



Research Article

In Silico Inhibition Studies of AXL Kinase by Curcumin and its Natural Derivatives

Ghrifi Fatima*, Allam Loubna, Lakhlii Wiame and Ibrahim Azeddine

Abstract

The last few years have seen the intensive investigation efforts for understanding the wide relevance of AXL activation in multiple aspects of oncogenesis, invasion, metastasis and drug resistance. Therefore, targeting AXL can be considered as one of the promising approaches for the treatment of cancer. A series of curcumin derivatives which are natural polyphenolic compounds were well-reported as an anti cancer and chemopreventive agents. We have investigated them as an ATP-competitive inhibitors of AXL kinase by using a docking studies. We built a model of AXL catalytic domain from the crystal structure of proto-oncogene tyrosine-protein kinase MER (MERTK) and the modeling of the three-dimensional (3D) structure of the AXL was performed by SWISS-MODEL homology modeling program. The quality and validation of the model were performed using PROCHECK and VERIFY 3D softwares. The Ramachandran plot was used to validate the overall stereochemical property of the protein. All curcuminoids formed a hydrogen bond with hinge region by Methionine (Met 623) and lysine (Lys 567) indicating that these compounds can be utilized therapeutically as a natural AXL inhibitors.

Keywords

AXL kinase; Curcumin; Docking; Homology modeling

Introduction

The AXL receptor tyrosine kinase, also known as UFO, Tyro7 and Ark, is a member of the tyrosine kinases receptors family TAM (Tyro3, AXL and MERTK) [1]. AXL, like the other TAM members, is activated via the interaction with the growth arrest-specific protein 6 (GAS6) ligand [2]. The receptor has been implicated in a number of oncogenic processes. AXL signaling enhances many essential biological functions for cancer formation and progression, including invasion, migration, survival, angiogenesis, cell transformation and proliferation [3]. The increase of AXL activity or overexpression indicates a poor prognosis for the patients and has been reported to be associated with metastasis in several types of cancer [4-6]. AXL was demonstrated to confer resistance to chemotherapies when overexpressed in gastrointestinal stromal tumor [7] and breast cancer cells [8]. AXL was first cloned from myeloid leukemia cells in 1991 [9].

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Received: August 08, 2017 Accepted: November 07, 2017 Published: November 13, 2017

No crystal structure for Axl catalytic domain has been reported. Just the extracellular Ig-like domain was crystallized as fragments in complex with Gas6 in 2006 [10].

During the last decades, inhibition of AXL tyrosine kinases has emerged as an important approach for cancer therapy. Until this date, no inhibitor was clearly designed and marketed to inhibit AXL. However, Some clinical molecules, developed to inhibit other kinases, were evaluated against AXL given its interest, but these molecules continue to be inadequate having either limited efficacy, prohibitive toxicities or often exhibit less potency for AXL. All these are due to the similarity of the kinases catalytic domains, to which classical ATP competitive inhibitors (type I and II) tend to bind, particularly, with c-MET and MERTK kinases. Recently, the first AXL specific small molecule inhibitor, BGB324 originally discovered by BergenBio Company as R-428, entered phase 1 clinical trials [11].

Thus, there is a need for other therapies that can deactivate AXL kinase and keep the cancer in remission and increase survival. Previous studies have focused on the anticancer effects of natural products, for designing novel molecules ATP competitors with low toxicity.

Curcumin, has been shown to be effective against different cancers [12-14] in both in vitro and in vivo assays through a variety of mechanisms [15,16]. In addition, human clinical trials did not demonstrate any toxicity for curcumin at doses up to 10 g/day [17]. Curcumin, isolated from *turmeric Curcuma longa* contains curcumin as major component (77%) but it also contains demethoxycurcumin (DMC) (17%) and bisdemethoxycurcumin (BDMC) (3%) that have a remarkable anti-carcinogenesis effect. A study showed that demethoxycurcumin induced the G2/M step of cellular cycle arrest in human glioma U87cells [18]. In another study, BDMC was observed to suppress the growth and activity in human adenogastric carcinoma [19].

