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Research Article

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Pharmacophore modelling of 2-chlorobenzimidazole and its specific docking to the active site c-Met Kinase: A search for potent c-Met Kinase inhibitor

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Abstract In this paper, we demonstrate how pharmacophore modelling was used to design the analogues of 2-chlorobenzimidazole and the application of molecular docking studies in the evaluation of the ligand affinity for the target. The lead molecule (1-benzyl-2-chloro-1*H*-benzimidazole) had the highest docking score of -8.0 kcal/mol and was observed to interact with 17 amino acids within the pocket of c-Met Kinase (ALA159, VAL39, TYR32, MET144, ASN142, ARG141, ALA154, ASP155, ALA49, LYS51, LEU90, TYR92, MET93, GLY32, ILE, ASP97 and GLY96). Meanwhile, the ADME characteristics of 1-benzyl-2-chloro-1*H*-benzimidazole showed approving properties of the lead molecule. Hence, we recommend 1-benzyl-2-chloro-1*H*-benzimidazoleas promising candidates with high potential to inhibit c-Met Kinase.

Keywords molecular docking, ADMET, bioactive compound, c-Met Kinase, 2-chlorobenzimidazole

Introduction

The genus *Melia dubia* belongs to family Meliaceae is widely found in India, Srilanka, Malaysia, Australia and Angola [1]. This tree plant is about 25 m tall, it is known to flower around March and produce fruit in April [2]. *Melia dubia* is a medicinal plant known to house Huge amount of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene lactones and oil. Hence, *Melia dubia* may contain secondary metabolites having essential bioactive characteristics that can be used in health care. On the other hand, report on this tree plant revealed that different parts of *Melia dubia* showed pharmacological activities like hepatoprotective activity, antiulcer activity, antimicrobial activity, anti-inflammatory activity, antifeedant activity, analgesic activity, anti-urolithic activity, antidiabetic activity, larvicide activity, anticancer activity, antibacterial activity, ovicidal and biopesticidal activity was reported [1, 3].

c-Met is a tyrosine kinase receptor that plays vital role in cell proliferation, migration, and invasion [4]. Apparently, when deregulated, the c-Metpath way leads to tumorigenesis and metastasis[5]. Meanwhile, an investigation carried out on MET gene, revealed that mutations in the c-Met kinase domain have been associated with human cancers [6]. Hence, the inhibition of c-Met is considered as an innovative therapeutic intervention against tumorigenesis and metastasis [7]. In other to meet the need for a novel therapeutic agent with the capacity to impede tumorigenesis, it is imperative to employ isolated bioactive compounds from medicinal plants as a therapeutic agent. The extraction,

separation, and characterization of a bioactive compound from *Melia dubia* plant leaves were performed and 2-chlorobenzimidazole was reported to be significantly present in the plant extract [8].

We herein report the pharmacophore modelling of 2-chlorobenzimidazole and molecular docking simulation of the modelled ligands against c-Met Kinase (ID: 3cd8). The drug-likeness, ADME (Adsorption, Distribution, Metabolism and Excretion) and pharmacokinetics characteristics of the lead model ligand was also investigated.

Methodology

Receptor and Ligand Preparation

The chemical structure of the 2-chlorobenzimidazole and its analoguewere built using ACD/ChemSketch 2018.2.5 Freeware version. The 2D conformation of 2-chlorobenzimidazole-analogs was designed by the addition of different functional group to the nitrogen atom at position 1 (see Fig 1). The functional groups attached to the nitrogen are expected to alter the biological activity of the ligands on the target receptor. The chemical structures of 2-chlorobenzimidazole and its analogue were optimized using the MMF94 force field on Avogadro interface [9]. The dock-prep tools on the UCSF Chimera interface were used to prepare chemical structures before the molecular docking step. The crystallized structure of c-Met Kinase in complex triazolopyridazines inhibitor having 2.00 Å resolution was retrieved from the Protein Data Bank (https://www.rcsb.org) with ID: 3cd8. The crystallized structure consists of a single distinct chain A bounded to small chemical residues (L5G). There after, the preparation of the receptor (3cd8) was performed on the UCSF Chimera interface [10].

Molecular Docking

All the molecular docking experiments were achieved using AutoDock Vina software [11]. This docking tool offers better accuracy in predicting ligand-protein interaction, it also gives better accuracy for ligand consisting of more than 20 rotatable bonds and shorter docking duration. This could be attributed to its multiple core processors. Specific docking of the 2-chlorobenzimidazole and its analogue to the active site c-Met Kinase (ID: 3cd8) was achieved by generating a grid box coordinate of the ligand to be substituted on the receptor. The grid box that defines the pocket of ID: 3cd8 protein was designed by making use of AutoDock Vina functionality on UCSF Chimera interface [10]. The grid box size and centre coordinates for the 3cd8 were x(21.9998, 10.00), y (10.9379,11.4149) and z (56.9596,7.7653)respectively. The 2-chlorobenzimidazole-analogue with the highest binding affinity for the c-Met Kinase (ID: 3cd8) was selected for further *in silico* ADME assay.

Validation and ADME analysis of lead molecule

The drug-likeness, solubility uniqueness and pharmacokinetics characteristics of the lead molecule selected among the analogues of 2-chlorobenzimidazolewere assessed using the online web server, SWISS-ADME (Adsorption, Distribution, Metabolism and Excretion) (https://www.swissadme.ch).

