\Delta G_{Nonpolar}(\text{kcal/mol})$	$\Delta G_{MMPBSA}(\text{kcal/mol})$
MolPort-050-872-358	-65.29 ± 1.61	-18.21 ± 1.89	38.15 ± 2.12	-3.67 ± 0.06	-49.02 ± 1.22
MolPort-050-884-068	-56.90 ± 1.47	7.44 ± 1.45	16.95 ± 4.46	-3.54 ± 0.02	-36.05 ± 4.88
MolPort-051-135-630	-49.88 ± 1.64	-12.95 ± 10.58	31.65 ± 9.57	-3.63 ± 0.06	-34.81 ± 5.56
MCC950	-46.21 ± 2.68	-51.16 ± 12.64	94.32 ± 13.67	-3.63 ± 0.04	-6.68 ± 4.09

Data expressed in mean $\pm$ S.D. (n = 6).

5.4. Conclusion

Neuroinflammation is closely linked to the neurodegenerative processes in AD. Exploring novel approaches to interconnected pathways is essential for developing new strategies for the treatment and management of the disease. A promising method to mitigate the harmful effects of neuroinflammation is the pharmacological inhibition of the NLRP3 inflammasome.

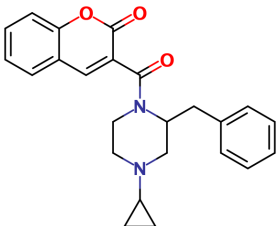
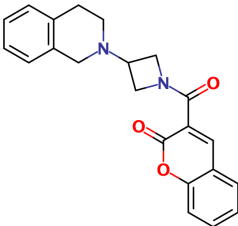
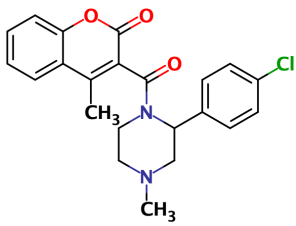
In this study, a comprehensive dataset of 9559 coumarin derivatives with diverse chemical structures was meticulously evaluated to identify potential inhibitors of the NLRP3 inflammasome. Through rigorous molecular docking studies and subsequent molecular dynamics simulations three promising coumarin derivatives were identified: **MolPort-050-872-358**, **MolPort-050-884-068**, and **MolPort-051-135-630**. They displayed strong binding affinities and favourable interactions with the NLRP3 inflammasome. The 2D structures, IUPAC names, and SMILES of the identified virtual hits are summarized in **Table 5.8**.

The carefully selected hits, resulting from a comprehensive analysis and rational filtration of chemical structures, represent valuable candidates for developing NLRP3 inhibitors. The removal of non-drug-like molecules and those with undesirable fragments ensured a refined selection, increasing the likelihood of successful experimental validation and optimization in the future. Additionally, the stability of the protein-ligand complexes for the final hits was thoroughly investigated using post-molecular dynamics simulation studies. Analyses, including RMSD, RMSF, H-bond analysis, Rg, and SASA, provided valuable insights into the stability and dynamics of the complexes.

These hits serve as a promising starting point for developing potential NLRP3 inhibitors. Further research and experimental validation, including *in vitro* and *in vivo* studies, are

necessary to establish the inhibitory potential and elucidate the mechanisms of action of the identified compounds. The optimization and refinement of these compounds based on the computational insights provided in this study may lead to the development of effective therapeutics for NLRP3-driven inflammatory pathways in AD.

Table 5.8 MolPort ID, 2D structure, IUPAC name, and SMILES of identified hits

MolPort ID	MolPort-050-872-358	MolPort-050-884-068	MolPort-051-135-630
2D Structure			
IUPAC name	3-(2-benzyl-4-cyclopropylpiperazine-1-carbonyl)-2H-chromen-2-one	3-[3-(1,2,3,4-tetrahydroisoquinolin-2-yl)azetidine-1-carbonyl]-2H-chromen-2-one	3-[2-(4-chlorophenyl)-4-methylpiperazine-1-carbonyl]-4-methyl-2H-chromen-2-one
SMILES	<chem>O=C(N1CCN(CC1Cc1cccc1)C1CC1)c1cc2cccc2oc1=O</chem>	<chem>O=C(N1CC(C1)N1CCc2cccc2C1)c1cc2cccc2oc1=O</chem>	<chem>CN1CCN(C(C1)c1ccc(Cl)cc1)C(=O)c1c(C)c2cccc2oc1=O</chem>
InChI key	WXGGKFVIWUPQDW-UHFFFAOYSA-N	NKOWDVGJMTUJBC-UHFFFAOYSA-N	JVZTWJQPPYGAGB-UHFFFAOYSA-N