RESEARCH ARTICLE

Virtual screening of curcumin analogues as DYRK2 inhibitor: Pharmacophore analysis, molecular docking and dynamics, and ADME prediction

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Abstract

Background: Curcumin reduces the proliferation of cancer cells through inhibition of the DYRK2 enzyme, which is a positive regulator of the 26S proteasome.
Methods: In the present work, curcumin analogues have been screened from the MolPort database using a pharmacophore model that comprised a ligand-based approach. The result of the screening was then evaluated by molecular docking and molecular dynamics based on binding the free energy of the interaction between each compound with the binding pocket of DYRK2. The hit compounds were then confirmed by absorption, distribution, metabolism, and excretion (ADME) prediction.
Results: Screening of 7.4 million molecules from the MolPort database afforded six selected hit compounds. By considering the ADME prediction, three prospective curcumin analogues have been selected. These are: 2-[2-(1-methylpyrazol-4-yl)ethyl]-1H,5H,6H,7H,8H-imidazo[4,5-c]azepin-4-one (Molport-035-369-361), methyl 4-(3-hydroxy-1,2-oxazol-5-yl)piperidine-1-carboxylate (Molport-000-004-273) and (1S)-1-[5-(furan-3-carbonyl)-4H,6H,7H-pyrazolo[1,5-a]pyrazin-2-yl]ethanol (MolPort-035-585-822).
Conclusion: Pharmacophore modelling, combined with molecular docking and molecular dynamics simulation, as well as ADME prediction were successfully applied to screen curcumin analogues from the MolPort database as DYRK2 inhibitors. All selected compounds that have better predicted pharmacokinetic properties than that of curcumin are considered for further study.

Keywords

Curcumin analogues, DYRK2, pharmacophore, docking, molecular dynamics simulation, ADME

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Introduction

Curcumin is a compound derived from turmeric (*Curcuma longa*), which is responsible for the yellow rhizome extract coloration. Traditionally, a large number of people in India, China, Indonesia and other Asian countries have applied turmeric powder in therapeutic herbs and as a food additive.[1–5] The curcumin (diferuloylmethane) constituent is a tautomeric compound known to exist as an enolic form in organic solvents, and in keto form in water.[6] The wide range of biological activities are currently being tested *in vivo* and *in vitro* to develop the numerous potentials in treating various diseases. These include the application of curcumin as an antioxidant, antibacterial, antifungal, antiviral, anti-inflammatory, and anti-angiogenic agent. Furthermore, there are reports on the promising anti-Alzheimer, as well as anticancer properties of curcumin, and its antagonistic effects against other degenerative diseases.[7] The curcumin component is also a non-toxic compound, as no toxicity has been reported following the administration of high doses to animals.[8] Previous reports have shown biological activities related to cancer, including lymphomas, breast, prostate, cervical, lung and colorectal cancers, alongside leukemia.[1,4] There are numerous pathways involved in regulation, including p53, BAX, cyclin D1, various BCL, p21, p27, AKT, COX-2, protein kinase, and others.[9]

The facts and opinions on the biological effects of curcumin as a drug candidate indicate that it is a PAIN (pan-assay interference) compound or IMP (invalid metabolic panacea). Moreover, this unstable compound is known to easily degrade into others,[10] and a total of eight have been reported to date. These include vanillin, ferulic acid,[11,12] feruloyl methane, 2-hydroxy-6-(4-hydroxy-3-methoxyphenyl)-4-oxohexa-2,5-dienal,[11] bicyclopentadione,[13] ferulic aldehyde, vanillic acid,[12] and 4-[(1e)-3-(propan-2-yloxy)prop-1-en-1-yl)guaiacol.[14] In addition, numerous pharmacokinetic evaluations have indicated the poor absorption, low solubility, rapid metabolism and elimination as well as poor bioavailability properties of curcumin.[15]

There have been several suggestions of methods to solve these challenges, including co-administration with adjuvants. In addition, studies have shown the possible development into a nanoparticle form, complexations with metallic and radioactive elements, using the derivatives or analogue products, and application in the bioconjugation form.[4,16] One of the current research strategies involves the screening of compounds from a large database to obtain analogues with the pharmacophore features of curcumin.

One of the purposes of studying curcumin and its analogues is to find analogues that are targeted to reduce cell proliferation by interacting with dual-specificity tyrosine-regulated kinase 2 (DYRK2). This is achieved through the positive regulation of the 26S proteasome, particularly in cancer cells. The inhibition property is observed in terms of cell proliferation with IC_{50} 5 nM.[17] DYRK2 is a family of protein kinases with members involved in cellular growth and development.[18] In addition, there have also been reports on its function as a tumor suppressant by regulating cell survival, differentiation, proliferation and apoptosis.[19] The mechanism adopted to control further involves the regulation of CDK14 expression.[19] Furthermore, DYRK2 as an enzyme is capable of phosphorylating serine substrates and threonine residues. This action regulates apoptotic cell death in response to DNA damage by impacting the phosphorylation effect of Ser46 on p53.[19,20] Furthermore, reports have shown the negative regulatory impact on breast cancer formation through the transcriptional downregulation of Kruppel-like factor 4 (KLF4).[20]

Therefore, the aim of this study is to explore the curcumin analogues for their potential application as a DYRK2 inhibitor through virtual screening by using pharmacophore molecular modelling as well as docking and molecular dynamics. The compounds of screening results are expected to be applied as lead compounds in discovering and developing a prospective anticancer molecule through DYRK2 inhibition.

Methods

Dataset preparation

Figure 1 shows the chemical structure of curcumin and the analogues[21] used to model the dataset for ligand-based pharmacophore. The 2D chemical configuration was constructed with MarvinSketch 19.2, prior to an analysis with LigandScout 4.3 win64 evaluation version[22] (the analysis can be replicated using PharmaGist Webserver). Subsequently, each structure's geometry was optimized using the energy minimize module with MMFF94[23] force field set at a default setting.

Protein preparation

5ZTN was the Protein Data Bank accession number of the DYRK2 protein used in this study,[17] and curcumin acted as the native ligand. In addition, the target for molecular docking and dynamics was prepared using Molecular Operating Environment (MOE) 2014.0901 software (this can be replicated using MGLTools 1.5.6 and USCF Chimera 1.13.1) in order to correct the break residues, charging, and protonation of the protein structure. The protein molecule was opened in

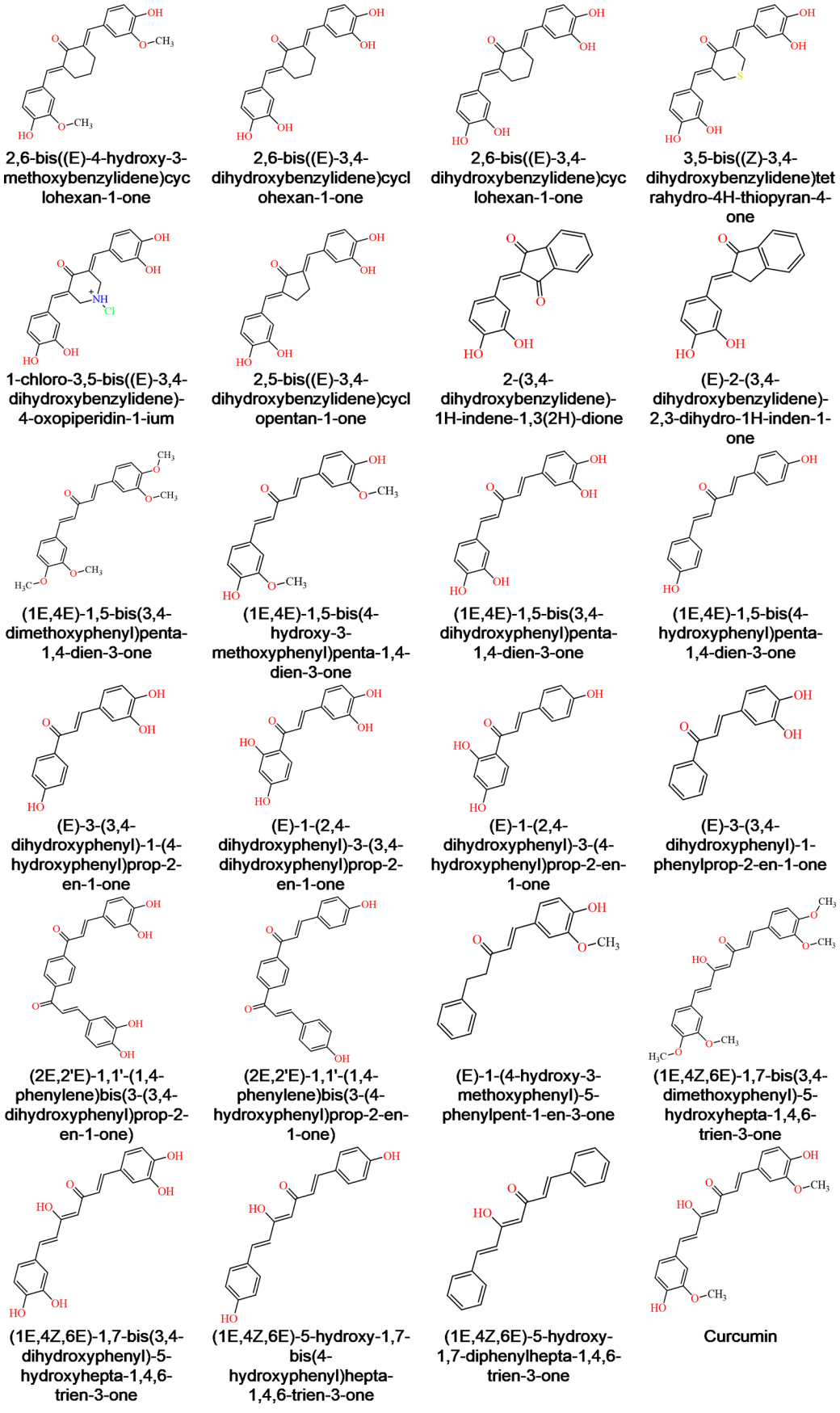

Figure 1. Chemical structure of dataset compounds.

AutoDockTool (ADT) 1.5.6, and the water molecule(s) were then removed. In grid menu, macromolecule was chosen, and the protein structure was then saved as a.pdbqt extension.

Ligand-based pharmacophore modeling

The ligand-based pharmacophore observed in this work was analyzed through multiple flexible alignment. Therefore, the model was generated from 24 dataset compounds using LigandScout 4.3 software (or PharmaGist Webserver). This was achieved through the 3D superposition of chemical features constructed by the flexible conformation alignments of all dataset compounds. In addition, the enrichment factor (EF) and receiver operating characteristics (ROC) analysis were used to validate the pharmacophore model using ROC Analysis: Web-based Calculator for ROC Curves. Active compounds were all of the 24 dataset compounds, and decoy compounds were obtained from zinc decoy database generated via DecoyFinder 2.0.

Filtering the compounds database

MolPort provided a large database with over 7.4 million catalogue compounds. The process of curcumin analogue filtration from the compounds database was performed on the Pharmit webserver. Filtering the compounds from database was then conducted using the pharmacophore query file as obtained from the above pharmacophore modeling.

Molecular docking

The goal of molecular docking was to assess the binding affinity of compound(s) upon interaction with the receptor. Therefore, all results obtained from the database filtering process were docked to the DYRK2 protein. In addition, the docking module of MOE was used for docking protocol detection and also for the docking score calculation of all hits (this can be replicated using AutoDock 4.2.6). Moreover, the molecular docking protocol was evaluated through virtual screening with the alpha triangle methods, London dG scoring and GridMin refinement.

Molecular dynamics

The aim of molecular dynamics was to evaluate the physical movement of molecules and atoms. This activity was intended to stimulate the interaction stability between the ligand and DYRK2, and was further investigated in combination with protein-ligand complexes obtained from the docking score calculation and characterized by the highest binding affinity. In addition, the interaction dynamics between ligands and receptors was measured using Gromacs 2018.3.[24–29] The stability of ligands in the binding pocket of DYRK2 protein were simulated by the molecular dynamic for 50 ns. In addition, Gormos96 54a7 force field was used to prepare the protein topology, while the PRODRG webserver was applied for ligand topology, using Gromos forcefield. The complex protein-ligand was solvated in a dodecahedron with 1 nm dimensions. Moreover, an aqueous environment was created in the system with the simple point charge (SPC) water model, and this was neutralized by adding Na^+ and Cl^-. The electrostatic interaction and periodic boundaries were calculated in all conditions using Particle-mesh Edward (PME) methods. Meanwhile, the cut-off radius for short-range van der Waals and Coulomb interactions was set to 0.9 nm. Furthermore, the linear constraint solver for molecular simulations (LINCS) method was used to constraint all bond lengths, while minimization, NPT and NVT equilibration as well as system production were performed at constant temperature (300K) and pressure (1 atm). The minimization process was conducted for 50 ps, NPT and NVT were collectively simulated for 100 ps, while the production process for 50 ns were saved at every 2 ps with coordinates of each simulation.

The interaction of ligand-receptor was visualized with LigandScout 4.3 (and can be replicated in USCF Chimera 1.13.1[30] and Discovery Studio Visualiser v20. The energy from ligand-receptor interactions were further estimated using the g-mmpbsa[31] platform.

ADME calculation

The absorption, distribution, metabolism and excretion (ADME) prediction values for hit compounds were calculated using ADMET Prediction by ADMETLab Webserver. Furthermore, ADME properties applied in the current research include caco-2 permeability, bioavailability 30% (F30), plasma protein binding (PPB), blood-brain barrier (BBB), Cyp450 1A2 inhibitor, Cyp450 1A2 substrate, half-life ($T_{1/2}$) and clearance (CL).

Results

Ligand-based pharmacophore modeling

The hypothetic pharmacophore was grouped based on the number of features, comprising 3 to 7, and each has 10 models, totaling to 50. Figure 1 shows the structure of the dataset molecules used to construct the pharmacophore model, where the validation process including ROC and EF analysis were implemented. These were performed on 24 active compounds and 717 decoy compounds obtained from zinc decoy database generated by DecoyFinder 2.0. Table 1 summarizes the values of area under the curve (AUC), ROC curve and EF for all models.

Table 1. Pharmacophore model candidates. Groups of model3, model4, model5, model6, and model7 have 3, 4, 5, 6 and 7 chemical features, respectively. Every group has 10 model candidates. AUC, area under the curve; EF, enrichment factor.