Here, we focused on examining the interactions between curcumin as well as DMC and BDMC with AXL domain kinase using *in silico* approach and molecular docking. This study could be applied to develop new potential AXL inhibitors ATP competitors from curcumin and natural derivatives in the future. Since no data are available on AXL crystal structure. Herein we used homology modeling to this end.

Materials and Methods

AXL kinase homology modeling

We used the Swiss model server [20] to build the AXL model by homology modeling, a proto-oncogene tyrosine-protein kinase MER (MERTK PDB code:3BRB, resolution 1.9Å) was used as template.

The template was selected based on sequence identity and the coverage of the sequence obtained by alignment using Basic Local Alignment SearchTool [21] NCBI BLAST. AXL and MERTK sequences were obtained from UniProt database (ID: P30530; ID: Q12866 respectively) Uniprot.

Model validation

Structural refinement was further assessed by Structural Analysis

and Verification Server (version3) (nih server) This meta server runs six programs for checking and validating protein structures. Verify3D analyzes the compatibility of an atomic model (3D) with its own amino acid sequence (1D) [22]. The structure was analyzed by Ramachandran's plot using PROCHECK software [23].

Active site validation

In order to evaluate the model catalytic cavity and critical residues involved in AXL active site, molecular docking studies of ATP with the AXL model was performed with AutoDock vina 4.0 [24]. The best conformation with best binding affinity in kcal/mol was selected.

Docking approach

Binding mode and selectivity of AXL kinase with curcumin and natural derivatives were studied by AutoDock vina 4.0 [24] which required the ligand and receptor in pdbqt format and the configuration file as txt format. The AutoDock Vina also produces files containing the ligands docked pose as output which contain the score of the binding affinity, these files were analyzed to study interactions and binding energy of the docking. The results were visualized using Pymol molecular viewer v.1.7.4 schrodinger.

Ligands collection and preparation

Curcumin and two natural analogues namely demethoxycurcumin (DMC), bisdemethoxycurcumin (BDMC) compounds were selected on the basis of their anticarcinogenic activity experimentally proved and metabolic stability in comparison with native curcumin. The structures of these compounds were downloaded from NCBI Pubchem (CID:96516; 5469424; 5315472 respectively) in PDB format. The Pymol was used to verify the presence or absence of hydrogen, the stereochemistry of chiral carbons, number of rotatable bonds, number of hydrogen acceptors and donors.

For all ligands, Gasteiger charges were designated and the torsions for ligands were permitted to rotate during docking procedure using AutoDock tools v.1.5.6. The ligands were saved in pdbqt format.

Target preparation

The receptor was prepared in AutoDock tools v.1.5.6, polar hydrogens were assigned to the receptor, the grid-box was centered around the AXL active site, the dimensions were set at 30,30,30 Å (x, y and z) consisting of active site residues within the box. The receptor was saved in pdbqt format.

Result

Sequence analysis

The amino acid sequence of AXL catalytic domain was retrieved from the Uniprot database. The selected sequence for physicochemical analysis and 3D modeling is given in Figure 1.

Sequence alignment by BLAST showed, 70% identity between a sequence of the AXL catalytic domain and the template.

3D structure prediction and model validation

The 3D structure prediction (Figure 2) was carried out by alignment of target sequences with template structure (PDB ID 3BRB), using a Swiss Model Server. Figure 3 presents the superposition between the constructed model and the template complexed with ADP ligand. Figure 4 shows the VERIFY-3D graph determining that 95.96% of

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>sp|P30530|536-807
VALGKTLGEGEFGAVMEGQLNQQDSSILKVAVKTMKIAICTRSELEDFLSEAVCMKEFDHP
NVMRLIGVCFQGSERESFPAPVVILPFMKHGDLSFLLYSRLGDQPVVLPVPTQMLVKFMAD
IASGMEYLSTKRFIHRDLAARNCLNENMNSVCVADFGLSKKIYNGDYRQRIAKMPVKW
IAIESLADRVTYSKSDVWSFGVTMWEIATRGTPTYPGVENSEIYDYLQGNRLKQPADCL
DGLYALMSRCWELNPNQDRPSFTELRDLENTL
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Figure 1: Amino acid sequence of AXL catalytic domain.