Results and Discussion

An intensive effort has been made to find the therapeutic agent for cancer, unfortunately, there is no drugs/vaccine available to efficiently combat its spread. Hence, it is imperative to find a cost-effective anticancer drug. An efficient approach to the discovery of novel anticancer agent is to reposition phytochemicals sourced from folk medicine as a therapeutic agent for the treatment of cancer. The conventional drug discovery methods are very expensive, time-taking and less efficient (Hui et al., 2020). Considering the aforementioned facts, the present study mainly focused on the pharmacophore modelling of 2-chlorobenzimidazole (see Fig 1) and specific molecular docking of the analogues against c-Met Kinase catalytic site (see Fig 2).

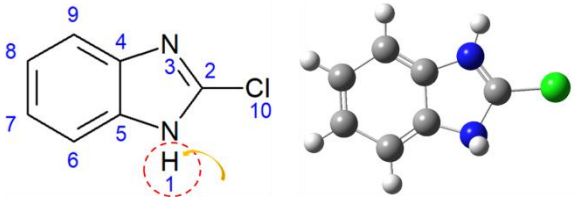


Figure 1: The 2D and 3D chemical structure of 2-chlorobenzimidazole

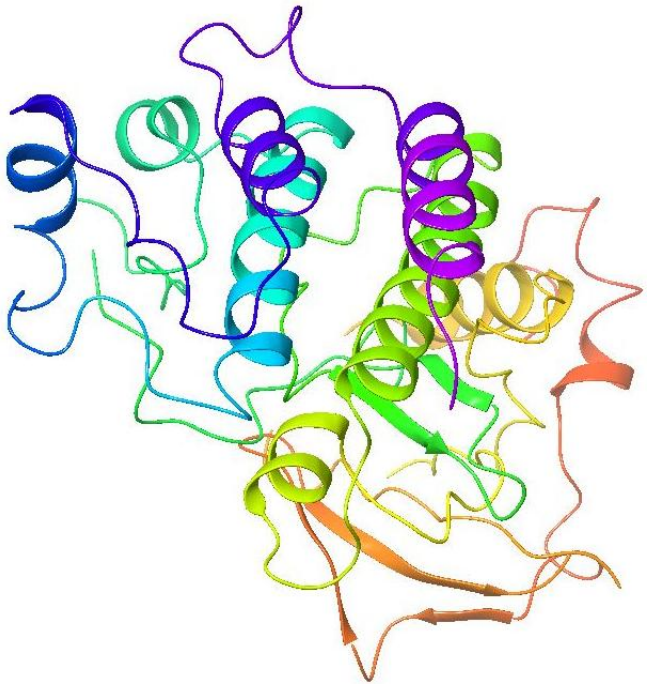
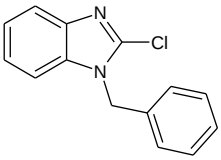
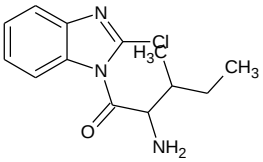
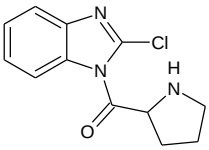
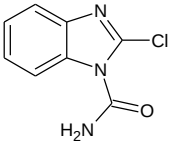
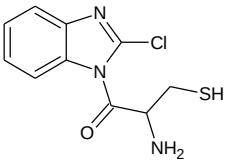
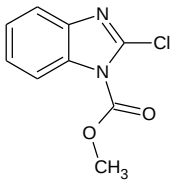
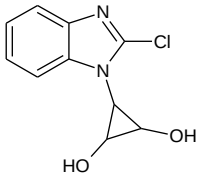
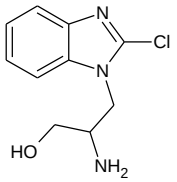
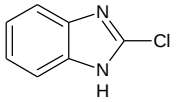


Figure 2: The crystalized structure of c-Met Kinase (ID: 3cd8)

Table 1: 2D representation of 2-chlorobenzimidazole-derivatives and their corresponding Glide score (G-Score) value calculated for the related query

Code	Score *ΔG (Kcal/mol)	Structure
CBD1	-8.0	
CBD2	-7.8	
CBD3	-7.4	
CBD4	-7.1	
CBD5	-6.9	