Model	*AUC*				*EF*				Numbers of hits
	1%	5%	10%	*100%*	*1%*	5%	10%	100%	
Model3-1	1	1	1	0.78	30.9	12.5	6.3	3.8	122
Model3-2	1	1	1	0.79	30.9	12.5	6.3	4.3	108
Model3-3	1	1	1	0.93	26.5	17.5	8.8	6.4	102
Model3-4	1	1	1	0.93	30.9	14.2	7.9	4.1	166
Model3-5	1	1	1	0.93	30.9	14.2	7.1	3.9	172
Model3-6	1	1	1	0.93	30.9	14.2	7.1	4	169
Model3-7	1	1	1	0.93	30.9	14.2	7.5	4.2	163
Model3-8	1	1	1	0.93	30.9	14.2	7.1	3.9	174
Model3-9	1	1	1	0.93	30.9	14.2	7.5	4.2	162
Model3-10	1	1	1	0.93	30.9	14.2	7.5	4.1	164
Model4-1	1	1	1	0.86	30.9	12.5	9.7	9.7	57
Model4-2	1	1	1	0.86	30.9	12.5	9.4	9.4	59
Model4-3	1	1	1	0.92	30.9	14.2	8.8	6.9	94
Model4-4	1	1	1	0.92	30.9	14.2	8.8	6.8	95
Model4-5	1	1	1	0.92	30.9	14.2	8.8	6.4	101
Model4-6	1	1	1	0.92	30.9	14.2	8.8	6.8	95
Model4-7	1	1	1	0.92	30.9	14.2	8.8	7	92
Model4-8	1	1	1	0.92	30.9	14.2	8.8	6.8	96
Model4-9	1	1	1	0.92	30.9	14.2	8.8	6.3	103
Model4-10	1	1	1	0.85	30.9	14.2	11.7	11.7	45
Model5-1	1	1	1	0.89	30.9	15	7.9	2	322
Model5-2	1	1	1	0.96	30.9	16.7	9.6	2.6	269
Model5-3	1	1	1	0.92	30.9	15.9	8.8	7	93
Model5-4	1	1	1	0.92	26.5	15	8.8	3.6	181
Model5-5	1	1	1	0.92	30.9	14.2	8.8	6.7	97
Model5-6	1	1	1	0.92	30.9	14.2	8.8	6.5	99
Model5-7	1	1	1	0.92	30.9	14.2	8.8	6.9	94
Model5-8	1	1	1	0.92	26.5	15.9	8.3	5	129
Model5-9	1	1	1	0.95	30.9	14.2	8.8	2.8	256
Model5-10	1	1	1	0.85	26.5	14.2	7.5	4.9	114
Model6-1	1	1	1	0.87	30.9	15	7.5	3.7	159
Model6-2	1	1	1	0.87	30.9	15	10.3	10.3	54
Model6-3	0.86	0.97	0.99	0.87	26.5	14.2	7.9	4	148
Model6-4	1	1	1	0.96	30.9	16.7	9.2	2.8	256
Model6-5	1	1	1	0.92	30.9	16.7	8.3	6.7	97
Model6-6	1	1	1	0.92	30.9	15.9	8.3	6.4	101
Model6-7	1	1	1	0.96	30.9	15	9.6	2.8	257
Model6-8	1	1	1	0.97	30.9	16.7	9.6	2.8	254
Model6-9	1	1	1	0.96	30.9	15.9	9.2	2.8	257
Model6-10	1	1	1	0.91	26.5	15	8.3	3.6	181

Table 1. *Continued*

Model	*AUC*				*EF*				Numbers of hits
	1%	5%	10%	*100%*	*1%*	5%	10%	100%	
Model7-1	1	1	1	0.87	26.5	15	7.5	4.2	139
Model7-2	1	1	1	0.91	30.9	14.2	7.9	3.1	211
Model7-3	1	1	1	0.87	30.9	15	10.5	10.5	53
Model7-4	1	1	1	0.87	30.9	15	7.5	4.4	134
Model7-5	1	1	1	0.9	26.5	14.2	8.3	2.6	252
Model7-6	1	1	1	0.9	26.5	14.2	8.3	2.5	258
Model7-7	1	1	1	0.87	30.9	14.2	7.5	4.1	143
Model7-8	1	1	1	0.87	26.5	14.2	7.5	3.8	155
Model7-9	1	1	1	0.87	26.5	15	7.5	4.1	142
Model7-10	1	1	1	0.91	30.9	15.9	8.3	3.3	196

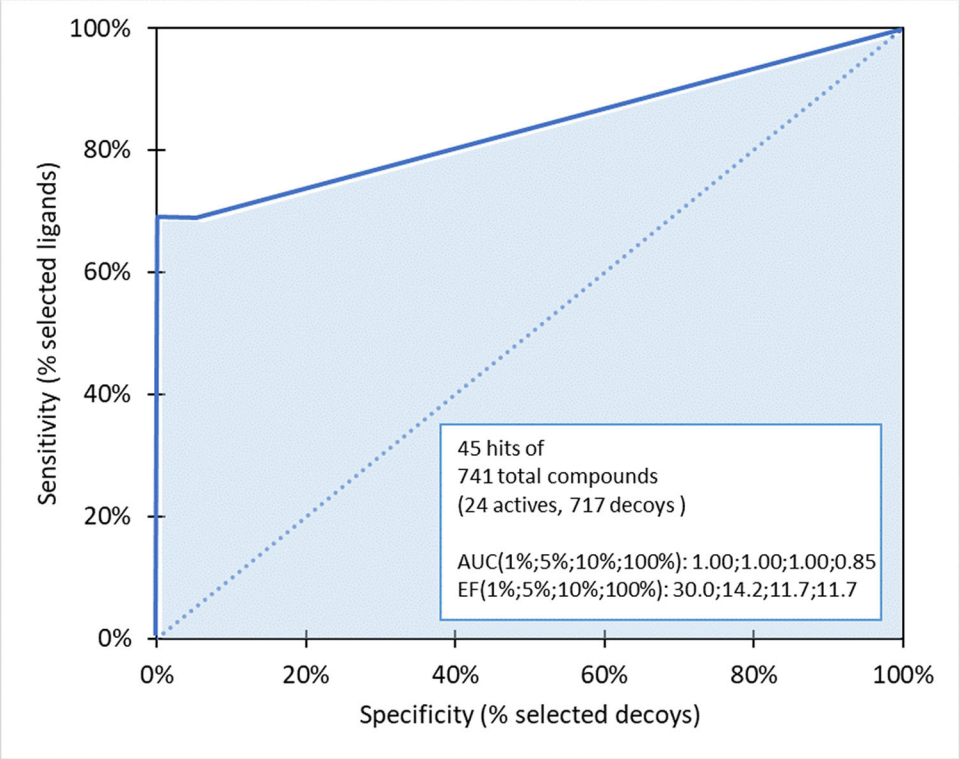

Figure 2. Receiver operating characteristics (ROC) curve with the area under the curve (AUC) and enrichment factor (EF) values in 1%, 5%, 10% and 100% of selected pharmacophore model.

Table 1 shows the adequacy of AUC values for all models, where the highest EF value of $EF^{100\%}$ was observed in model4-10 and was consequently selected as the best model. Figure 2 shows the ROC curve indicating the composition of 45 hit compounds, including 17 active and 28 decoy. Meanwhile, AUC values shown on 1%, 5%, 10% and 100% were 1.00, 1.00, 1.00 and 0.85, respectively, while the corresponding EF values were 30.8, 14.2, 11.7, and 11.7.

Filtering the curcumin analogues from database

The output file of the selected pharmacophore model generated from LigandScout 4.3 (or PharmaGist Webserver) was used to filter the compound database. Furthermore, the Pharmit interface was used for 3D visualization of the features, including details on the coordinate position and radius. The presence of hydrophobic, H-bond acceptor and donor features with radius 1.5 Å, and 0.9 Å for the aromatic variant were observed in the default setting. Moreover, filtering on Pharmit

allowed the users to modify the feature's radius, and consequently increase or decrease the amount of hit(s) as a result. However, manually changing this value is also possible by modifying the hit reduction and screening criteria. The Pharmit developers enter some criteria to set up the maximum hits per conformation, and molecule number, as well as the total limit for reduction, molar weight, rotated bond, logP, polar surface area (PSA), aromatic ring, H-bond donor and acceptor for screening.

This novel work involves database filtering with default settings for pharmacophore features and hit screening, with the exception of reduction. The model ultimately produced 1,130 hits, and a lot more compounds exist for continued screening. Therefore, the feature radius and screening procedure were modified. In particular, the hydrophobic, H-bond acceptor and donor radius were reduced to 1.4 Å for each, while the Rule of Three (RO3)[32] was applied during hit screening. These rules include molar weight (300 Dalton), rotated bond (3), logP (3), aromatic ring (3), H-bond donor (3) and H-bond acceptor (3). Meanwhile, PSA was set to the maximum value according to another reference, at 90 Å,[33] and these filtering protocols collectively produced 566 hits.

Molecular docking

Binding affinity is an indicator of the connection strength between ligand and receptor. This was determined by a docking score calculated using the MOE software (and can be replicated using AutoDock 4.2.6). This procedure was then validated by redocking the native ligand of DYRK2 protein (5ZTN) present in curcumin. The lowest RMSD value for successful docking was 0.7788, indicating the propensity to apply this protocol to other ligands.

This protocol docking validation process involved calculating the binding affinity (docking score) for each curcumin analogue complex and DYRK2 protein. Moreover, the value obtained for curcumin during redocking was –12.46 kcal/mol. This was used to filter the hits, as the specimens with greater and relatively close values between the native ligand and the binding pocket of target were selected. Figure 3 shows the chemical structure of ligand (hit compounds) and ligand-DYRK2 interaction with docking score lower than –10 kcal/mol, and Figure 4 indicates the overlay of each, with pose characterized by the highest value.

Molecular dynamics

The complexes (ligand-protein) motion during simulation were expressed in Root Mean Square Deviation (RMSD) (Figure 4(a)), while the movements of protein backbone during simulation was estimated in a Root Mean Square Fluctuation (RMSF) curve (Figure 4(b)). Figure 4(a) showed that the interactions are stable after 30 ns of simulation.

ADME Prediction

The ADME (absorption, distribution, metabolism, and excretion) provide a description for drug disposition within an organism. These pharmacokinetic parameters influence the overall level and kinetics in the tissues, and consequently influence the pharmacological effect of the active compounds. In addition, it is possible to represent the ADME of active compounds as a prediction value.

The results obtained with the selected compounds in this current investigation were caco-2 permeability and F30 for absorption; PPB and BBB for distribution, Cyp450 1A2 inhibitor and Cyp450 1A2 substrate for metabolism; while $T_{1/2}$ and CL represented excretion. Table 2 shows the summary of ADME prediction for selected compounds in contrast with curcumin.

Discussion

Ligand-based pharmacophore modeling

The pharmacophore is the physicochemical feature of a molecule known to interact with a specific target receptor. This is modelled in a 3D pattern and the basic characteristics are also shared by a set of molecules. The ligand-based pharmacophore model was constructed without needing a protein target structure, through a 3D superposition of the ligand conformed physicochemical features.

The chemical characteristics of a selected pharmacophore model include three H-bond acceptors provided by the oxygen in keto, methoxy and hydroxyl groups, a H-bond donor from the hydroxyl, an aromatic feature and hydrophobicity contributed by aromatic group. Figure 5(a) shows the 2D visualization of pharmacophore model, while Figure 5(b) and 5(c) demonstrates it in 3D.

Filtering and interaction study of curcumin analogue

There is rule of three (RO3) beside Lipinski's rule of five (RO5), which applies in searches pertaining to drug-likeness. Particularly, RO5 stipulates the criteria to be satisfied, including the presence of no more than five hydrogen bond donors, 10 hydrogen bond acceptors, a molecular weight (MW) of less than 500 Daltons, and an octanol-water partition coefficient

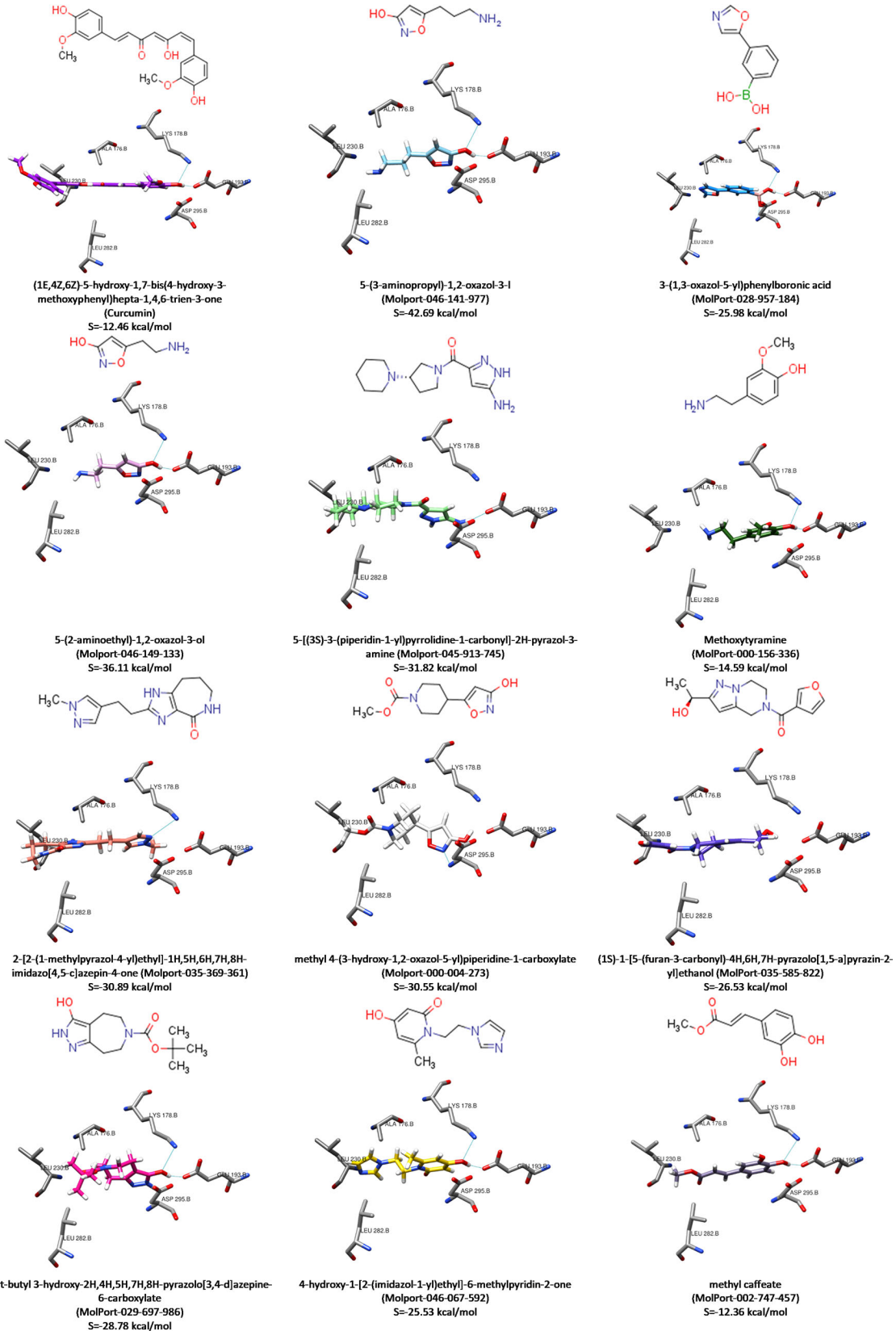

Figure 3. Chemical structure of hit compounds, its interaction with dual-specificity tyrosine-regulated kinase 2 (DYRK2), and their docking score S (< –10 kcal/mol).

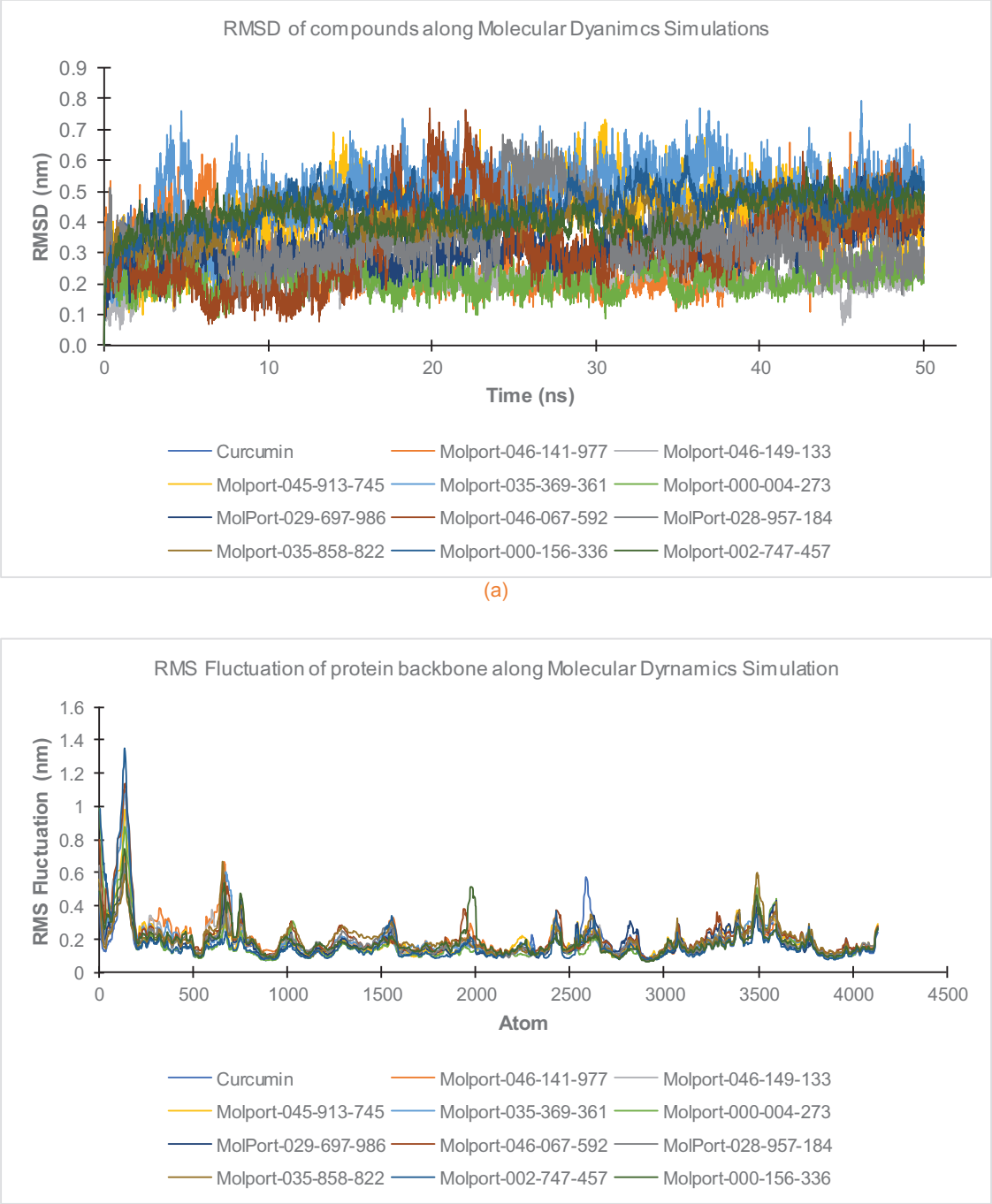

Figure 4. Root Mean Square Deviation (RMSD) of ligand (a), and Root Mean Square Fluctuation (RMSF) of protein backbone (b) of all complexes in 50 ns simulation.