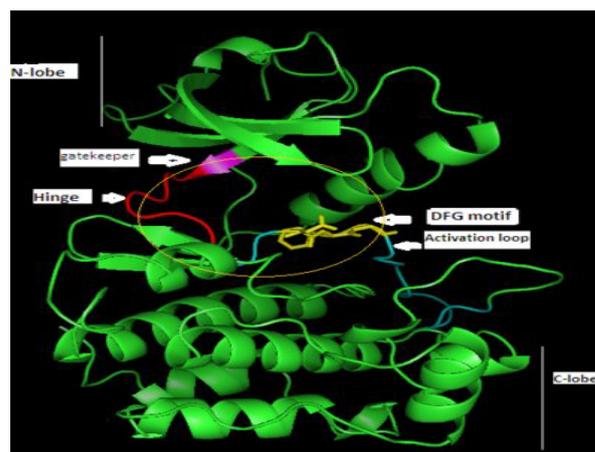


Figure 2: Constructed 3D model of AXL receptor generated by Swiss Model Server.

Notes: The model is oriented with the N-lobe at the top and the hinge is colored in red. The C-lobe is at the bottom of the figure, the activation loop in cyan, gatekeeper in magenta and the active site is surrounded in yellow.

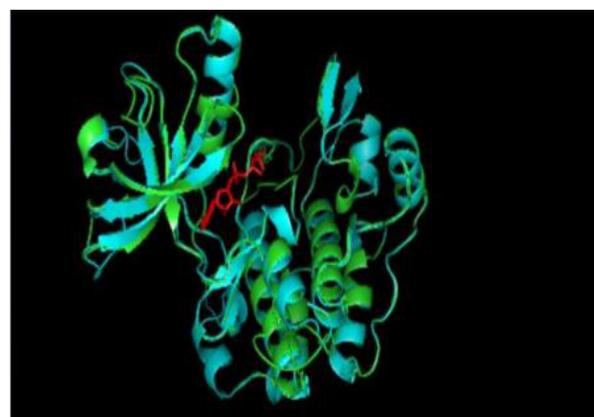


Figure 3: Superposition of AXL (green color) and template MERTK(3 BRB) (cyan color), binding the ADP ligand of template (red color).

AXL residues have an average score of 3D-1D ≥ 0.2 confirming the compatibility of the 3D structure and the primary sequence of AXL.

The stereochemical quality and accuracy of the predicted model was evaluated after the refinement process using the Ramachandran plot calculated with the PROCHECK program [23]. The π and ψ distributions of the Ramachandran plots of non-glycine, non-proline residues are represented in Figure 5, showing 93.3% (252 amino-acids) of residues in the favoured regions, 6.3% (17 amino acids) in allowed region and 0.4% (1 amino acids) in outlier region Figure 6 shows the residue phenylalanine (Phe613) found in the outlier region is at very long distances from the pocket suggesting that this amino

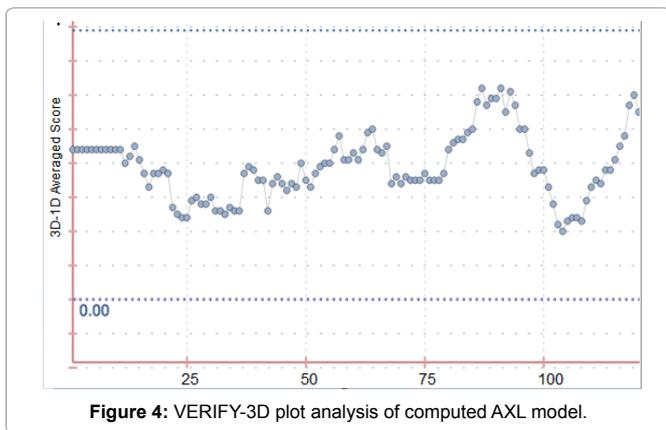


Figure 4: VERIFY-3D plot analysis of computed AXL model.