CBD6	-6.7	
CBD7	-6.7	
CBD8	-6.4	
2-chlorobenzimidazole	-6.0	

Molecular Docking

By using Autodock Vina tools, molecular docking was performed tofind out the best candidates among the analogues of 2-chlorobenzimidazole based on their binding scores. Binding scores of CBD1 (1-benzyl-2-chloro-1*H*-benzimidazole), CBD2 (2-amino-1-(2-chloro-1*H*-benzimidazol-1-yl)-3-methylpentan-1-one), CBD3 (2-chloro-1*H*-benzimidazol-1-yl)(pyrrolidin-2-yl)methanone), CBD4 (2-chloro-1*H*-benzimidazole-1-carboxamide) CBD5 (2-amino-1-(2-chloro-1*H*-benzimidazol-1-yl)-3-sulfanylpropan-1-one), CBD6 (methyl 2-chloro-1*H*-benzimidazole-1-carboxylate), CBD7 (3-(2-chloro-1*H*-benzimidazol-1-yl)cyclopropane-1,2-diol), CBD8 (2-amino-3-(2-chloro-1*H*-benzimidazol-1-yl)propan-1-ol) and 2-chlorobenzimidazole were estimated as -8.0 kcal/mol, -7.8 kcal/mol, -7.4 kcal/mol, -7.1 kcal/mol, -6.9 kcal/mol, -6.7 kcal/mol, -6.7 kcal/mol, -6.4 kcal/mol and -6.0 kcal/mol respectively (see Table 2). A comparison of the minimum energies showed that CBD1 had the highest affinity for c-Met Kinase, hence, was selected as the lead molecule. The lead molecule was observed to interact with 17 amino acids within the pocket c-Met Kinase and they include ALA159, VAL39, TYR32, MET144, ASN142, ARG141, ALA154, ASP155, ALA49, LYS51, LEU90, TYR92, MET93, GLY32, ILE, ASP97 and GLY96. This amino acid residues are positively and negatively charged, polar and predominantly hydrophobic. The lead molecule was noticed to have some degree of solvent exposure within the pocket of c-Met Kinase indicating an ideal environment for biochemical interactions. The modelled ligand (2-chlorobenzimidazole) was observed to interact with 12 amino acid within the pocket of the receptor. The lead molecule was better exposed within the sphere of the pocket than 2-chlorobenzimidazole, hence, the high the affinity of -benzyl-2-chloro-1*H*-benzimidazole), CBD2 (2-amino-1-(2-chloro-1*H*-benzimidazol-1-yl)-3-methylpentan-1-one for c-Met Kinase. Meanwhile, the three-dimensional representation of intermolecular ligand-receptor interaction of all the analogues isdisplayed in Figures 3 to11.

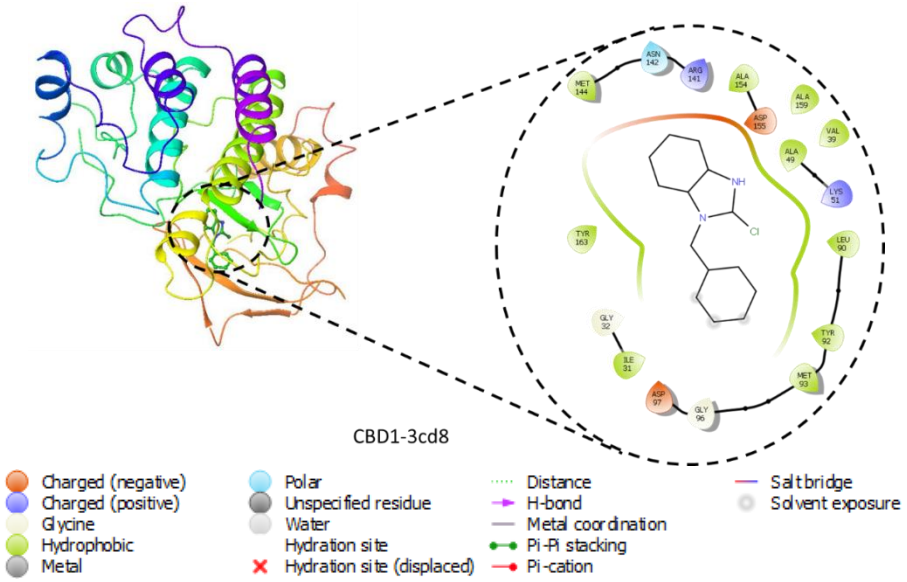


Figure 3: The3D X-ray crystal structure of 3cd8 complex with CBD1showing also the binding site region and the residues that constitute this binding site region

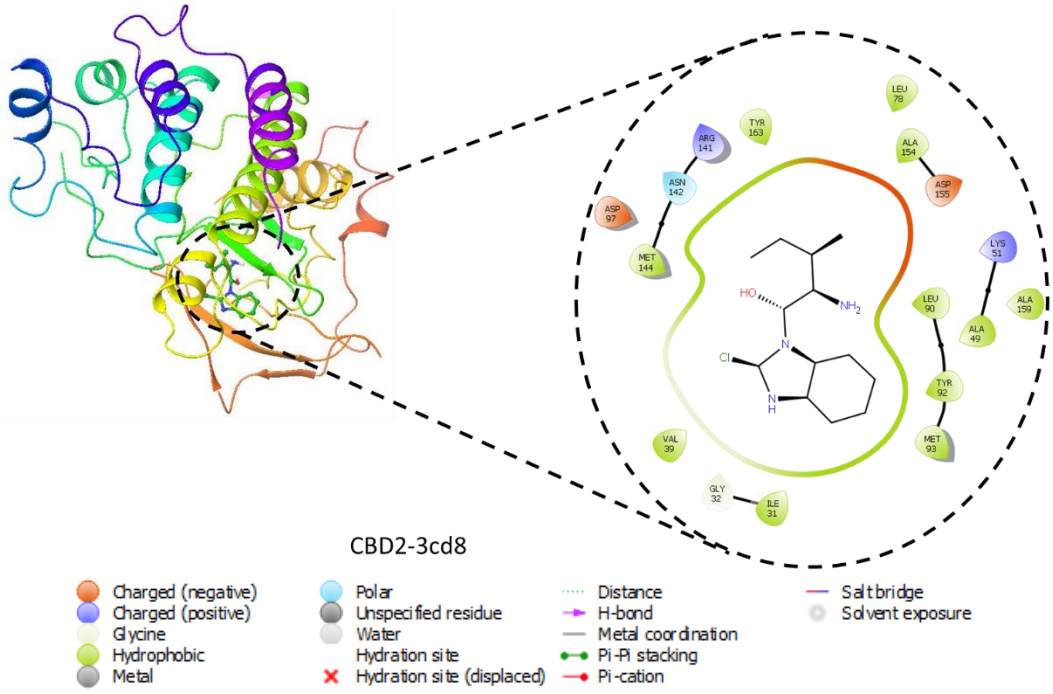


Figure 4: The3D X-ray crystal structure of 3cd8 complex with CBD2 showing also the binding site region and the residues that constitute this binding site region