(logP) below 5.[34,35] Furthermore, all numbers are in the multiple of five, hence the RO3 criteria requires a multiple of three. These criteria include logP not greater than 3, MW less than 300 Daltons, the presence of no more than three hydrogen bond donors and acceptors, respectively, and no more than three rotatable bonds.[36]

The Pharmit platform allows users to customize the pharmacophore feature criteria based on RO5 or RO3. In addition, the value modification potentially increases or decreases the amount of hits. The application of RO3 in this research instigated a decline in the number of hit compounds from 1,130 (default criteria) to 566 (modified criteria). The latter was determined to be more rational during molecule screening.

Table 2. The absorption, distribution, metabolism, and excretion (ADME) prediction of hit compounds and curcumin. F30, 30% bioavailability; PPB, plasma protein binding; BBB, blood-brain barrier, $T_{1/2}$, half-life CL, clearance.

No.	Compound	caco2	F30		PPB	BBB	
			Category	Probability		Category	Probability
1	Curcumin	-5.133	0	0.452	88.84	1	0.579
2	Molport-046-141-977	-4.969	1	0.628	20.79	0	0.214
3	Molport-046-149-133	-4.993	1	0.628	17.27	0	0.277
4	Molport-045-913-745	-4.914	1	0.748	34.89	1	0.855
5	Molport-035-369-361	-4.658	1	0.692	40.05	1	0.890
6	Molport-000-004-273	-4.869	1	0.531	36.79	1	0.931
7	MolPort-029-697-986	-5.029	0	0.495	44.41	1	0.830
8	Molport-046-067-592	-5.007	1	0.638	54.75	1	0.789
9	MolPort-028-957-184	-4.968	1	0.652	50.57	1	0.986
10	MolPort-035-585-822	-4.906	1	0.672	60.69	1	0.845
11	MolPort-000-156-336	-4.933	1	0.536	41.09	0	0.183
12	MolPort-002-747-457	-4.951	1	0.652	48.23	1	0.726

No.	Compound	CYP1A2-inhibitor		CYP1A2-substrate		$T_{1/2}$	CL
		Category	Probability	Category	Probability		
1	Curcumin	0	0.449	0	0.456	1.653	1.560
2	Molport-046-141-977	1	0.586	0	0.354	0.906	1.421
3	Molport-046-149-133	1	0.567	0	0.362	1.069	1.291
4	Molport-045-913-745	0	0.037	0	0.460	1.260	1.910
5	Molport-035-369-361	0	0.158	1	0.678	1.310	1.991
6	Molport-000-004-273	0	0.103	0	0.462	0.910	1.756
7	MolPort-029-697-986	0	0.103	0	0.410	0.953	1.953
8	Molport-046-067-592	1	0.655	1	0.578	0.860	1.727
9	MolPort-028-957-184	1	0.910	1	0.542	0.847	1.572
10	MolPort-035-585-822	0	0.133	1	0.552	1.034	1.776
11	MolPort-000-156-336	0	0.034	1	0.608	0.972	1.801
12	MolPort-002-747-457	1	0.837	0	0.462	0.765	1.686

Molecular docking

Molecular docking is one of the *in silico* approaches of interaction studies between ligand(s) and receptor. The 566 hits are further filtered through this means, and 11 compounds were selected after using the specified protocol. In addition, the binding affinity (docking score) of samples in each hit were better than or close to curcumin (native ligand). Figure 6 shows the respective overlay with the binding pocket of DYRK2 protein. It was observed that all selected hit compounds occupy the DYRK2's binding site.

Molecular dynamics

Figure 4(a, b) shows the stability performance of RMSD for each ligand, as well as the RMSF for the respective protein backbone on each complex, after the initial 25 ns simulation. Therefore, an average of the system binding energy was calculated at the end. The van der Waals, electrostatic, polar solvation, non-polar (Solvent-Accessible Surface Area) and binding energy were calculated every 200 ps, in order to obtain an average value for each complex. Table 3 shows the summarized system energy report obtained from 200 snapshots.

The native ligand (curcumin) has a binding energy of –53.058 kJ/mol with the DYRK2 protein. Table 3 showed six selected hit compounds have more negative values compared to that of Curcumin, i.e. Molport-035-369-361 (–71.35±24.85), Molport-000-004-273 (–83.56±18.24), MolPort-029-697-986 (–61.69±14.89), MolPort-035-585-822 (–74.24±15.55), MolPort-000-156-336 (–54.49± 15.09), and MolPort-002-747-457 (–69.36±12.58).

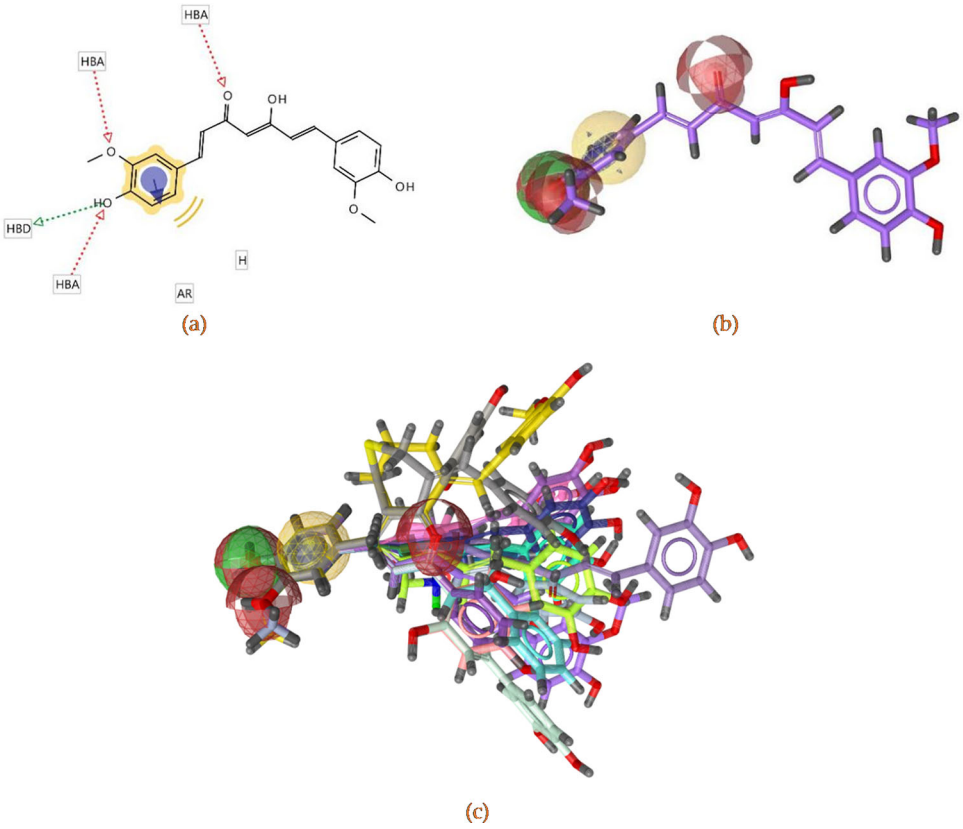

Figure 5. 2D visualizations of the selected pharmacophore model (a). 3D visualization of pharmacophore model (red balls are H-bond acceptors, the green are H-bond donors and the yellow are aromatic and hydrophobic features) (b). Alignment of 17 hits of active compounds in the pharmacophore features (c).

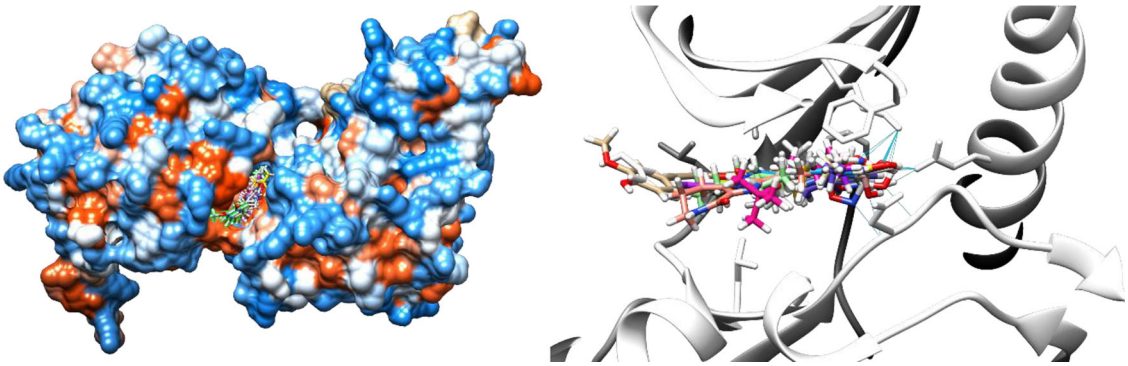

Figure 6. Overlay of selected hit compounds and curcumin (native ligand) in the binding pocket of dual-specificity tyrosine-regulated kinase 2 (DYRK2) protein.

This shows that van der Waals and polar solvation energy are the main impact on binding factors for curcumin and all selected compounds. The respective values were negative and positive. This indicates the tendency to generate more positive effect on binding energy at more negative van der Waals. The binding energy of all six selected compounds were larger (more negative) than that of curcumin. This discrepancy was attributed to the lesser polar solvation characteristics. Moreover, significantly higher values of positive polar solvation energy tend to decrease binding energy.

Figure 7 showed the position of curcumin and other selected hit compounds in the binding pocket of DYRK2 during the molecular dynamic simulation. These results confirm those of our docking study. The interaction of 5ZTN-curcumin (Figure 8(a)) and 5ZTN-Molport 000-004-273 (Figure 8(b)) show the binding mode which involves H-bonds and hydrophobic interactions.

Table 3. Binding energy of curcumin and hit compounds upon interaction with dual-specificity tyrosine-regulated kinase 2 (DYRK2) protein obtained from molecular dynamics simulation. SASA, Solvent-Accessible Surface Area.

No.	Compound	van der Waals (E_{VDW})(kJ/mol)	Electrostatic (E_{Elec})(kJ/mol)	Polar solvation (E_{polar})(kJ/mol)	SASA ($E_{nonpolar}$)(kJ/mol)	Binding energy (E_{bind}) (kJ/mol)
1	Curcumin	–112.43 ± 18.69	–151.33 ± 22.12	229.65 ± 25.56	–18.95 ± 1.38	–53.06 ± 20.80
2	Molport-046-141-977	–64.33 ± 16.76	–97.88 ± 13.93	141.26 ± 20.85	–10.81 ± 0.76	–31.75 ± 12.41
3	Molport-046-149-133	–60.31 ± 12.01	–79.93 ± 14.52	115.43 ± 19.96	–9.55 ± 0.58	–34.37 ± 13.61
4	Molport-045-913-745	–141.79 ± 12.87	–71.03 ± 14.65	180.21 ± 19.52	–16.46 ± 0.97	–49.07 ± 14.99
5	Molport-035-369-361	–139.94 ± 13.10	–33.42 ± 30.50	117.32 ± 42.56	–15.31 ± 1.19	–71.35 ± 24.85
6	Molport-000-004-273	–128.37 ± 12.70	–69.26 ± 8.73	128.63 ± 19.28	–14.56 ± 0.78	–83.56 ± 18.24
7	MolPort-029-697-986	–104.59 ± 18.68	–116.96 ± 13.94	174.61 ± 15.54	–14.75 ± 0.92	–61.69 ± 14.89
8	Molport-046-067-592	–102.19 ± 15.47	–98.01 ± 22.78	177.08 ± 31.05	–13.44 ± 0.98	–36.55 ± 18.86
9	MolPort-028-957-184	–83.91 ± 11.48	–107.11 ± 28.64	179.02 ± 46.31	–12.68 ± 1.10	–24.68 ± 25.28
10	MolPort-035-585-822	–135.86 ± 11.74	–80.71 ± 15.28	158.27 ± 18.93	–15.94 ± 0.82	–74.24 ± 15.55
11	MolPort-000-156-336	–83.75 ± 10.68	–84.63 ± 16.67	126.46 ± 19.53	–12.58 ± 0.66	–54.49 ± 15.09
12	MolPort-002-747-457	–86.45 ± 13.99	–103.17 ± 26.38	133.69 ± 15.44	–13.43 ± 1.06	–69.36 ± 12.58

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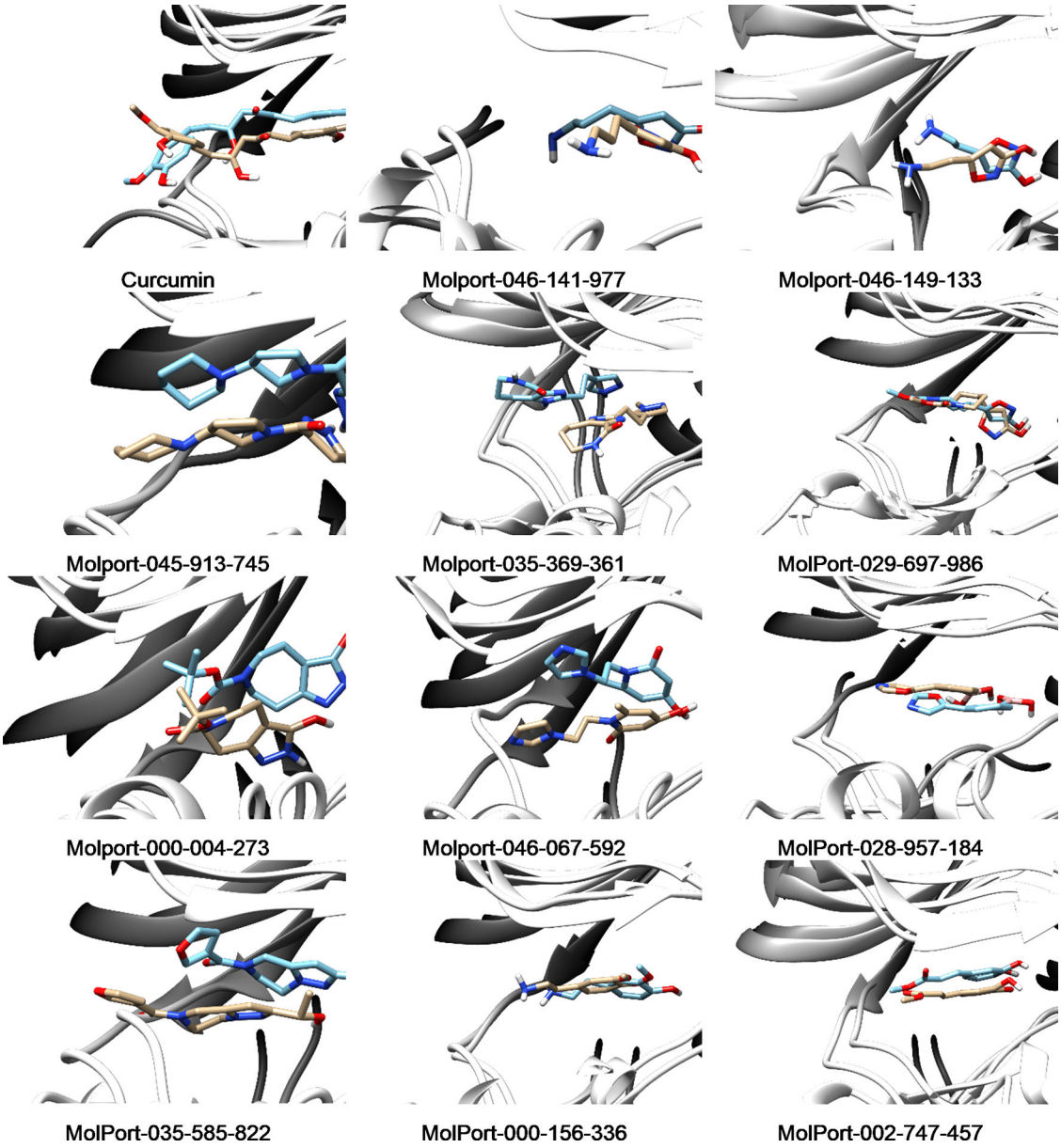

Figure 7. Overlay binding pose of hits in dual-specificity tyrosine-regulated kinase 2 (DYRK2) binding pocket at 0 ns (brown) and 50 ns (blue).