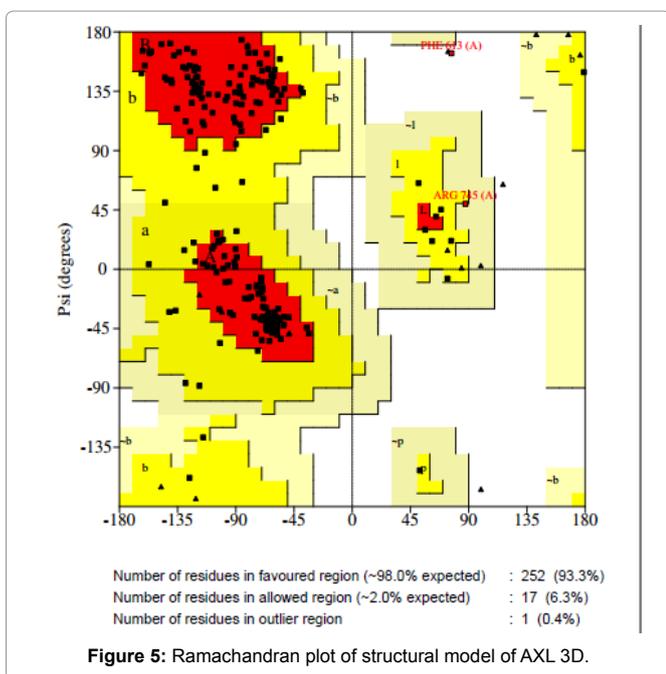


Figure 5: Ramachandran plot of structural model of AXL 3D.

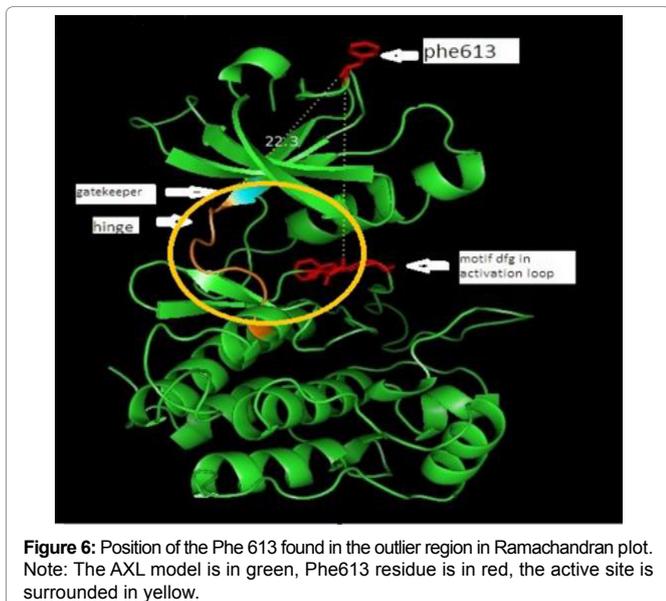


Figure 6: Position of the Phe 613 found in the outlier region in Ramachandran plot. Note: The AXL model is in green, Phe613 residue is in red, the active site is surrounded in yellow.

acid is structurally and functionally irrelevant to the ligand-binding site.

Active site validation by ATP

Among the nine conformations results of docking between AXL kinase and ATP, we have selected the best binding position with an energy of -8.7 kcal/mol. Figure 7 shows that AXL kinase interacts with ATP principally by seven hydrogen bonds which are summarized in Table 1.

Interaction between AXL and curcumin compounds

The 2D structures of curcumin compounds used in this study are illustrated in Figure 8. As showed in Figure 9(a,b), the three compounds occupied the common binding site of the AXL kinase. The binding energy of AXL- curcumin, AXL-DMC and AXL-BDMC complexes was respectively -9.8 kcal/mol, -9.4 kcal/mol and -9.0 kcal/mol. Table 2 resemble the binding affinity and the hydrogen bond distances of the key residues that interact with inhibitors.

Figures 10 (a, b and c) shows that all curcuminoids formed a hydrogen bonds with the same residues, Met 623, Lys 567, Ala 689 and Asp 690. The Met 623 of the hinge region makes a hydrogen bonds of 2.3Å, 2.7Å and 2.4Å between their nitrogen backbone with curcumin,

Table 1: Molecular docking results of AXL receptor with ATP using Vina docking software.