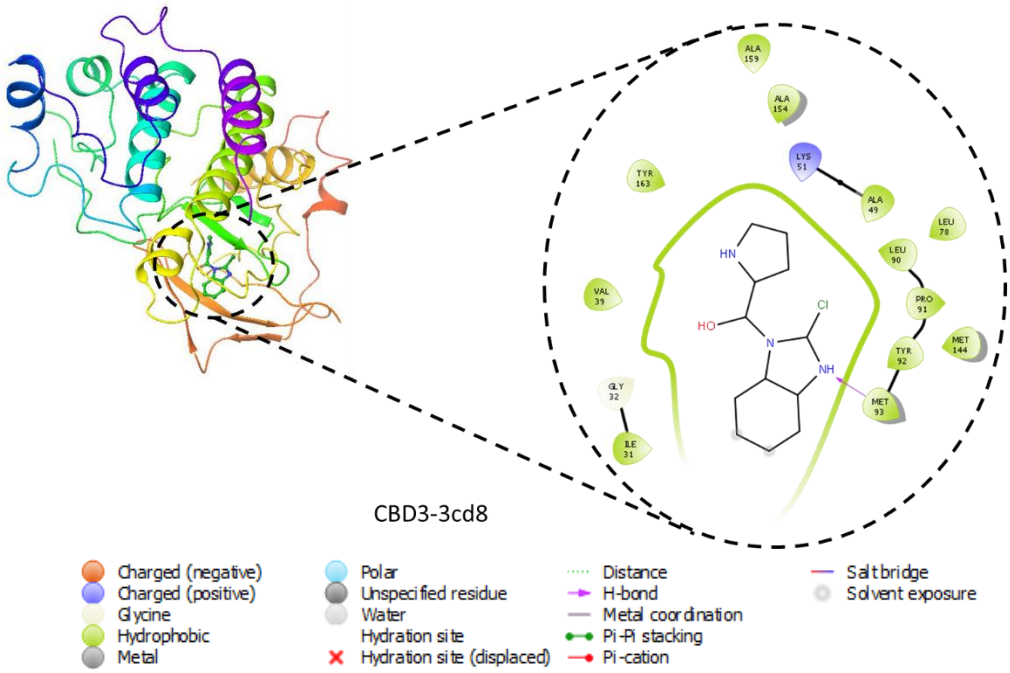


Figure 5: The 3D X-ray crystal structure of 3cd8 complex with CBD3 showing also the binding site region and the residues that constitute this binding site region

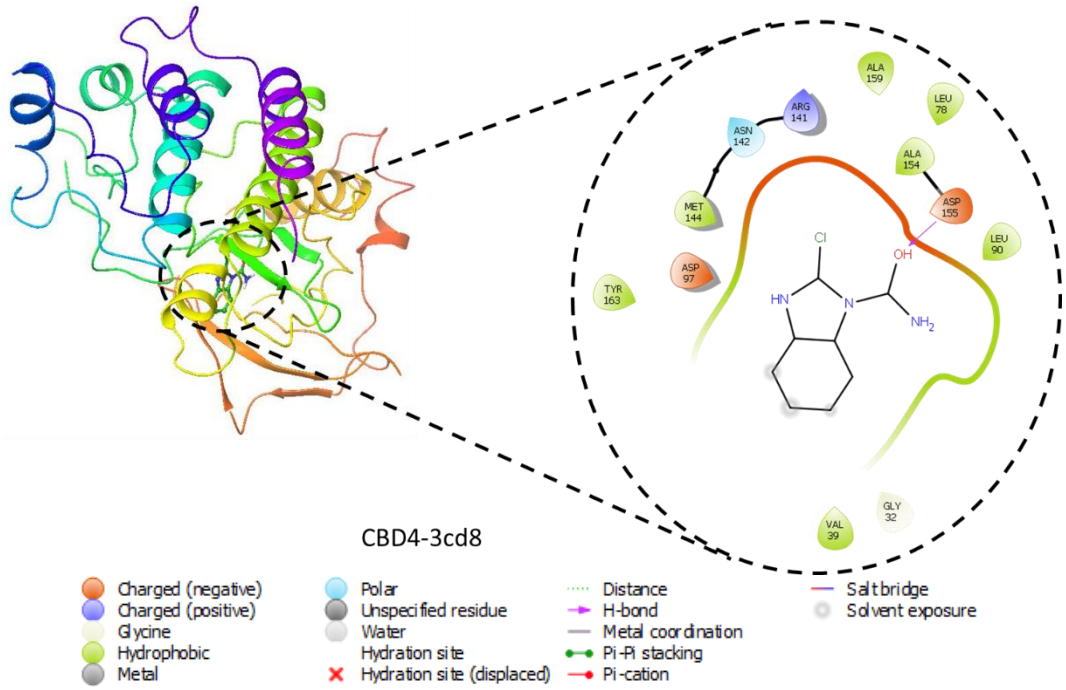


Figure 6: The 3D X-ray crystal structure of 3cd8 complex with CBD4 showing also the binding site region and the residues that constitute this binding site region