Table 4 summarizes the percentage of H-bond occupancy between the selected hit compounds and residues of DYRK2. It was observed that LYS178, GLU193, ASP295 have important roles in the ligand interaction in the DYRK2 binding pocket.

ADME prediction

The absorption prediction results recognized all 11 selected compounds to be better than curcumin in terms of Caco-2 permeability, where the optimal output for active compound is suggested to be more than –5.15 log cm/s.[37] The calculated values were above –5.0, whereas a value of –5.13 was recorded for curcumin. In addition, the bioavailability (F30) was also better, as the curcumin was categorized 0 (<30), while the selected compounds were attributed as 1 (>30%) except MolPort-029-697-986 or tert-butyl-3-hydroxy-2H,4H,5H,7H,8H-pyrazolo[3,4-d] azepine-6-carboxylate.[38]

The PPB was higher in curcumin, at 88.84% compared to the others. Hence, the native ligand is considered to have a more significant bond with plasma protein, although less than the suggested 90%, and consequently has a lower therapeutic

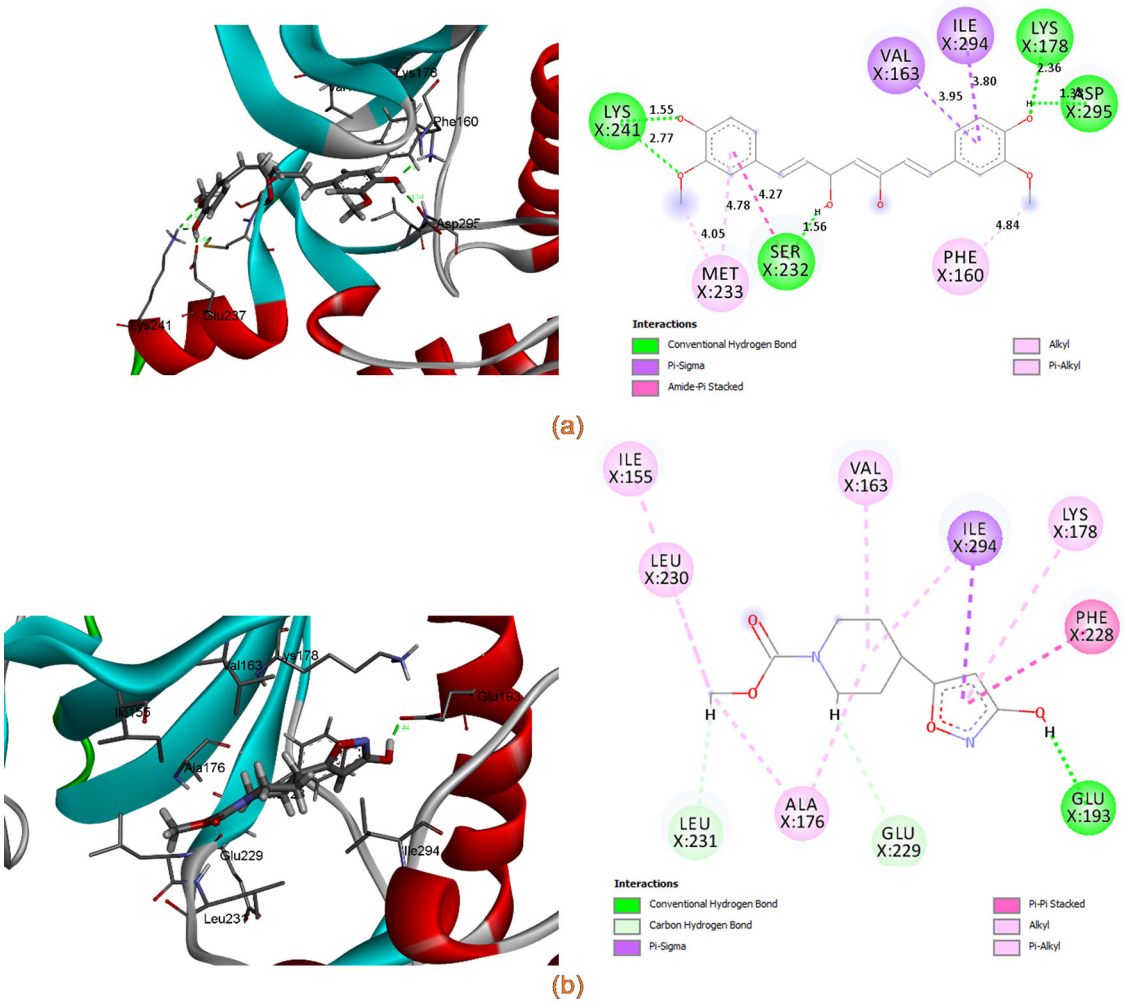

Figure 8. Receptor-ligand interaction; curcumin (a) and MolPort 000-004-273 (b) are in the 5ZTN's binding pocket (both were captured on 50 ns trajectory).

index.[39] Conversely, the selected compounds have a greater propensity to attach to the target receptors and provide the desired therapeutic effect.

The BBB is a highly selective semipermeable border separating the circulating blood from the brain and other extracellular fluids in the central nervous system (CNS).[40] This parameter is calculated as a ratio of compounds, and is improved by H-bond numbers as well as molecular weight.[41] The ADMETLab webserver categorizes the BBB value of a drug as 0 (BBB negative) indicating the inability to penetrate, whereas 1 (BBB positive) demonstrates a barrier permeation potential. In addition, all compounds, including the curcumin were categorized with a BBB value of 1, and a positive BBB is derived in instances where the ratio probability was above 0.1.

The cytochrome P450 (CYP) gene family are responsible for drug metabolism. Here, the ADMETLab webserver was used to predict the interaction potentials of curcumin and all selected compounds as an inhibitor and substrate of the cytochrome P450 enzyme. However, the drugs were unable to act as inhibitors but could act as a substrate of cytochrome P450. The prediction results showed the tendency for 2-[2-(1-methylpyrazol-4-yl)ethyl]-1H,5H,6H,7H,8H-imidazo[4,5-c]azepin-4-one (Molport-035-369-361), (1S)-1-[5-(furan-3-carbonyl)-4H,6H,7H-pyrazolo[1,5-a]pyrazin-2-yl] ethanol (MolPort-035-585-822), and methoxytyramine (MolPort-000-156-336) to act as a substrate and not as CYP1A2 inhibitor. Furthermore, 4-hydroxy-1-[2-(imidazol-1-yl)ethyl]-6-methylpyridin-2-one (Molport-046-067-592), and 3-(1,3-oxazol-5-yl) phenylboronic acid (MolPort-028-957-184) potentially act as substrate and inhibitor. Curcumin or (1E,4Z,6Z)-5-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,4,6-trien-3-one, Methyl 4-(3-hydroxy-1,2-oxazol-5-yl)piperidine-1-carboxylate (Molport-000-004-273), and tert-butyl 3-hydroxy-2H,4H,5H,7H,8H-pyrazolo[3,4-d]azepine-6-carboxylate (MolPort-029-697-986)

Table 4. **Occupancy of hydrogen bond during 50 ns simulations.**

	Acceptor-Donor	Occupancy (%)
Curcumin	O16-SER232	66.64
	O41-ASP295	53.44
	O4-GLU237	75.10
	LYS178-O41	37.11
	LYS241-O4	18.67
	ASN234-O2	3.80
Molport-046-141-977	LYS178-O1	13.11
	O1-ASP295	86.55
	LYS178-N2	1.04
Molport-046-149-133	O1-GLU193	88.90
	LYS178-O1	2.32
	ASP295-O1	14.07
	PHE296-O1	1.52
	LYS178-N2	5.90
Molport-045-913-745	LYS178-O1	29.65
	N1-GLU193	3.32
Molport-035-369-361	N4-LEU230	9.06
	ASN234-O1 LYS178-N2	11.76 80.34
Molport-000-004-273	O3-GLU193	86.88
	PHE296-O3	1.86
	LYS178-O3	1.78
	ASP295-O3	3.30
MolPort-029-697-986	O3-GLU193	78.81
	LYS178-O3	21.21
	N3-GLU193	11.24
Molport-046-067-592	O1-GLU193	75.01
	LYS178-O1	13.91
	LYS178-O2	17.21
	GLN189-O1	3.18
	ASP295-O1	3.52
MolPort-028-957-184	LYS178-O1	14.32
	O2-ASP295	66.42
	O1-ASP295	56.60
	O1-ASP275	9.86
MolPort-035-585-822	LYS178-O3	52.82
	LEU231-O1	21.69
	O3-GLU193	18.84
	O3-ASP295	36.86
MolPort-000-156-336	LYS178-O2	39.70
	O2-GLU193	56.06
MolPort-002-747-457	O3-GLU193	47.72
	O4-ASP295	67.69

Table 4. *Continued*

	Acceptor-Donor	Occupancy (%)
	LYS178-O3	27.33
	LEU231-O2	12.59
	LYS178-O4	5.06

were predicted as being unable to act as inhibitor and a substrate of cytochrome. The three other compounds can only act as a cytochrome inhibitor but cannot act as a substrate.

CL refers to the volume of plasma of the drug freed per unit time, where ($T_{1/2}$) is half-life in plasma. The results indicate lower values with hits and curcumin at < 5 mL/min/kg and < 3 hours, respectively.[39] Curcumin possessed the comparable CL and the highest $T_{1/2}$. Thus, selected hit compounds are considered to have relatively better excretion properties.

Finally, the selected compounds demonstrated similar or better overall pharmacokinetic parameters than that of curcumin, as observed with the ADME discussed above.

Conclusions

A combined pharmacophore model and molecular docking for virtual screening has been conducted to find a potential DYRK2 inhibitor.

Based on a gradual virtual screening process using a ligand-based pharmacophore model and molecular docking, 11 hit compounds have been selected. Further detailed study using molecular dynamics simulation afforded six hit compounds with better binding interaction with DYRK2 compared to that of curcumin, i.e. Molport-035-369-361 (-71.35±24.85) kJ/mol, Molport-000-004-273 (-83.56±18.24) kJ/mol, MolPort-029-697-986 (-61.69 ±14.89) kJ/mol, MolPort-035-585-822 (-74.24±15.55) kJ/mol, MolPort-000-156-336 (-54.49±15.09) kJ/mol, and MolPort-002-747-457 (-69.36±12.58) kJ/mol.

The six compounds obtained after a gradual virtual screening process have similar pharmacophore characteristics. Considering the pharmacokinetic properties, Molport-035-369-361, MolPort-035-585-822 as well as Molport-000-004-273 are now under *in vitro* study for further investigation as lead compounds, and the results will be reported elsewhere.

Data availability

Source data

Protein Data Bank: DYRK2 data from Protein Data Bank. Accession number PBD5ZTN; https://identifiers.org/structure/5ztn.

Dataset of compounds with biological activity for ligand-based pharmacophore modeling were obtained from[21] and are shown in Figure 1.

Acknowledgements

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Version 1

Reviewer Report 04 October 2021

https://doi.org/10.5256/f1000research.31013.r95613

✗ **Hrvoje Rimac**
Department of Medicinal Chemistry, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia

In the article entitled "Virtual screening of curcumin analogues as DYRK2 inhibitor: Pharmacophore analysis, molecular docking and dynamics, and ADME prediction" the authors performed *in silico* analysis of the curcumin analogues as DYRK2 inhibitors and have decided on 3 compounds which will undergo further *in vitro* study as possible lead compounds. While the research seems to be performed correctly, there are several issues that require attention and need to be corrected and more thoroughly explained before the article is approved:

1. English should be corrected in the entire manuscript, especially in the title "Virtual screening of curcumin analogues as DYRK2 inhibitors..."

2. Sentences where the authors explain in which programs can the analyses be replicated are irrelevant. The authors should just write how they did what they did, not all the ways it is possible to do it.

3. In Methods section a lot of details concerning the filtering and docking parameters are missing.

4. The authors said they performed MD simulation in total length of 50 ns and that the complexes are stabilize after 30 ns. 50 ns is not enough time to draw any conclusion about the stability of the complexes, the simulations should be at least 100 ns long, if not more.

5. Why did the authors check if the ligands are CYP1A2 substrates or inhibitors? Why just 1A2? If the influence on CYP metabolism is evaluated, CYP3A4 should definitely be included.

6. Correct writing of ZINC Directory of Useful Decoys (DUD), Caco-2 etc. should be used.

7. On page 8 the authors write about the possibilities of Pharmit. This should be removed as this manuscript is not the point of this article.

8. MD results are not explained. What are the conclusions from RMSF? Which are the most important interactions and the most important amino acids? Is this in agreement with the literature and the known inhibitors? There is no real discussion and comparison with other research.

9. The authors should analyze and discuss more thoroughly the 3 best compounds.

10. What is the point of Figure 7? It takes a lot of space and the same is shown in Figure 6.

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

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?
No

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: Computational chemistry, CYP enzymes

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 25 August 2021

https://doi.org/10.5256/f1000research.31013.r90128

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Mohd Athar

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The manuscript uses standard procedure for computational modelling and can be considered for indexing underlying below mentioned concerns:

1. As per reference paper (JMC, 1998, 41, 21), the 24 ligands are HIV-1 IN inhibitors. Hence these ligands can be active ligands against HIV-1 IN and may not be against DYRK2. Please check.

2. Is pharmacophore a 'physicochemical feature' or '3D structural feature'?

3. What kind of alignment/superposition was used for the pharmacophore mapping?

4. Figure should be numbered in order of their description in text. Pharmacophore model should come first before pharmacophore screening results.

5. Better to number the ligand mentioned in the text for making the discussion simpler.

6. Discussion section can be combined without any subheading.

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?
Partly

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: Computational 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, however I have significant reservations, as outlined above.

RESEARCH ARTICLE

Virtual screening of curcumin analogues as DYRK2 inhibitor: Pharmacophore analysis, molecular docking and dynamics, and ADME prediction [version 1; peer review: 1 approved with reservations, 1 not approved]

La Ode Aman[1,2], Rahmana Emran Kartasasmita[1], ✉ Daryono Hadi Tjahjono (mailto:daryonohadi@fa.itb.ac.id) (http://orcid.org/0000-0002-9675-0134)[1]

PUBLISHED 17 May 2021

OPEN PEER REVIEW DETAILS

REVIEWER STATUS ? ✕

Abstract

Background: Curcumin reduces the proliferation of cancer cells through inhibition of the DYRK2 enzyme, which is a positive regulator of the 26S proteasome.

Methods: In the present work, curcumin analogues have been screened from the MolPort database using a pharmacophore model that comprised a ligand-based approach. The result of the screening was then evaluated by molecular docking and molecular dynamics based on binding the free energy of the interaction between each compound with the binding pocket of DYRK2. The hit compounds were then confirmed by absorption, distribution, metabolism, and excretion (ADME) prediction.

Results: Screening of 7.4 million molecules from the MolPort database afforded six selected hit compounds. By considering the ADME prediction, three prospective curcumin analogues have been selected. These are: 2-[2-(1-methylpyrazol-4-yl)ethyl]-1H,5H,6H,7H,8H-imidazo[4,5-c]azepin-4-one (Molport-035-369-361), methyl 4-(3-hydroxy-1,2-oxazol-5-yl)piperidine-1-carboxylate (Molport-000-004-273) and (1S)-1-[5-(furan-3-carbonyl)-4H,6H,7H-pyrazolo[1,5-a]pyrazin-2-yl]ethanol (MolPort-035-585-822).

Conclusion: Pharmacophore modelling, combined with molecular docking and molecular dynamics simulation, as well as ADME prediction were successfully applied to screen curcumin analogues from the MolPort database as DYRK2 inhibitors. All selected compounds that have

better predicted pharmacokinetic properties than that of curcumin are considered for further study.

READ LESS

Keywords

Introduction

Methods

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Acknowledgements

COMMENTS (0)

Author details

Competing interests

Grant information

Article Versions (1)

Copyright

OPEN PEER REVIEW

CURRENT REVIEWER STATUS: ✗ ?

Key to Reviewer Statuses VIEW

VERSION 1 PUBLISHED 17 May 2021

REVIEWER REPORT 04 Oct 2021

Hrvoje Rimac
Department of Medicinal Chemistry, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia

In the article entitled “Virtual screening of curcumin analogues as DYRK2 inhibitor: Pharmacophore analysis, molecular docking and dynamics, and ADME prediction” the authors performed *in silico* analysis of the curcumin analogues as DYRK2 inhibitors and have decided on 3 compounds which will undergo further *in vitro* study as possible lead compounds. While the research seems to be performed correctly, there are several issues that require attention and need to be corrected and more thoroughly explained before the article is approved:

1. English should be corrected in the entire manuscript, especially in the title “Virtual screening of curcumin analogues as DYRK2 inhibitors…”

2. Sentences where the authors explain in which programs can the analyses be replicated are irrelevant. The authors should just write how they did what they did, not all the ways it is possible to do it.