Residues	H Bond length (Å)
Glu 546	3.0
Lys 567	1.9
Lys 624	2.1
Asp 627	2.4
Arg676	3.3
Asn677	2.1
Asp690	3.3

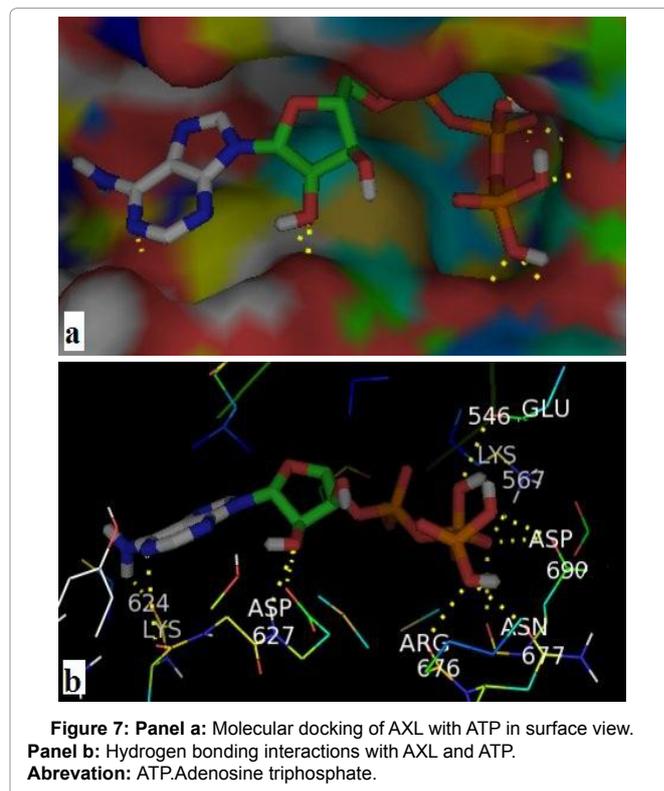


Figure 7: Panel a: Molecular docking of AXL with ATP in surface view. Panel b: Hydrogen bonding interactions with AXL and ATP. Abreviation: ATP.Adenosine triphosphate.

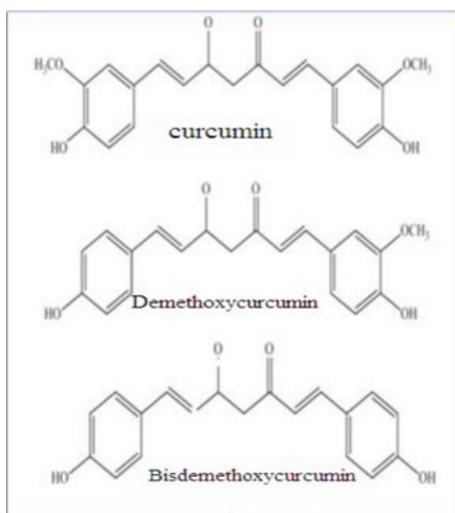


Figure 8: 2D Structures of curcumin natural derivatives.