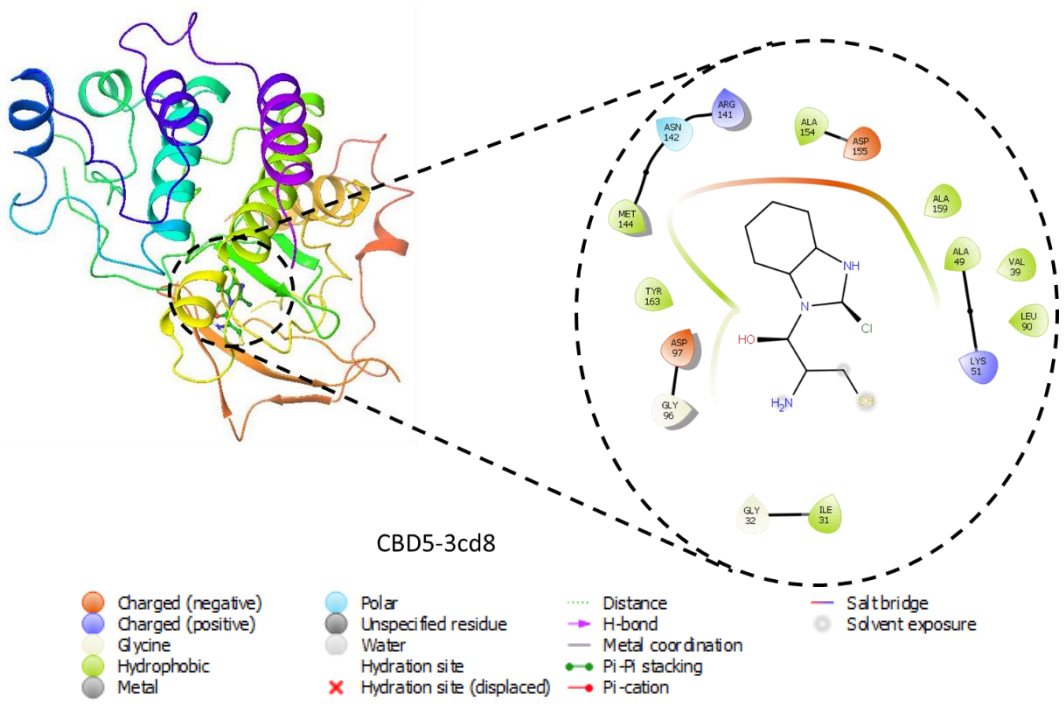


Figure 7: The 3D X-ray crystal structure of 3cd8 complex with CBD5 showing also the binding site region and the residues that constitute this binding site region

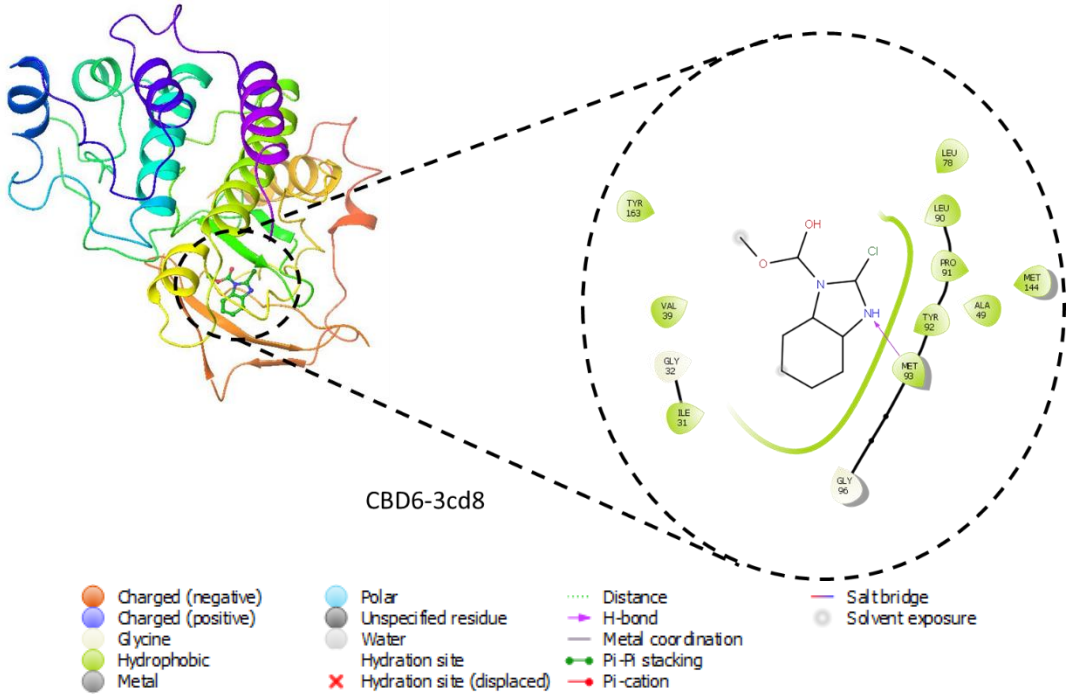


Figure 8: The3D X-ray crystal structure of 3cd8 complex with CBD6 showing also the binding site region and the residues that constitute this binding site region