3. In Methods section a lot of details concerning the filtering and docking parameters are missing.

4. The authors said they performed MD simulation in total length of 50 ns and that the complexes are stabilize after 30 ns. 50 ns is not enough time to draw any conclusion about the stability of the complexes, the simulations should be at least 100 ns long, if not more.

5. Why did the authors check if the ligands are CYP1A2 substrates or inhibitors? Why just

5. Why did the authors check if the ligands are CYP 1A2 substrates or inhibitors? Why just 1A2? If the influence on CYP metabolism is evaluated, CYP3A4 should definitely be included.

6. Correct writing of ZINC Directory of Useful Decoys (DUD), Caco-2 etc. should be used.

7. On page 8 the authors write about the possibilities of Pharmit. This should be removed as this manuscript is not the point of this article.

8. MD results are not explained. What are the conclusions from RMSF? Which are the most important interactions and the most important amino acids? Is this in agreement with the literature and the known inhibitors? There is no real discussion and comparison with other research.

9. The authors should analyze and discuss more thoroughly the 3 best compounds.

10. What is the point of Figure 7? It takes a lot of space and the same is shown in Figure 6.

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

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

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If applicable, is the statistical analysis and its interpretation appropriate?
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Reviewer Expertise: Computational chemistry, CYP enzymes

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

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REVIEWER REPORT 25 Aug 2021

Mohd Athar
School of Chemical Sciences, Central University of Gujarat, Gandhinagar, India; University of Cagliari, Cagliari, Italy

VIEWS 0

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Universitas Negeri Gorontalo UNGmail

La Ode Aman <laode_aman@ung.ac.id>

Komen reviewer F1000Research

2 messages

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To: La Ode Aman <laode_aman@ung.ac.id>
Cc: daryonohadi <daryonohadi@gmail.com>

Assalamu'alaikum Laode,

Semoga Laode sekeluarga sehat selalu ya. Aamien
Sudah mulai perkuliahan semester baru?

Di ITB baru mulai Senin kemarin.

Anyway, ini ada komentar dari Reviewer.
Tolong dibaca komentar nomer 1-5, di bawah ini.
Silahkan kalau ada jawaban ya.

Insya Alloh nanti minggu depan saya rangkum, krn minggu ini masih penuh jadwal.
Jaga selalu kesehatan ya.
Nuhun
dht

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Are the conclusions drawn adequately supported by the results?
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La Ode Aman <laode_aman@ung.ac.id> Sun, Sep 5, 2021 at 8:10 AM
To: Daryono Hadi <daryonohadi@fa.itb.ac.id>

Alhamndulillah saat ini saya dan keluarga baik-baik Pak. Semoga Bapak dan keluarga juga sehat-sehat selalu.

Walaupun sekitar 3 pekan sejak awal agustus saya dan keluarga harus menjalani isolasi mandiri karena satu rumah (saya, istri, anak-anak, ponakan) memiliki gejala yang hampir sama (flu, batuk, demam, badan meriang, hilang indra penciuman dan rasa) dengan tingkat keparahan yang berbeda. Walaupun tanpa tes swab atau PCR kami memutuskan untuk isolasi mandiri dan alhamdulillah setelah 3 pekan, kondisi kami semua pulih kembali.

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Oh iya pak, tentang komentar/pertanyaan reviewer, jawaban atau tanggapan diuraikan sebagai berikut sesuai nomor pertanyaan reviewer:

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Thus, the pharmacophore is a '3D structural feature'.

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Multiple flexible alignment

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Penjelasan untuk nomor 4 dan 5:

Mungkin yang dimaksudkan oleh reviewer adalah mengapa Table 1 dan Figure 2 ditempatkan setelah Sub Bab *Filtering the Curcumin Analogues from Database* (pada Bab Results) padahal sub bab ini mendeskripsikan tentang pharmacophore screening results. Reviewer menginginkan Table 1 dan Figure 2 dipindahkan dari tempat pada draft manuskrip saat ini, menjadi sebelum Sub Bab *Filtering the Curcumin Analogues from Database* (pada Bab Results).

Atau mungkin yang dimaksudkan dari pertanyaan no. 4 adalah **Table 1**. Kolom terakhir pada **Table 1** menunjukan ***number of hits***. Number of hits disini menunjukan tentang jumlah senyawa hasil screening menggunakan model farmakofor yang sesuai (bukan model farmakofor yang terpilih/terbaik) dari database 24 senyawa aktif dan 717 senyawa decoy.

Hasil screening berdasarkan farmakofor terpilih (terbaik) secara text diuraikan pada Bab *Results* Sub Bab *Filtering the Curcumin Analogues from Database*, lalu dilanjutkan dengan screening dengan menerapkan tehnik molecular docking pada Sub Bab Molecular Docking. Hasil screening berdasarkan model farmakofor terpilih (terbaik) hanya diuraikan secara text setelah model farmakofor terpilih/terbaik diperoleh. Figure 3 adalah daftar senyawa hasil screening setelah menerapkan model farmakofor terbaik/terpilih dan molecular docking. Senyawa hasil screening (Figure 3) diperoleh setelah model farmakofor diperoleh (Table 1 dan Figure 2).

Untuk sementara demikian yang dapat saya jelaskan pak. Mohon arahan dan bimbingannya Bapak. Terima kasih pak.

Hormat saya,

La Ode Aman
Lecturer/Researcher on Pharmacy Dept.
Universitas Negeri Gorontalo
Jl. Jend Sudirman 6 Gorontalo 96128
INDONESIA

Contact Mobile: +62-811-434-084

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Universitas Negeri Gorontalo UNGmail

La Ode Aman <laode_aman@ung.ac.id>

Komen reviewer F1000Research

2 messages

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Mungkin yang dimaksudkan oleh reviewer adalah mengapa Table 1 dan Figure 2 ditempatkan setelah Sub Bab *Filtering the Curcumin Analogues from Database* (pada Bab Results) padahal sub bab ini mendeskripsikan tentang pharmacophore screening results. Reviewer menginginkan Table 1 dan Figure 2 dipindahkan dari tempat pada draft manuskrip saat ini, menjadi sebelum Sub Bab *Filtering the Curcumin Analogues from Database* (pada Bab Results).

Atau mungkin yang dimaksudkan dari pertanyaan no. 4 adalah **Table 1**. Kolom terakhir pada **Table 1** menunjukan ***number of hits***. Number of hits disini menunjukan tentang jumlah senyawa hasil screening menggunakan model farmakofor yang sesuai (bukan model farmakofor yang terpilih/terbaik) dari database 24 senyawa aktif dan 717 senyawa decoy.

Hasil screening berdasarkan farmakofor terpilih (terbaik) secara text diuraikan pada Bab *Results* Sub Bab *Filtering the Curcumin Analogues from Database*, lalu dilanjutkan dengan screening dengan menerapkan tehnik molecular docking pada Sub Bab Molecular Docking. Hasil screening berdasarkan model farmakofor terpilih (terbaik) hanya diuraikan secara text setelah model farmakofor terpilih/terbaik diperoleh. Figure 3 adalah daftar senyawa hasil screening setelah menerapkan model farmakofor terbaik/terpilih dan molecular docking. Senyawa hasil screening (Figure 3) diperoleh setelah model farmakofor diperoleh (Table 1 dan Figure 2).

Untuk sementara demikian yang dapat saya jelaskan pak. Mohon arahan dan bimbingannya Bapak. Terima kasih pak.

Hormat saya,

La Ode Aman
Lecturer/Researcher on Pharmacy Dept.
Universitas Negeri Gorontalo
Jl. Jend Sudirman 6 Gorontalo 96128
INDONESIA

Contact Mobile: +62-811-434-084

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artico1998.pdf
254K

Universitas Negeri Gorontalo UNGmail

La Ode Aman <laode_aman@ung.ac.id>

Minta diperbaiki gambar 3 dan Gambar 7

3 messages

Daryono Hadi Tjahjono <daryonohadi@gmail.com> Sun, Sep 19, 2021 at 6:28 PM
To: La Ode Aman <laode_aman@ung.ac.id>

Assalamu'alaikum Laode,

Minta tolong diperbaiki gambar 3 terlampir.
Perbaikannya hanya menambahkan nomor saja, misal:
(Molport-046-141-977 = **1**)

Terus, S = -42.69 kcal/mol ----> sebelum dan sesudah tanda =
diberi spasi ya.

Untuk gambar 7, juga ditambahi nomor, Molport-046-141-977 (**1**)
Nomor senyawa dalam kurung.
Penomorannya harus sama dengan Gambar 3 ya.

Ditunggu ya, nuhun.
dht

2 attachments

Figure3.tif
1750K

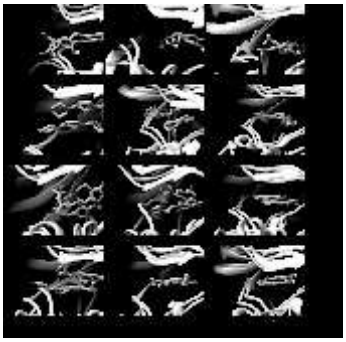

Figure7.tif
2344K

La Ode Aman <laode_aman@ung.ac.id> Mon, Sep 20, 2021 at 5:42 AM
To: Daryono Hadi Tjahjono <daryonohadi@gmail.com>

Waalaikum salam wr wb.
Iya pak, bersama ini terlampir hasil perbaikan Fig. 3 dan Fig. 7. Terima kasih pak.

La Ode Aman
Lecturer/Researcher

Chemistry Dept.
Universitas Negeri Gorontalo
Jl. Jend Sudirman 6 Gorontalo 96128
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Contact Mobile: +62-811-434-084

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2 attachments

Figure3v2.tif
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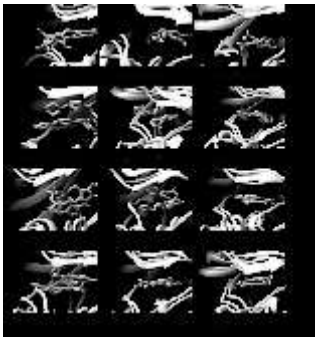

Figure7v2.tif
5796K

La Ode Aman <laode_aman@ung.ac.id> Mon, Sep 20, 2021 at 7:14 PM
To: Daryono Hadi Tjahjono <daryonohadi@gmail.com>

Assalamu alaikum Pak.
Terkirim revisi gambar 3. Semoga bisa tidak ada masalah. Kalau buka di laptop saya ditampilkan normal pak. Terima kasih.

La Ode Aman
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Figure3v5.tif
7030K

Universitas Negeri Gorontalo UNGmail

La Ode Aman <laode_aman@ung.ac.id>

Re: Regarding your article published in F1000Research

1 message

Daryono Hadi <daryonohadi@fa.itb.ac.id> Wed, Jan 5, 2022 at 6:24 PM
To: editorial <editorial@f1000research.com>
Cc: daryonohadi <daryonohadi@gmail.com>, La Ode Aman <laode_aman@ung.ac.id>

Dear Kate,

Thank you for your email along with information about our manuscript.
Yes, we are still running some calculations as suggested by reviewer 2.
Once we finishing the revision, we will submit it.
Thank you.
Best regards,
daryono hadi

From: "editorial" <editorial@f1000research.com>
To: "Daryono Hadi" <daryonohadi@fa.itb.ac.id>
Sent: Wednesday, January 5, 2022 9:15:44 AM
Subject: Regarding your article published in F1000Research

Do not delete (filing code): F1KR00CDE F1R-VER31013-A (end code)

Dear Daryono Hadi

Virtual screening of curcumin analogues as DYRK2 inhibitor: Pharmacophore analysis, molecular docking and dynamics, and ADME prediction
Aman LO, Kartasasmita RE and Tjahjono DH

It's been a while since we've heard from you, so we wanted to check whether you were aware that your article has not yet passed peer review.

Your article has received peer review reports with the following status(es):

1 Approved with Reservations
1 Not Approved

To pass peer review, and be indexed in PubMed and Scopus, the article must receive at least two peer review reports with the status 'Approved' or at least two reports with the status 'Approved with Reservations' and one with the status 'Approved'.

Until now, we have assumed that you are in the process of revising your article in response to the peer review reports. However, as we have yet to receive your revisions, please can you update us on whether you are in the process of revising or intending to do so in the future. For information on how to submit a new version, please visit Article Guidelines (new versions).

If you need any assistance, please let us know and we will be happy to help - we look forward to hearing from you.

Best regards

Kate
The Editorial Team, F1000Research

Virtual Screening of Curcumin Analogues from the Zinc15 Natural Product Database Using Ligand-based Pharmacophore Model

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La Ode Aman

Roles: Data Curation, Investigation, Methodology, Validation, Writing – Original Draft Preparation

Rahmana Emran Kartasasmita

Roles: Formal Analysis, Supervision, Validation, Writing – Review & Editing

Daryono Hadi Tjahjono

Roles: Conceptualization, Formal Analysis, Funding Acquisition, Methodology, Resources, Supervision, Validation, Writing – Review & Editing

Abstract

Background: Curcumin reduces the proliferation of cancer cells through inhibition of the DYRK2 enzyme, which is a positive regulator of the 26S proteasome.

Methods: In the present work, curcumin analogues have been screened from the Zinc15 natural product database using a pharmacophore model that comprises a ligand-based approach. The result of the screening was then evaluated by molecular docking and molecular dynamics based on interaction energy, the average of binding free energy and interaction stability between ligand and active site of DYRK2.

Results: Screening of 270.547 molecules from the Zinc15 natural product database yielded 110 selected hit compounds. By considering the docking and molecular dynamics simulation, three prospective curcumin analogues have been selected which are ZINC000085597244, ZINC000217945958, and ZINC000217643970. These molecules have better criteria than curcumin in some criteria, such as interaction energy, free binding energy, and interaction stability with the target.

Conclusion: ZINC000085597244, ZINC000217945958, and ZINC000217643970 compounds are predicted to be potential candidates for anticancer drugs with a specific mechanism of action against DYRK2, which is a positive regulator of the 26S proteasome.

Keywords

Curcumin analogues, DYRK2, Pharmacophore, Docking, Molecular dynamics simulation

Introduction

Many studies have been reported on the anticancer effects of curcumin, derivatives or curcumin analogues [1–3]. Anticancer activity of curcumin act by inducing apoptosis, inhibiting the proliferation and invasion of tumors by suppressing various cellular signal lines [4]. This secondary metabolite of turmeric rhizome (Curcuma longa) also have mechanisms as antitumor by disrupting different cellular pathways and inducing/inhibiting the production of various types of cytokines, enzymes or growth factors such as MAPK, EGF, NFkB, PKD1, COX-2, STAT3, TNF-α, and IκKβ [1], as well as by inhibiting dual-specificity tyrosine-regulated kinase 2 (DYRK2) which is a positive regulator of 26S proteasome [5]. Some studies report curcumin's antitumor activity in breast cancer, lung cancer, head and neck squamous cell carcinoma, prostate cancer, and brain tumors [6], demonstrating its ability to target multiple lines of cancer cells [7]. Some of the discoveries of new compounds that are derivatives or analogues of curcumin that have better pharmacokinetics than curcumin, such as increasing solubility, stability in water, slowing metabolism and selective accumulation in the pancreas and prostate tissue, show that there is selectivity in certain types of cancers and even provide better anticancer efficacy than curcumin [3,7].

Investigation of curcumin derivative or analogues compounds is still a challenging and prospective job today in the context of finding anticancer drug candidates in the form of both new compounds and existing compounds. A pharmacophore studies of curcumin analogs in the context of ligand-based virtual screening using 24 compounds (curcumin and curcumin analogues) that have been reported by Artico et al [8] as active set have never been conducted.. In addition, curcumin has been proven in vitro to be able to selectively inhibit DYRK2 [5]. However, there have been no reported curcumin analogues studied as potential anticancer agents through DYRK2 inhibition.

One of the approaches used in searching for drug candidate compounds and studying their pharmacological potential is the application of computational chemistry, which includes pharmacophore studies, virtual screening, docking, and molecular dynamics. Pharmacophore study means studying the steric and electronic factors possessed by a drug compound that play a role in molecular interactions with specific biological target structures. Hypothetical pharmacophore models can be performed in both ligand-based and protein structure-based. Virtual screening means the process of screening a compound data bank by applying certain criteria or parameters so that a part of the compound list from the total data bank is selected. To obtain a small number of selected compounds, usually the virtual screening process is continued with the application of docking and molecular dynamics.