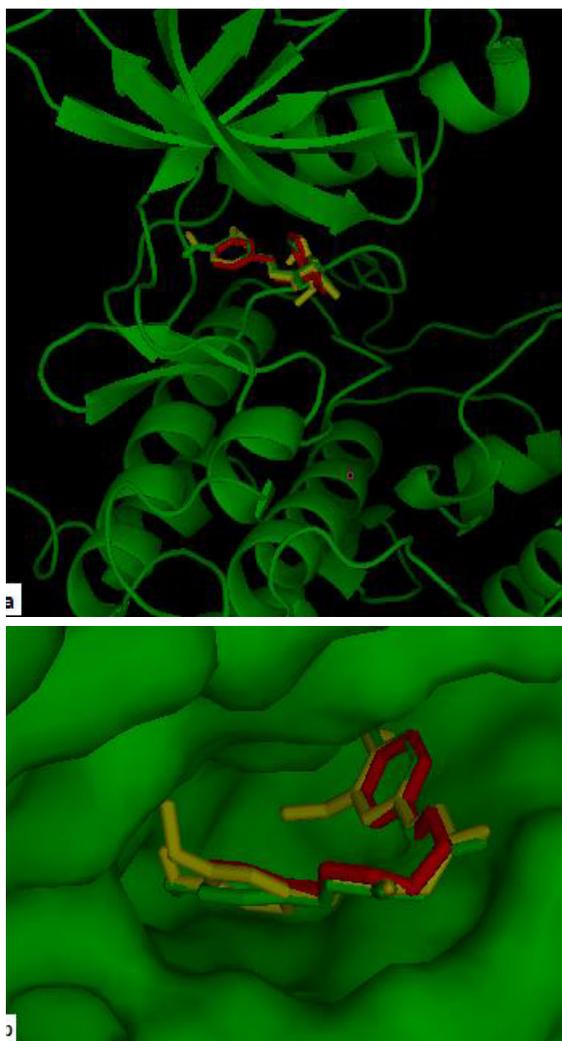


Figure 9: Panel a: ligand binding pose of curcumin (yellow color), BDMC (green color), and DMC (red color) superimposed in the active site of AXL. Panel b: ligand binding pose of curcumin (yellow color), BDMC (green color), and DMC (red color) superimposed in the active site of AXL in surface view.

Table 2: The binding affinity and the hydrogen bond distances of the key residues that interact with inhibitors.

Ligands docked With AXL	binding affinity Kcal /mol	Interaction amino acid Residues	H Bond distance (Å)
Curcumin	-9.8	Lys567	2.27
		Met623	2.30
		Ala689	2.50
		Asp690	3.122
Demethoxycurcumin (DMC)	-9.4	Lys 567	1.3
		Met623	2.7
		Ala689	3.2
		Asp690	3.4
Bisdemethoxy curcumin (BDMC)	-9.0	Lys 567	2.20
		Met 623	2.40
		Ala 689	3.26
		Asp690	3.06

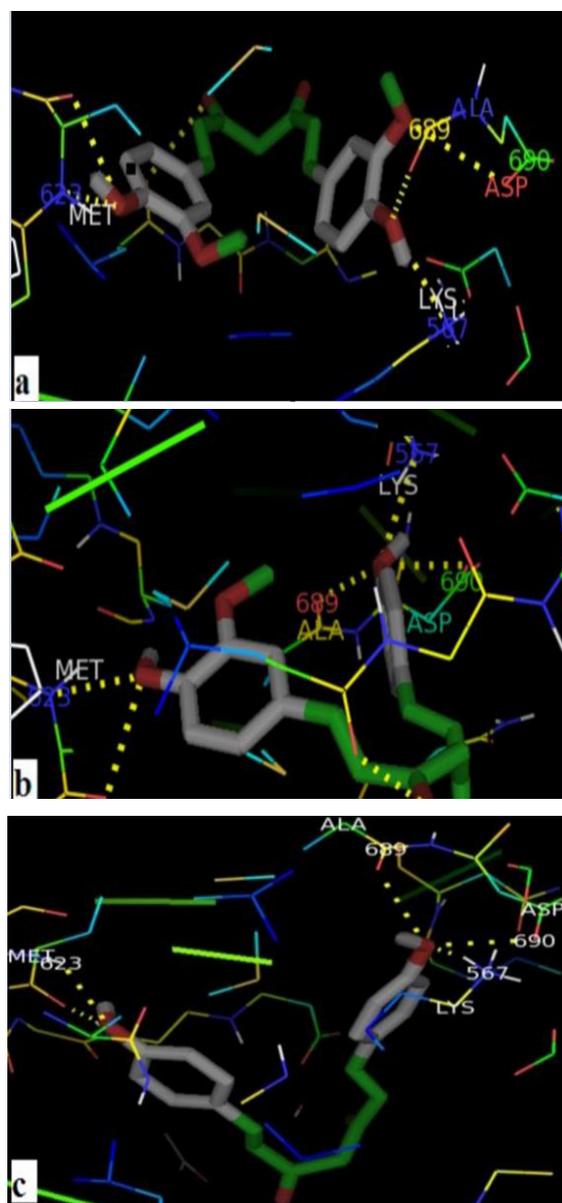


Figure 10: Panel a: Hydrogen bonded interactions of curcumin against AXL protein. Panel b: Hydrogen bonded interactions of DMC against AXL protein. Panel c: Hydrogen bonded interactions of BDMC against AXL protein.