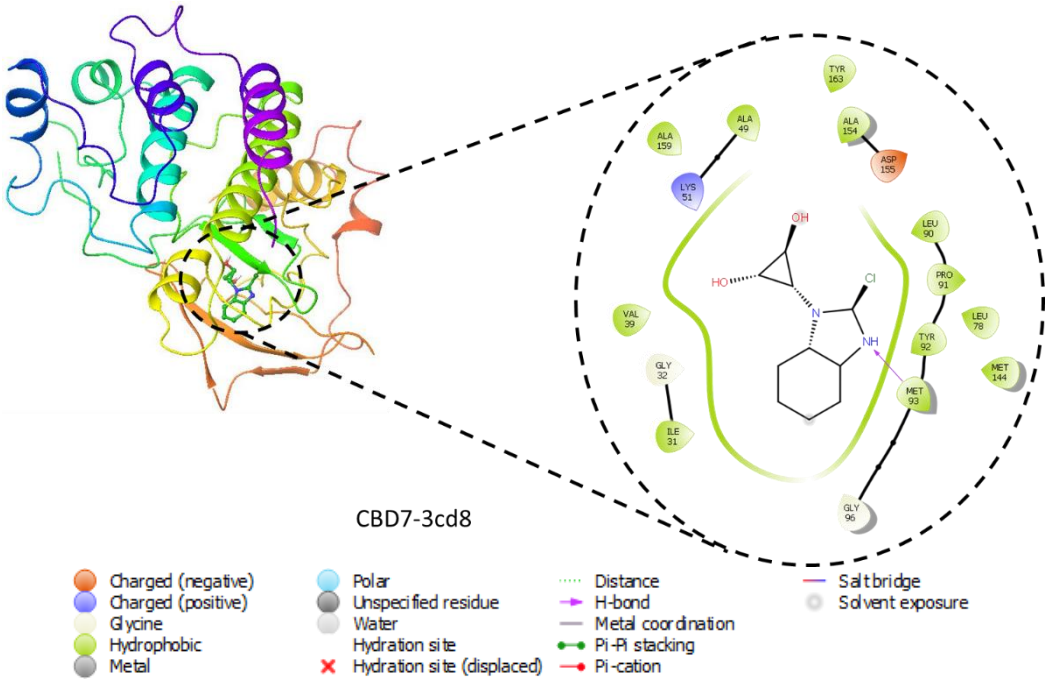


Figure 9: The 3D X-ray crystal structure of 3cd8 complex with CBD7 showing also the binding site region and the residues that constitute this binding site region

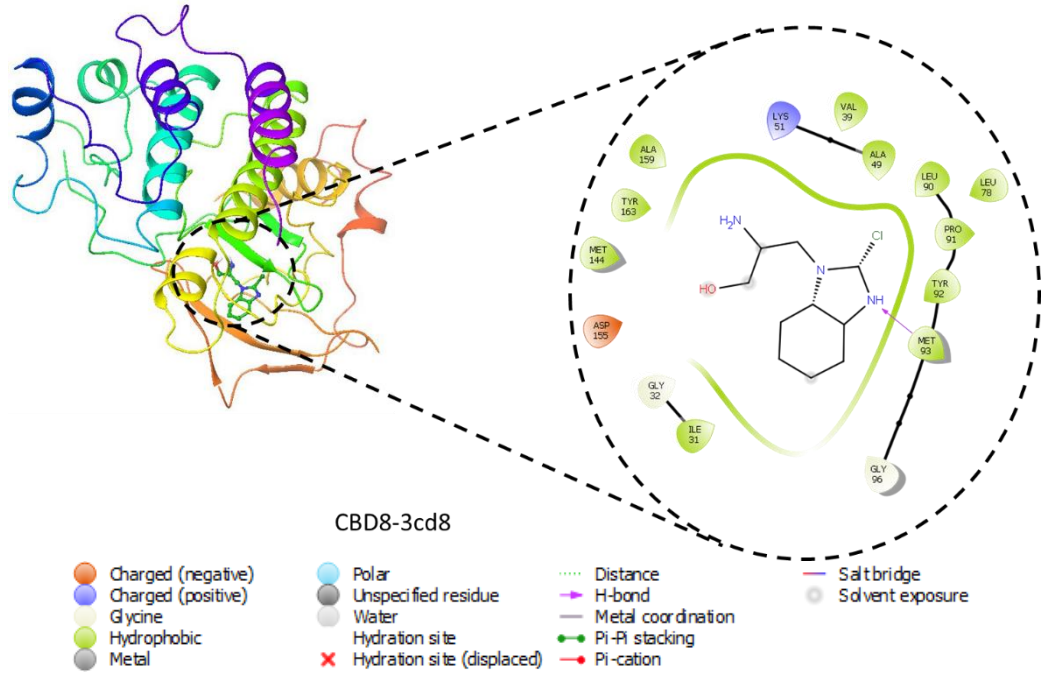


Figure 10: The 3D X-ray crystal structure of 3cd8 complex with CBD8 showing also the binding site region and the residues that constitute this binding site region