The advantage of this approach as a preliminary study in screening and studying the pharmacological potential of a compound is the savings in terms of cost and time. Thousands or even millions of compounds do not need to be tested *in vivo* or *in vitro* to select the best potential compounds. Virtual screening of curcumin analogs based on pharmacophore models will usher in a small number of curcumin analogues that have pharmacophore similarities to curcumin. Docking and molecular dynamics will lead to an advanced selection of compounds that have similar pharmacophores so that a much smaller number of selected compounds is obtained after knowing the energy and interaction patterns with the target molecule.

The purpose of this study was to obtain curcumin analog compounds that were predicted to have anticancer properties with a specific inhibiting mechanism of action of DYRK2. The steps taken to achieve this goal was to conduct a virtual screening of the database of natural material compounds provided by Zinc15 [9] with an approach to pharmacophore similarities with curcumin and curcumin analogues that have known anticancer activity. The list of compounds resulting from virtual screening is then re-selected by applying docking methods and molecular dynamics. Selected compounds are compounds that provide the best parameters in terms of interaction energy, average binding free energy, and stability of interaction with the target molecule.

Methods

Preparation of the Active and Decoy Compounds

The molecular structure of the active compound, totaling 24 compounds consisting of curcumin and curcumin analogues used in pharmacophore modeling, is shown in Fig 1 [8]. Decoy compounds were generated using Decoy Finder 2.0 [10]. The three-dimensional structure of active and decoy compounds is saved in the mol2 format.

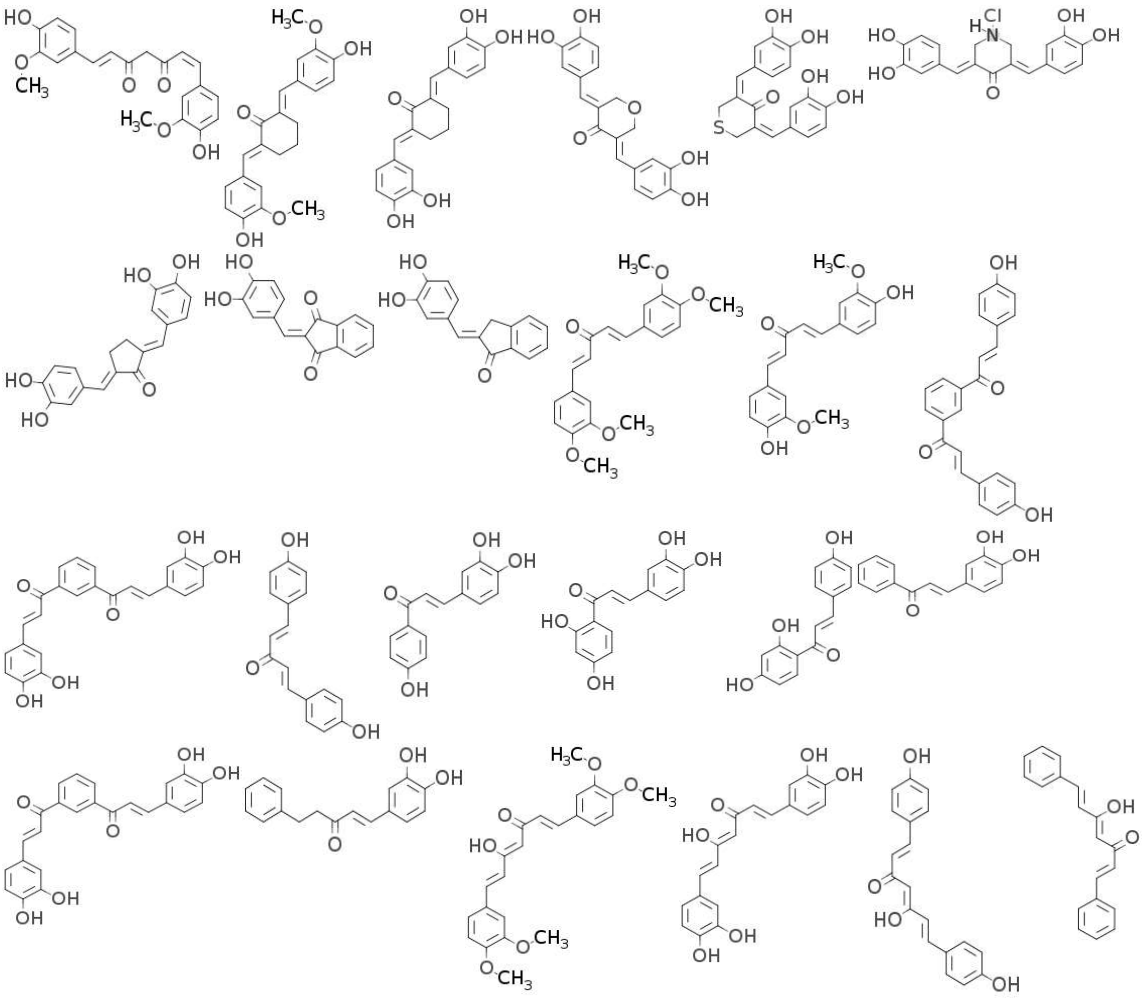


Figure 1 Curcumin and curcumin analogs as active compounds.

Protein Preparation

The target protein used in the study was DYRK2, which was obtained from the RCSB web server data bank protein [11] with PDB ID 5ZTN [5]. The crystal structure of the protein is in its complex with curcumin, which is an inhibitor of DYRK2. Protein preparation actions include removing water molecules and other atoms/molecules, selecting one of the chains, and separating protein structures and native ligands using Biovia Discovery Studio [12]. Repair of break residues in protein structures was carried out using the MODELLER module [13] integrated on UCSF Chimera [14]. Atomic repair, addition of hydrogen, and charge from proteins are machined using AutoDockTools 1.5.6 [15].

Pharmacophore Analysis

Pharmacophore analysis includes pharmacophore detection, generation, and validation of pharmacophore models performed using PharmaGist [16,17]. Pharmacophore detection was carried out on the Curcumin molecule, while pharmacophore models were generated from active compounds (Fig 1), where

Curcumin was selected as a key molecule (pivot). Pharmacophore model validation is done using the Screening Explorer application [18] to detect the best model with an AUC ROC value criterion of more than 0.5.

Screening Database

Natural product compounds totaling 270,547 compounds were provided by Zinc15 [9] selected as the database. The screening of database compounds is carried out using PharmaGist with a pharmacophore-based approach, namely by using the best pharmacophore model obtained from the pharmacophore analysis stage. The results of the database screening are designated as the selected compound of step 1 and called SCS1 (Supplement 1).

Molecular Docking

The screening process was continued by applying a molecular docking simulation using the Autodock4 program package [19]. The selected compounds from the screening database step were docked to the validated active side of DYRK2. The native ligand re-docking protocol was a tool used for DYRK2 active side evaluation where the active side was considered valid if the native ligand re-docking results show RMSD (room mean square distance) < 2 Å. The docking was carried out using criteria namely: rigid protein, genetic algorithm parameter with GA runs 20, maximum number of evals to long = 25000000 on the number of rotatable bonds. Based on the interaction energy of native ligands and SCS1, which is calculated with a molecular docking approach so that two selected compounds of stage 2 (SCS2) were produced with the highest interaction energy compared to other test ligands.

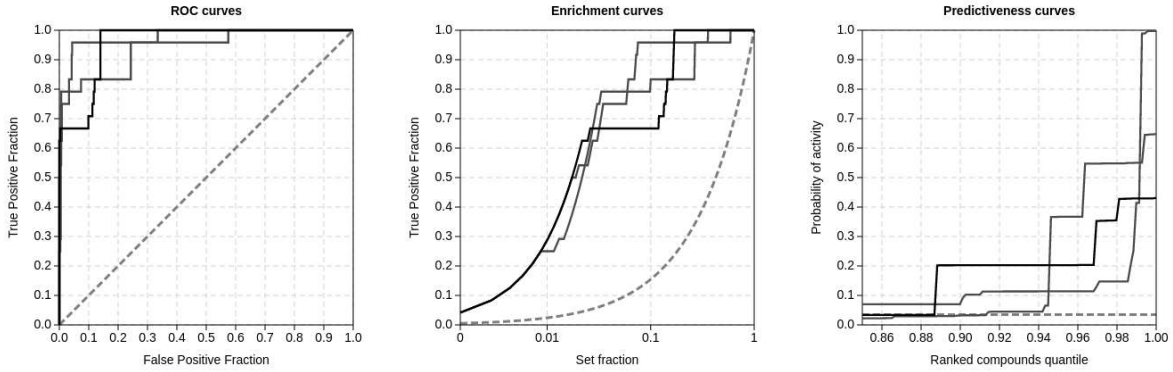
Molecular Dynamics

Molecular dynamics simulations were used to continue figuring out how much energy three compounds (two SCS2 and one native ligand) use to interact with each other. Molecular dynamics simulation was performed using the GROMACS 2022 program package [20]. The protein topology was prepared using the AMBER99SB-ILDN [21] generated by Gromacs 2021.3, and the ligands topology was prepared using the General AMBER Force Field (GAFF) [22] generated using the Antechamber program package from Ambertools 2021 [23] with the help of Acpype [24]. In the MD simulation, the first shape is a protein-ligand complex that comes from a simulation of molecular tethering with the lowest bond energy.
The complex was immersed in water in the form of a cubic box using the TIP3P water molecule model [25]. A neutral system was obtained after the addition of Na^+/Cl^- ions. An equilibrium system consisting of proteins, ions, and ligands was obtained after NVT and NPT simulations at 298.25 K for 100 ps each. The production system takes place at a temperature of 298.25K and a pressure of 1 bar for 100 ns. The simulation results were analyzed using RMSD and RMSF values, and binding free energy was calculated with the MMGBSA approach [26].

Results

Pharmacophore Analysis

Ligand-based pharmacophore analysis is performed with the help of the PharmaGist application. Pharmacophore detection was carried out using 24 compounds (Fig 1) as ligands with curcumin as a key molecule (pivot) so that three pharmacophore models were obtained, namely 1.pha, 2.pha and 3.pha (supplement 1). Validation of pharmacophore model candidates was carried out by applying retrospective evaluation of the performances analysis with a consensus scoring approach. The ROC (receiver operating characteristic), Enrichment and Predictiveness curves as well as a summary of the analysis results (global metrics) of each pharmacophore model are shown in Fig 2.



(a) (b) (c)

Set 1 - ROC AUC: 0.940 - Total Gain (TG): 0.681 - RIE: 11.676 - BEDROC: 0.810 - Avg rank of actives: 52.38
Set 2 - ROC AUC: 0.976 - Total Gain (TG): 0.780 - RIE: 12.400 - BEDROC: 0.860 - Avg rank of actives: 28.29
Set 3 - ROC AUC: 0.958 - Total Gain (TG): 0.753 - RIE: 10.989 - BEDROC: 0.763 - Avg rank of actives: 40.54

Compounds: 690 - Actives: 24 - Inactives: 666 - Hit-rate: 3.48% - Maximum reachable EF: 28.75

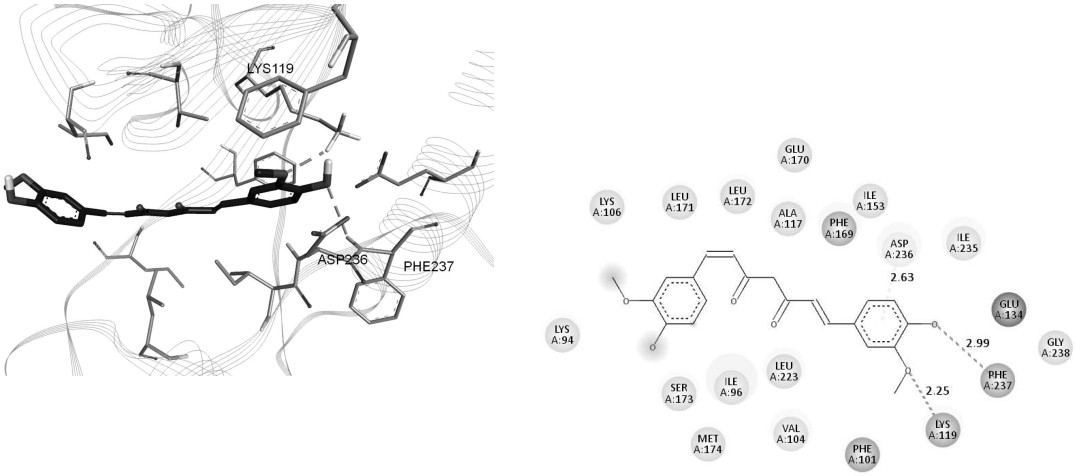
(d)

Figure 2. ROC (a), Enrichment (b) and Predictiveness (c) Curve of Set 1.pha in is red line, set 2.pha is green line, and Set 3.pha is black. Global metrics of calculation is shown in (d).

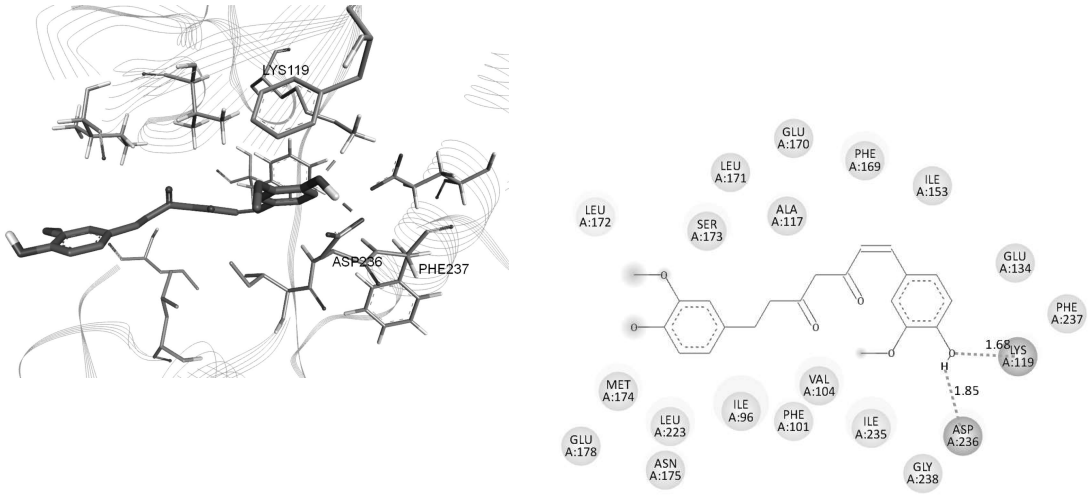
The 2.pha pharmacophore model was used for screening the database of natural material compounds from Zinc 15, which amounted to 270,547 compounds, so that 110 compounds were obtained. SCS1 assignments are based on the value of the pairwise alignment score > 40.00 of each database compound (Supplement 3).

Molecular Docking

The X-ray diffraction crystal structure of 5ZTN showed that the interaction of DYRK2 and Curcumin was formed by three hydrogen bonds (H-bonds) residues, LYS119, PHE237, and ASP236 respectively, with interaction distances of 2.25, 2.99 and 2.63Å (Fig 3a).



(a)



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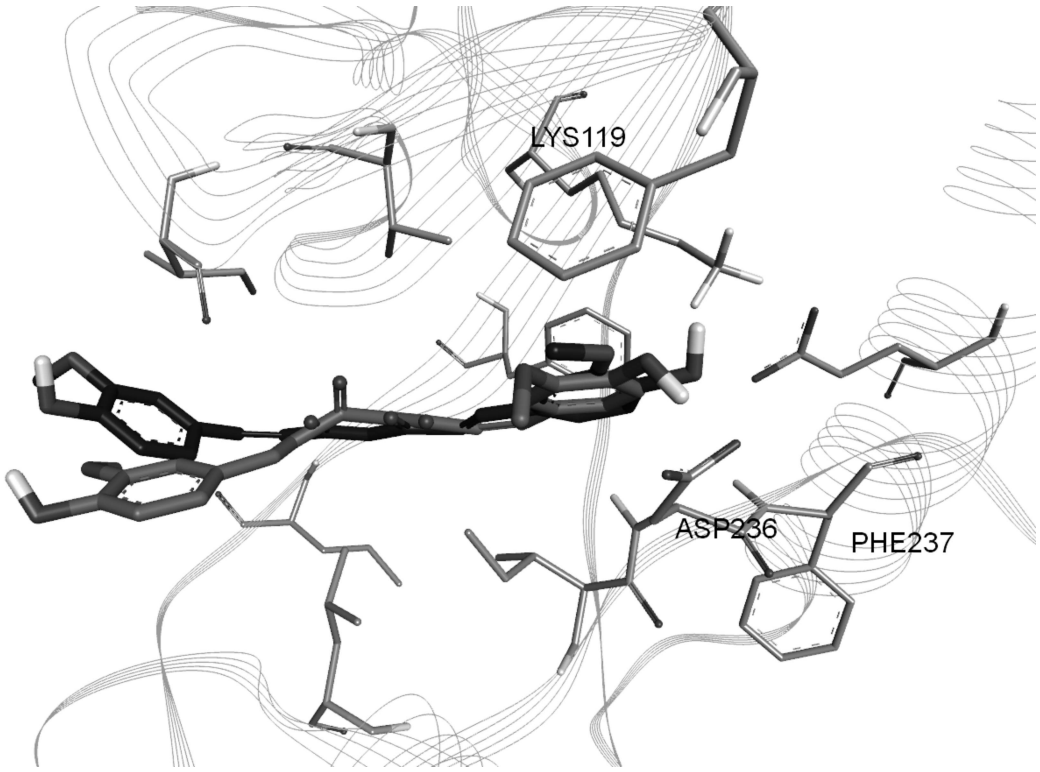


(c)

Fig 3. Curcumin in the active site of DYRK2: (a) original position, (b) best pose, (c) overlay the original (blue), and after redocking (red).