DMC and BDMC respectively. The Lys 567, one of the binding site key residues of ATP, interacts with curcuminoid compounds, the shorter one have a distance of 1.3 Å in their interaction with DMC. The shorter distance between Ala 689 of the activation loop and curcumin compound was 2.5 Å. Furthermore, the three compounds possess a hydrogen bond with DFG motif by their Asparatic acid residue. The hydrogen bonds were created between the side chain of Asp690 and curcumin, DMC and BDMC have a distance of 3.12Å, 3.4Å and 3.06Å respectively.

Discussion

In this paper, we used the *in Silico* approach and molecular docking to explain the binding mechanism of curcumin and its natural derivatives (DMC, BDMC) to the AXL kinase. We generated a model of AXL kinase using the MERTK as a template for the Swiss Model software and the model was evaluated by Verrify 3D and PROCHECK programs. The model validation indicated the acceptable quality of the structural model with 99.6% of the model's torsion angles in favorable positions. The molecular docking did confirm that the ATP molecule is positioned in their AXL kinase binding site. Thereafter, we studied the curcuminoids interaction with the AXL kinase to demonstrate if these molecules hold a position inside of the ATP binding site, the results obtained from the molecular docking demonstrated that the selected phytoconstituents are positioned into the active cavity of AXL and exhibits good binding mode with AXL.

The binding affinity of the curcuminoids with AXL is well above that of ATP. The curcuminoids create hydrogen bonds with three of the key residues in ATP binding site. The first one was with Met 623 of the hinge region; the second was with Lys 567 positioned on a β -sheet of N-lobe, and the third with Asp 690 of the DFG-motif. Curcumin was found to bind with the best affinity with AXL than DMC and BDMC. The difference in affinity may be due to the number of methoxy groups on the aromatic rings in the curcuminoids, both natural derivatives are two congeners of curcumin isolated from turmeric, furthermore curcumin has two symmetric phenyl-methoxy groups, DMC contains one and BDMC contains none. This suggests that the phenyl methoxy groups do contribute to the inhibiting power of compounds *Chen and al* demonstrated that the activity is also due to the ortho-methoxy phenolic functionality [25].

Curcumin, together with demethoxycurcumin and bisdemethoxycurcumin, are the three predominant active compounds derived from the turmeric root. Several reports have shown that curcumin could modulate each stage of cancer such as initiation, promotion and progression [26] Kim *et al.* indicate that curcumin has inhibitory effects on AXL expression as Gas6-dependent AXL phosphorylation, and AXL promoter activity in non-small cell lung cancer cells [27]. The present results will be the basis to develop new compounds ATP- competitive more selective and active against AXL kinase, derived from the curcumin molecule. These results encouraged us to perform the ongoing structural-activity relationship studies to evaluate the relationship of the methoxy-groups and the inhibitory effect against AXL.

Conclusion

Traditionally, the turmeric has been used commonly as a spice in curries to give a specific flavor and yellow color. This phytochemical and its derivatives have also been found to be effective against different cancers. Our results showed that these compounds have a strong binding affinity to the AXL receptor and could be useful as a source of

potential therapeutics or lead molecules for prevention or treatment of multiple cancers. Curcumin has a promising cytotoxic effects against cancer cell lines. But, further clinical research about the toxicity of the molecule is needed to bring in more clarity on human safety and how their activity could be exercised *in vivo* after human consumption.

Competing Interests

The authors declare that they have no competing interests.

Aknowledgments

This work was carried out under National funding from the Moroccan Ministry of Higher education & Scientific research (PPR program) to AI. This work was also supported by a grant from the NIH for H3Africa BioNet to AI.

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