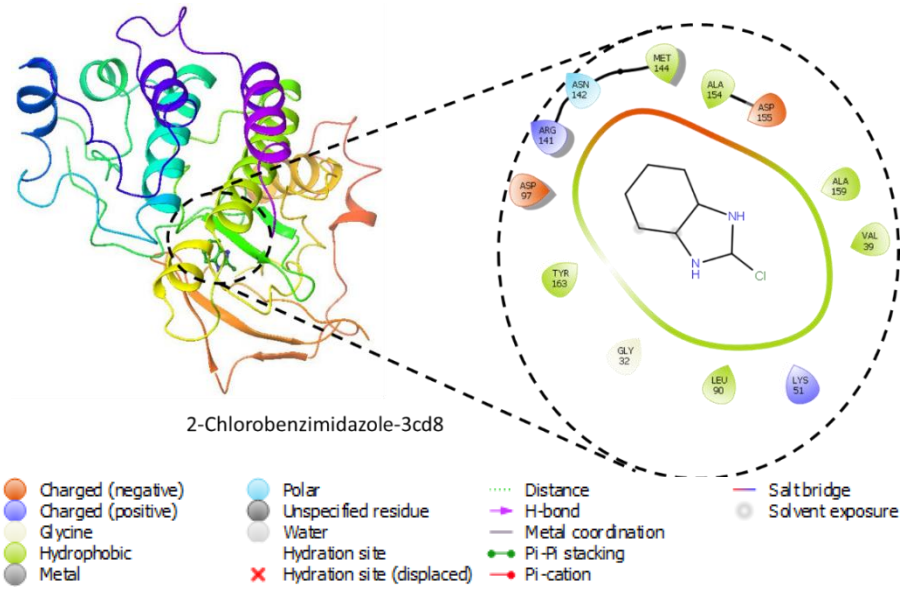


Figure 11: The 3D X-ray crystal structure of 3cd8 complex with 2-chlorobenzimidazoleshowing also the binding site region and the residues that constitute this binding site region.

ADME assessment of potential c-Met Kinase -3 inhibitor

The physicochemical space employed for the ADME prediction of 1-benzyl-2-chloro-1*H*-benzimidazole is displayed in the coloured zoneof the bioavailability radar (see Fig 12).

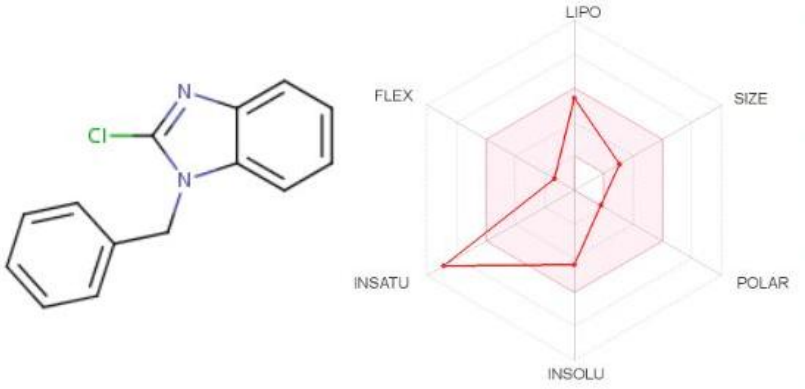


Figure 12: The bioavailability radar of 1-benzyl-2-chloro-1H-benzimidazoleusing Swiss ADME predictor

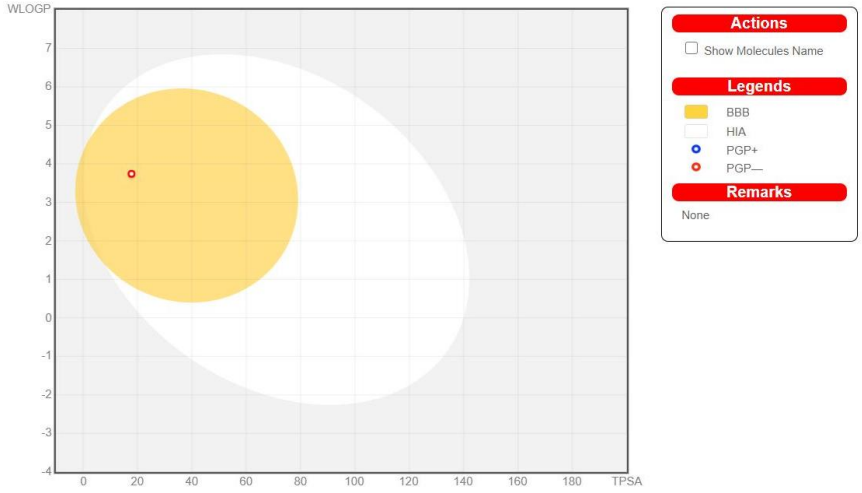


Figure 13: Molecule falling in egg's yolk prediction of 1-benzyl-2-chloro-1H-benzimidazole

The ADME prediction of the lead molecule was (1-benzyl-2-chloro-1*H*-benzimidazole) was obtained using SWISSADME online webserver. The physiochemical properties of the 1-benzyl-2-chloro-1*H*-benzimidazoleinclude: 17 heavy atoms, 1 hydrogen bond acceptors, 0 hydrogen bond donors, molar refractivity of 70.49 and topological polar surface area (TPSA) of the molecule is found to be 17.82 $Å^2$. On the other hand, the lipophilicity of 1-benzyl-2-chloro-1*H*-benzimidazole was estimated and the parameters obtained include: iLOGP is 2.50, XLOGP3 is 4.00, WLOGP is 3.74, MLOGP is 3.33, SILICOS-IT is 3.49 and Consensus P0/W is 3.41.