Validation of active site DYRK2 is done by implementing the curcumin re-docking protocol as a native ligand. Curcumin re-docking results obtained the best pose with RMSD = 1.57Å and binding free energy of -10.06 kcal/mol. Visualization of overlapping native ligand structures before and after redocking is shown in Fig 4. The pose was obtained at the setting number grid point = (34, 46, and 34), spacing 0.375 Å and grid center coordinate = (8.66, -22.126, and 20.789). The position of curcumin on the active site DYRK2 after redocking simulations was shown in Fig 3b where the H-bond interaction distance between curcumin and LYS119 and ASP236 became 1.68 and 1.85Å. Thus, site active of DYRK2 was valid so that it can be used to perform test ligand docking protocols on active DYRK2 sites.

The test ligand was SCS1 with contained amounts to 110 compounds. Each test ligand undergoes a docking protocol with a grid map following the site's active validation process settings. The results of docking for the test ligand with free energy binding of more than 10.00 kcal / mol were summarized in Fig 4. The three compounds that have the top binding free energy were selected as SCS2, namely ZINC000085597244, ZINC000217945958, and ZINC000217643970, with binding free energy of -12.09, -11.21, and -10.31 kcal/mol, respectively.

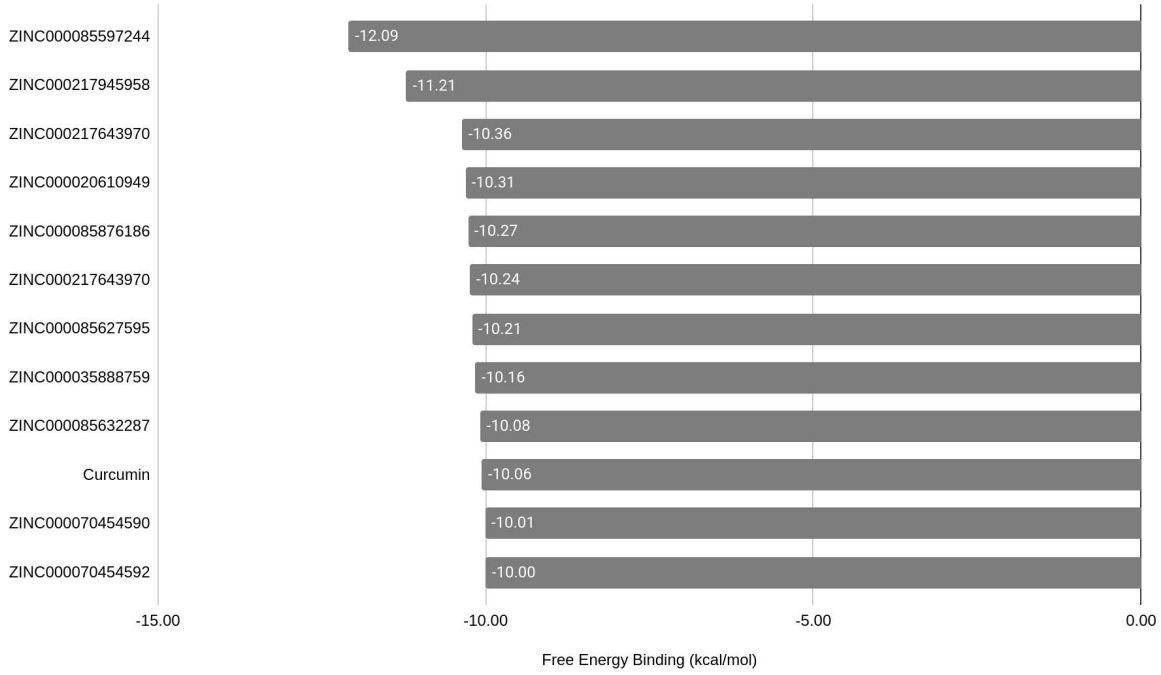
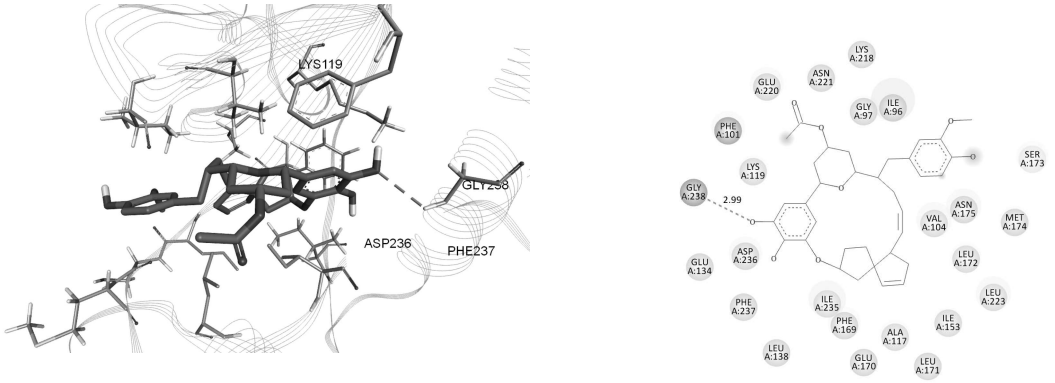
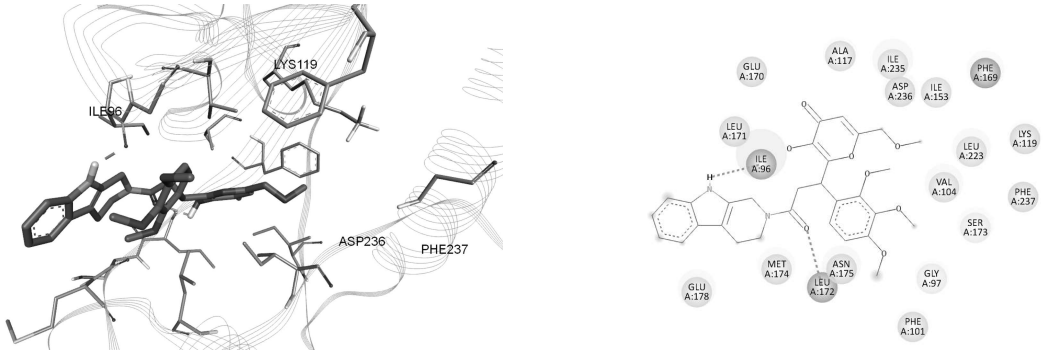


Fig 4. Docking result of test set



(a)



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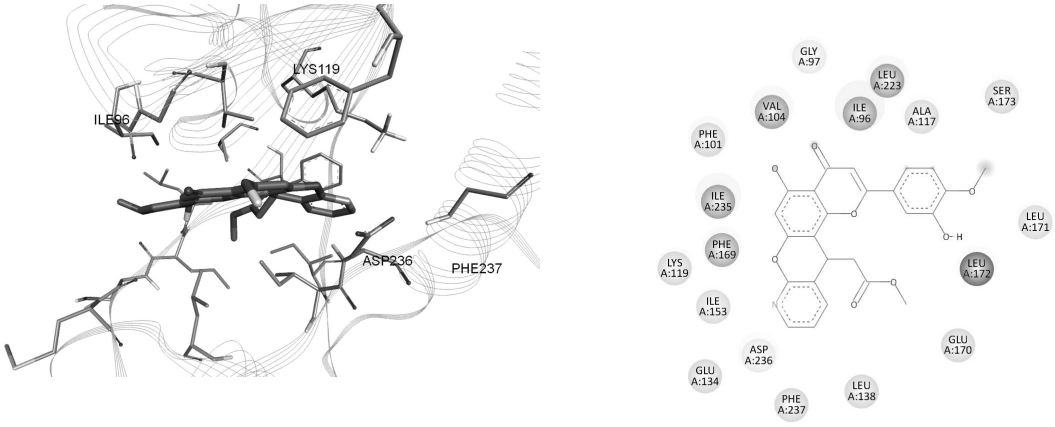

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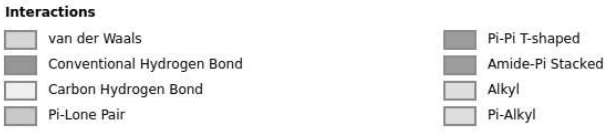


Fig 5. The interaction of SC2's best pose with DYRK2 active site: (a) ZINC000085597244, (b) ZINC000217945958, and (c) ZINC000217643970

Molecular Dynamics

MD simulations can be used to test how stable the interaction between two compounds is after docking simulation. Each compound of SCS2 (ZINC000085597244, ZINC000217945958, and ZINC000217643970) in a complex with the DYRK2 protein simulated MD for 100 ns. Root means square distance (RMSD) proteins and any compounds of SCS2 and Curcumin were summarized in Fig 6. Based on the RMSD chart, it can be seen that the movement of proteins and all four ligands during the dominant simulation was at a distance of less than 1.0 nm from the initial position.

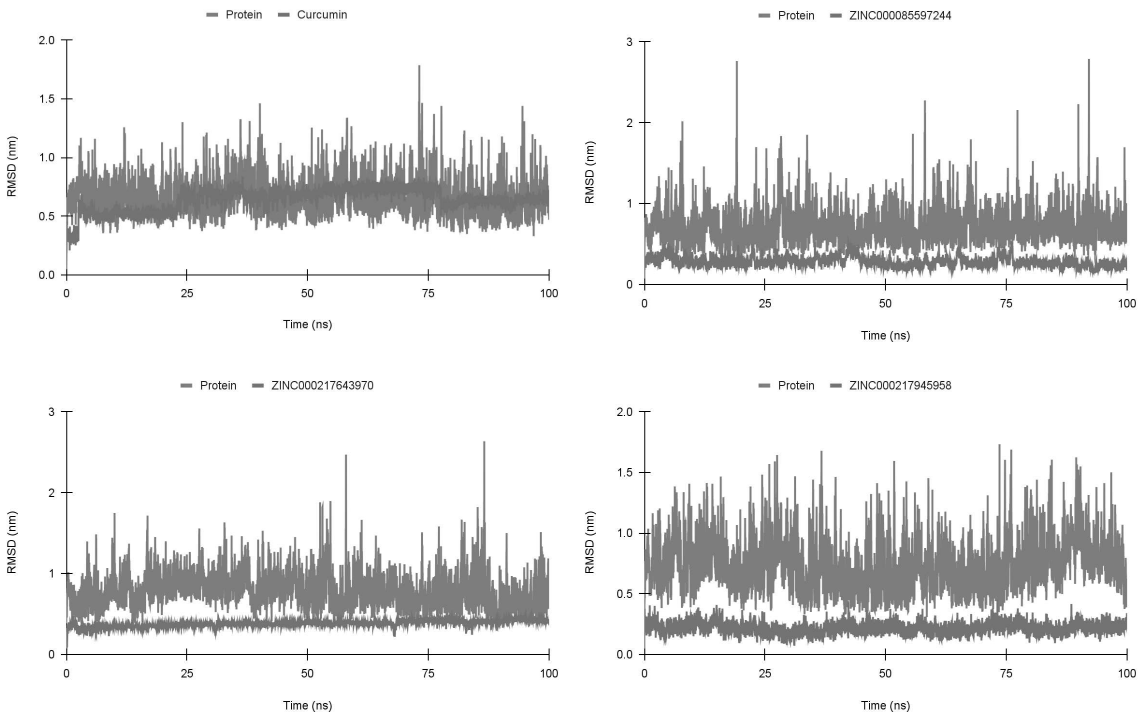


Fig 6. RMSD ligands and protein for 100 ns MD simulation

The strength of the interaction between DYRK2-ligands among them can be seen in the value of the interaction energy of the non-bond between the two. Interaction energy is different from free energy or connective energy[27]. The GROMACS program package outlines and calculates the energy of these non-bonded interactions derived from the short-range Coulombic interaction energy (Coul-SR) and the short-range Lennard-Jones (LJ-SR). The sum of the averages of Coul-SR and LJ-SR constitutes the total interaction energy value[28]. The total interaction energy of DYRK2 with Curcumin, ZINC000085597244, ZINC000217945958, and ZINC000217643970 is -45.808, -56.328, -62.335, and -55.240 kcal/mol, respectively. The Coul-SR and LJ-SR energies of each complex are aimed at Fig 7.

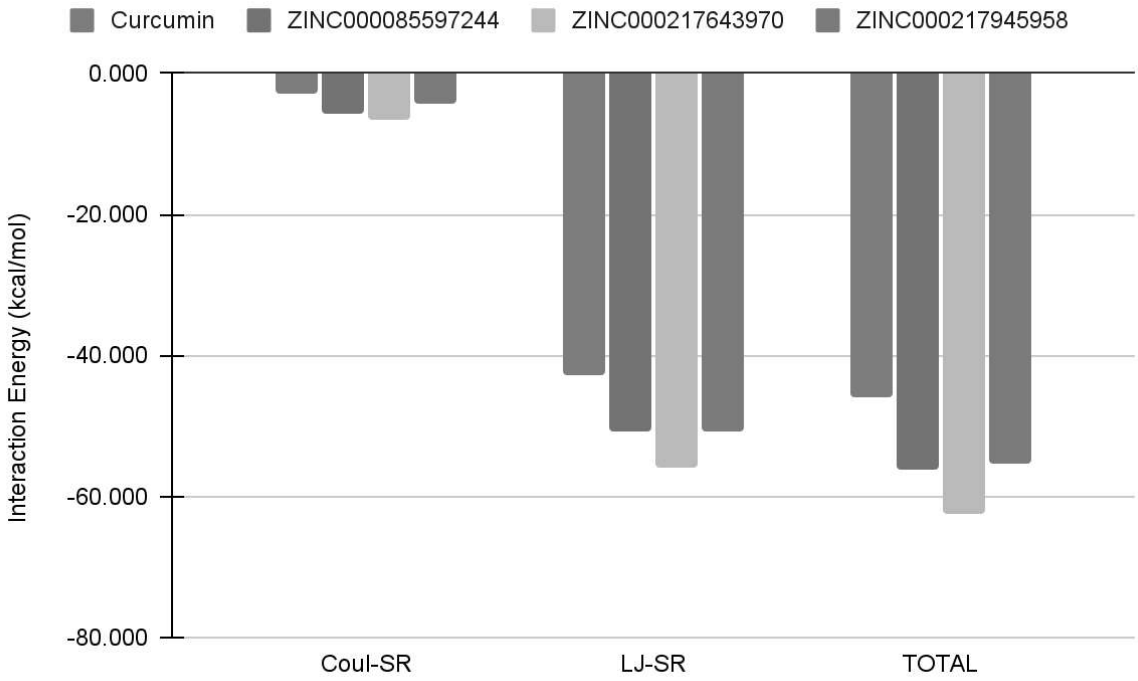


Fig 7. Total interaction energy

Evaluation of the stability of protein-ligand interactions can also be seen in the fluctuations and energy contributions of each active site receptor residue in the interaction with the ligand. Active site DYRK2 is an amino acid residue that is at a distance of 5Å from the ligand, so that the number of active site residues from curcumin ligands was 21 residues (ILE96, PHE101, VAL104, VAL105, ALA117, LYS119, GLU134, ILE153, PHE169, GLU170, LEU171, LEU172, SER173, MET174, ASN175, GLU178, LEU223, ILE235, ASP236, PHE237, GLY238), ZINC000085597244 is 26 residues (ILE96, GLY97, LYS98, GLY99, PHE101, VAL104, ALA117, LYS119, GLU134, ILE153, PHE169, GLU170, LEU171, LEU172, SER173, MET174, ASN175, GLU178, LYS218, GLU220, ASN221, LEU223, ILE235, ASP236, PHE237, GLY238), ZINC000217643970 are 23 residues (ILE96, GLY97, LYS98, PHE101, VAL104, ALA117, LYS119, GLU134, ILE153, PHE169, GLU170, LEU171, LEU172, SER173, MET174, ASN175, GLU178, GLU220, LEU223, ILE235, ASP236, PHE237), and ZINC000217945958 are 26 residues (96, GLY97, LYS98, GLY99, PHE101, VAL104, LYS106, ALA117, LYS119, GLU134, LEU138, ILE153, PHE169, GLU170, LEU171, LEU172, SER173, MET174, ASN175, GLU220, ASN221, LEU223, ILE235, ASP236, PHE237, GLY238). The contribution of each active site residue to binding free energy was summarized in Fig 8b. Fluctuations in active site DYRK2 residues during simulations were summarized in Fig 8a. Fig 8 only displays residues with a free energy contribution in interaction with one of the ligands of more than 0.5 kcal/mol.

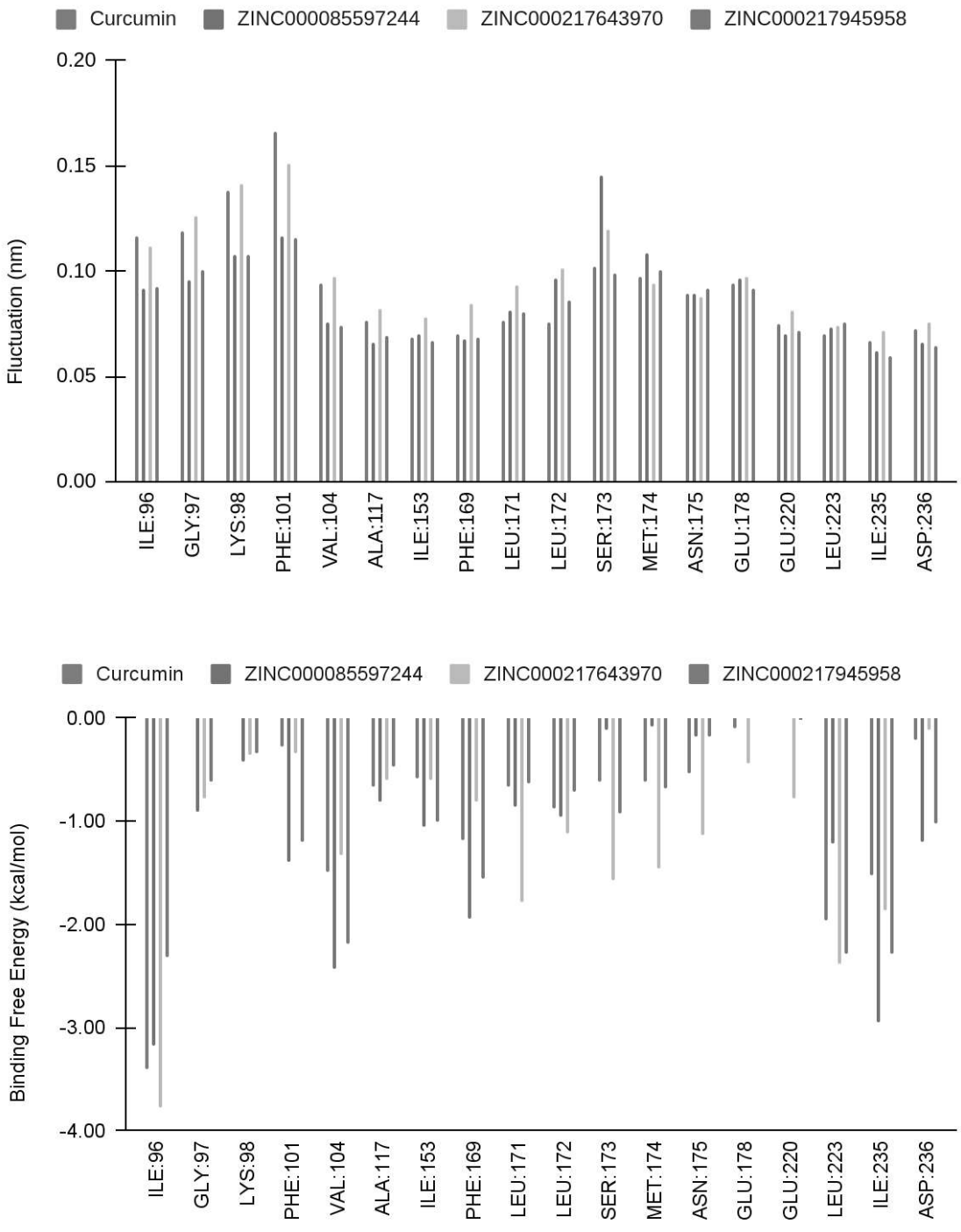
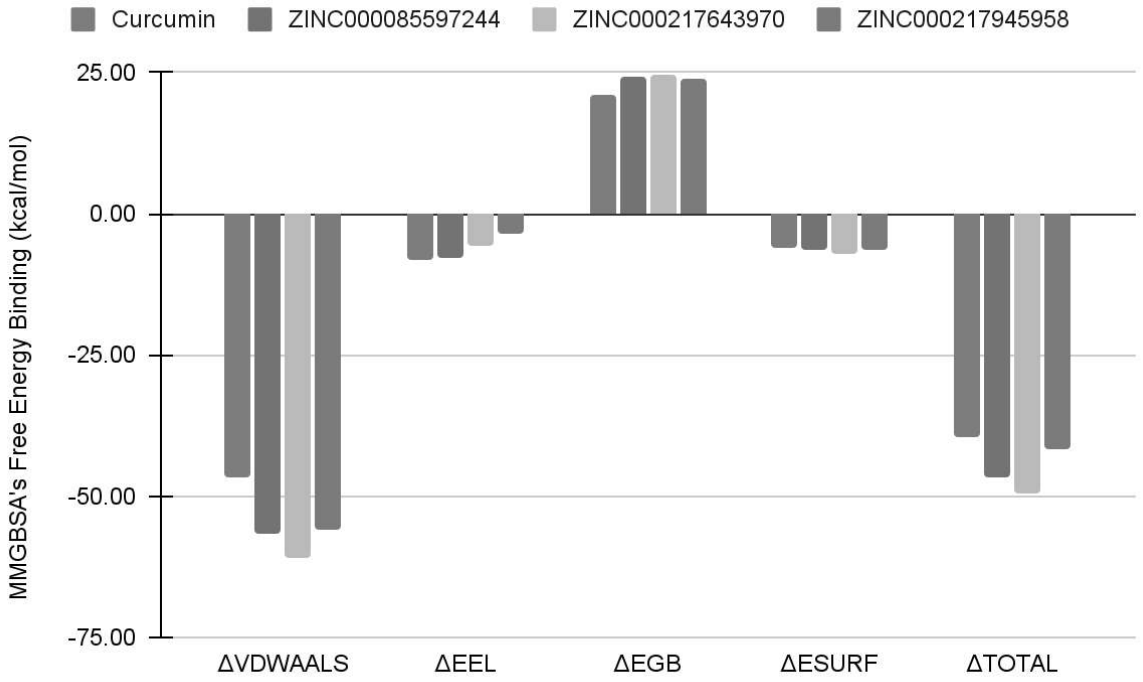


Fig 8. The residual contribution of the DYRK2 active site in ligand interactions: (a) fluctuation, (b) binding energy

The calculation of the average of binding free energy of the ligand-receptor interaction was carried out by applying the MMGBSA (Molecular mechanics/Generalized-Born Surface Area) method. The results of the calculation of binding free energy from each interaction of curcumin, ZINC000085597244,

ZINC000217945958, and ZINC000217643970 with DYRK2 were summarized in Fig 9.



Discussion

Pharmacophore Analysis

Pharmacophores are chemical features of molecules that interact with target receptors in various modes of interaction, such as H-bond, electrostatic, hydrophobic, steric, and or other non-bonding interactions [17]. Each molecule can have pharmacophore features different from other molecules in some ways, such as functional groups, distances between features, 3-dimensional spaces formed by features, and others. A group of compounds analogous to a certain pharmacological activity (an active set) can have a specific pharmacophore model. PharmaGist is a free pharmacophore detection tool that performs ligand-based pharmacophore analysis.

Pharmacophore-based virtual screening using PharmaGist consists of three stages, namely (i) detection of active set molecular pharmacophores, (ii) validation of pharmacophore models, and (iii) database screening [17]. Pharmacophore model detection has been carried out using 24 compounds consisting of curcumin and its analogues as active sets. *In vitro*, this series of compounds is proven to be able to reduce the exponential growth of MT-4 and KB cells [8]. Supplement 1 containing the pharmacophore detection output file of active set molecules, it can be seen that there are three pharmacophore models produced in pharmacophore detection using Curcumin as the pivot molecule, namely 1.pha, 2.pha, and 3.pha. All three

pharmacophore models need to go through a validation process to find out which one can be used to screen compounds in a database the best. The decoy compounds (decoy set) required in the validation process were obtained from the zinc database generated using DecoyFinder 2.0. The best modeling criteria for data mining classification, area under ROC (AUC) value [29] are grouped as follows:

AUC Value	Description
0.90 – 1.00	Excellent Classification
0.80 – 0.90	Good Classification
0.70 – 0.80	Fair Classification
0.60 – 0.70	Poor Classification
0.50 – 0.60	Failure

The results of the ROC curve analysis with calculations using the Screening Explorer application showed that the AUC ROC values of each pharmacophore model were: 1.pha = 0.940, 2.pha = 0.976, and 3.pha = 0.958. Based on the UAC ROC value, it can be concluded that the three models are included in the exolytic group. Nevertheless, 2.pha is the highest UAC ROC value, so this pharmacophore model was chosen for database screening. Of the 270,547 compounds of natural ingredients provided by Zinc15, 110 compounds (SCS1) of them have pharmacophore similarities with active sets based on the 2.pha model. The molecular structure of 110 SCS1 is available in the Supplement 2 folder.

Molecular Docking

The active site of a receptor can be determined easily if the target macromolecules are crystallized along with the native ligand [30] which in vivo or in vitro that the native ligand exerts a certain pharmacological effect associated with the target macromolecules. Protein with code 5ZTN is a DYRK2 enzyme that is in its complex with curcumin which acts as a DYRK2 inhibitor with an IC_{50} of 5 nM [5].

Molecular docking is an important stage in the virtual screening process because it produces and screens drug candidates more effectively than the physical assessment of thousands of compounds, thereby increasing the rate of drug discovery while reducing costly costs. Molecular docking predicts the mode and affinity of non-covalent bonds between a pair of molecules. Molecular docking predicts the preferred binding orientation of a single ligand molecule with macromolecules in a stable complex indicated by binding free energy [31]. Two approaches to docking are known, namely blind docking and site-specific docking. Blind docking means docking the ligand to the entire surface of the macromolecule because there is no information about the binding site of a macromolecule, while

site-specific docking is carried out after the binding residue has been determined in advance so that the test ligand is only bound to the residues of the binding site.

The existence of curcumin as the native ligand of DRYK2 in the 5ZTN complex makes the docking process test set ligand against DRYK2 apply the site-specific docking method. The DYRK2 amino acid residue designated as the binding residue is on the criteria of grid center coordinate = (8.66, -22.126, 20.789) with a three-dimensional grid box size = (34, 46, 34) and spacing = 0.375Å. The determination of the binding residue was validated because the RMSD of the curcumin atoms between the initial and after re-docking positions was valued at < 2.0Å which was 1.57Å. The binding of curcumin at the binding site of DYRK2 is also indicated by the negatively valued predictive binding free energy of -10.06 kcal/mol. The amino acid as binding pocket of DYRK2, which was at a distance up to 5Å from the ligand amounts to 21 residues, namely ILE96, PHE101, VAL104, VAL105, ALA117, LYS119, GLU134, ILE153, PHE169, GLU170, LEU171, LEU172, SER173, MET174, ASN175, GLU178, LEU223, ILE235, ASP236, PHE237, and GLY238.

A total of 110 test set compounds were docked at site active DYRK2 using Autodock4, which calculated the best predictive binding free energy of each ligand. Three SCS2 compounds (ZINC000085597244, ZINC000217945958, and ZINC000217643970) that have the top binding free energy of -12.09, -11.21, and -10.31 kcal/mol, respectively. Fig. 5 shows that the three ligands are in the same DYRK2 environment as where curcumin is located (Fig. 4). Negatively valued binding free energy suggests the DYRK2-ligand interaction is a spontaneous and stable process with stronger binding free energy than the DYRK2-curcumin interaction.

Molecular Dynamics

MD simulations are needed to evaluate the stability of macromolecular-ligand complexes in several observation parameters such as RMSD, RMSF (root mean square fluctuation) atomically or residual active site, interaction energy, as well as total and residual active site binding free energy. RMSD shows the distance of motion of each ligand atom and the target against the system during the simulation. RMSF shows the average distance of motion during the simulation of each atom or residue. The RMSF shown in this study was only the distance of fluctuations in active site residues from DYRK2, which was at a distance of 5Å from the ligand center. Interaction energy shows the strength of non-bond interactions between macromolecules and ligands, which was the total of coulombic interaction energy (Coul-SR) and Lennard-Jones energy. In contrast to interaction energy, binding free energy is calculated from the Molecular Mechanics Generalized Born and Surface Area (MMGBSA) and/or Molecular Mechanics Poisson-Boltzmann and Surface Area (MMPBSA). MMGBSA and MMPBSA are calculated from the same amount, namely the amount of gaseous state free energy (ΔG Gas) and free energy in the dissolved

state (ΔG Solv), where ΔG Gas is contributed by van der Waals energy (VDWAALS) and electronic energy (EEL). The difference is in the ΔG Solving component where in MMGBSA, it is the amount of EGB (Generalized Born energy) and ESUF (surface area energy), MMPBSA is contributed by EPB (Poisson-Boltzmann energy), ENPOLAR (non-polar solvation energy), and EDISPER (dispersion energy). EGB and EPB are polar energies, and the others are non-polar energies [32]. In this study, the total free energy of the bond using the MMGBSA approach was calculated using the GMX-MMPBSA application, in addition to the residual free energy of each DYRK2 site active amino acid.

The average binding free energy of three SCS2 compounds with DYRK2 during MD simulation was calculated with the MMGBSA approach. ZINC000085597244, ZINC000217643970, and ZINC000217945958 each have an average binding free energy of -46.69, -49.45 and -41.81 kcal/mol, respectively, while curcumin has a weaker bond strength with DYRK2, which was only -39.60 kcal/mol. Fig 9 shows ZINC000217643970 is the ligand with the strongest average binding free energy compared to other ligands.

The RMSD data of the ligands against the residual position of the amino acid of DYRK2 (Fig 6) during the simulation also confirmed the spontaneity and stability of the interaction, while the average binding free energy confirmed that all three compounds were predicted to have a better inhibition ability to DYRK2 than curcumin. Based on residual analysis, MD simulation results showed that the DYRK2 amino acid residue that established interactions with the most powerfully contributing ligands (average binding free energy <-2.00 kcal / mol) was ILE96, VAL104, LEU223, and ILE235.

The results of the MD simulation showed that the DYRK2 amino acid residue that established an interaction with the strongest contributing ligand (the average binding free energy of <-2.00 kcal / mol) was ILE96, VAL104, LEU223, and ILE235. ILE96 formed H-bonds with ZINC000085597244 and ZINC000217945958, while with ZINC000217643970, it formed a non-hydrophobic bonding interaction. VAL104 formed hydrophobic interactions with ZINC000085597244, ZINC000217945958, and ZINC000217643970. LEU223 formed a hydrophobic interaction with ZINC000085597244 and ZINC000217945958 whereas with ZINC000217643970 it is at a distance above 5Å so that the type of interaction that may occur is not detected. ILE235 forms alkyl hydrophobic interactions with all three ligands.

Conclusion

Three curcumin analogues obtained through pharmacophore-based screening, namely ZINC000085597244, ZINC000217945958, and ZINC000217643970, were predicted to have a better specific inhibition ability against DYRK2 than curcumin,

which was confirmed through molecular docking and molecular dynamics simulations. The inhibitory ability indicates that the three curcumin analogues have the potential to be anticancer with a mechanism of action to inhibit DYRK2, which was a positive regulator of proteasome 26S.

Data availability

Source data

Protein Data Bank: DYRK2 data from Protein Data Bank. Accession number PBD5ZTN; https://identifiers.org/structure/5ztn.

Dataset of compounds with biological activity for ligand-based pharmacophore modeling were obtained from[8] and are shown in Fig 1.

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Assalamu alaikum Pak. Mohon maaf baru dapat menyelesaikan revisi manuskrip yang di submit pada F1000Research seperti terlampir. Sekali mohon maaf dan terima kasih.

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