Table 2: Water solubility of 1-benzyl-2-chloro-1*H*-benzimidazole.

Log *S* (ESOL)		-4.39
	Solubility	$9.99e^{-03}$ mg ml^{-1}; $4.11e^{-05}$ mol ml^{-1}
	Class	Moderately soluble
Log *S* (Ali)		-4.08
	Solubility	$2.04e^{-02}$ mg ml^{-1}; $8.39e^{-5}$ mol ml^{-1}
	Class	Moderately soluble
Log *S* (SILICOS-IT)		-5.63
	Solubility	$5.70e^{-04}$ $mgml^{-1}$; $2.35e^{-06}$ mol ml^{-1}
	Class	Moderately soluble

Meanwhile, -[4-(diethylamino)butyl]-5-(prop-2-en-1-yl)phenol was observed to obeys Lipinski's Ghose, Egan, Veber and Muegge rules with no violation (see Table 4). It also obeys drug-likeness score rules such as and with 0.55 Bioavailability score. The blood-brain barrier (BBB) permeant and gastrointestinal absorption (GI) were feasible and high, respectively. Hence, for clarity, the BBB and GI were better demonstrated using the molecule falling in egg's yolk model (see Fig 13). As listed in Table 2, the solubility of 1-benzyl-2-chloro-1*H*-benzimidazole was moderately soluble in all the class solvent employed for the assay.Isoenzymes such asCYP1A2, CYP2C19 and CYP2D6 were inhibited by 1-benzyl-2-chloro-1*H*-benzimidazole. On the contrary, CYP3A4and CYP2C9 isoenzymes were not inhibited by the lead molecule. This indicates the possibility of drug-drug interaction that may lead to cytotoxicity. Hence, it is imperative to extensively evaluate 1-benzyl-2-chloro-1*H*-benzimidazole as a prospective therapeutic agent.

Table 4: Pharmacokinetics of 1-benzyl-2-chloro-1*H*-benzimidazole.

GI adsorption	High
BBB permeant	Yes
P-gp substrate	No
CYP 1A2	Yes
CYP2C19	Yes
CYP2C9	No
CYP2D6	Yes
CYP3A4	No
Log Kp (skin permeation)	-4.940 cm s^{-1}

Table 5: Druglikeness of 1-benzyl-2-chloro-1*H*-benzimidazole.

Lipinski	Yes, 0 violation:
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability score	0.55

Conclusion

The inhibition of c-Met Kinase protein with 2-chlorobenzimidazole and its analogue was successfully studied using pharmacophore modelling and molecular docking approach. 1-benzyl-2-chloro-1*H*-benzimidazole was noticed to have the highest minimum energy and was selected as the lead molecule. Further *in silico* ADME assay of the lead molecule, offered 71-benzyl-2-chloro-1*H*-benzimidazole as a probable drug candidate for any disease cancer. Hence, more focus should be channelled to study the *in-vivo* mode of 1-benzyl-2-chloro-1*H*-benzimidazole as a prospective therapeutic agent for cancer.

Acknowledgement

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Declaration of interest

The authors declare no conflict of interest.

References

[1]. Valentina, P., et al., *Preliminary phytochemical analysis and biological screening of extracts of leaves of Melia dubia Cav.* Int. J. Res. Ayurveda Pharm, 2013. 4(3): p. 417-419.

[2]. Gopal, V., G. Prakash Yoganandam, and P. Manju, *A concise review on melia dubia Cav.(Meliaceae).* Euro J Environ Ecol, 2015. 2: p. 57-60.

[3]. Murugesan, S., et al., *Phytochemical characterization of Melia dubia for their biological properties.* Der Chemica Sinica, 2013. 4(1): p. 36-40.

[4]. Giordano, S., et al., *Tyrosine kinase receptor indistinguishable from the c-met protein.* Nature, 1989. 339(6220): p. 155-156.

[5]. Birchmeier, C., et al., *Met, metastasis, motility and more.* Nature reviews Molecular cell biology, 2003. 4(12): p. 915-925.

[6]. Dharmawardana, P.G., A. Giubellino, and D.P. Bottaro, *Hereditary papillary renal carcinoma type I.* Current molecular medicine, 2004. 4(8): p. 855-868.

[7]. Albrecht, B.K., et al., *Discovery and optimization of triazolopyridazines as potent and selective inhibitors of the c-Met kinase.* Journal of medicinal chemistry, 2008. 51(10): p. 2879-2882.

[8]. Jeyaleela, G.D., et al., *Isolation of 2-Chlorobenzimidazole from Melia Dubia Leaf Extract and Its Structural Characterisation.* Mass spectrometry, 2017. 9: p. 10.

[9]. Hanwell, M.D., et al., *Avogadro: an advanced semantic chemical editor, visualization, and analysis platform.* Journal of cheminformatics, 2012. 4(1): p. 17.

[10]. Pettersen, E.F., et al., *UCSF Chimera—a visualization system for exploratory research and analysis.* Journal of computational chemistry, 2004. 25(13): p. 1605-1612.

[11]. Morris, G.M., et al., *Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function.* Journal of computational chemistry, 1998. 19(14): p. 1639-1662.