Binding Energy Prediction and Molecular Docking Studies of Falcarindiol and its Monosubstituted Analogues Against *Aspergillus fumigatus* Chitinase; The In *Silico* Pharmacokinetics

O.A Durojaye (lanre.durojaye@yahoo.com), P.S Ajuluchukwu (pajuluchuku@yahoo.com), S. Cosmas (Cos242@yahoo.com), Robert Igomu (igomur@yahoo.com), I.U Okagu (okagu.innocent@yahoo.com), S. B Sani (shaddysani@gmail.com).

Abstract

Background: *Aspergillus fumigatus* is the most common causative agent of invasive fungal infection in immunosuppressed individuals. These include patients receiving immunosuppressive therapy for autoimmune or neoplastic disease, organ transplant recipients, and AIDS patients. *A. fumigatus* is the primary cause of invasive infection in the lungs and represents a major cause of morbidity and mortality in infected individuals. Additionally, *A. fumigatus* can cause chronic pulmonary infections, allergic bronchopulmonary aspergillosis, or allergic disease in immunocompetent hosts. Falcarindiol is a polyacetylene found in carrot roots which has antifungal activity.

Materials and Methods: A molecular docking study was carried out on ten analogous structurally diverse falcarindiol against *Aspergillus fumigatus* chitinase using the Autodock Vina software. Extensive structure activity relationship study was also carried out with these molecules. The physicochemical analysis, lipophilicity, solubility, pharmacokinetics and Lipinski druglikeness of falcarindiol and its analogues were evaluated. These molecules were designed by substituting one of the OH functional groups of the falcarindiol with C=O, C2H5, CH3, CHO, COOH, C3H6O2, C4H8O2, NH2, OCH3 and C3H6O groups. The scoring function (empirical binding free energy) was used to estimate the inhibitory activity of the protein-ligand complex.

Results: The binding energy of falcarindiol was -6.2Kcal/mol, while the free binding energies of the C=O, C2H5, CH3, CHO, COOH, C3H6O2, C4H8O2, NH2, OCH3 and C3H6O analogues of falcarindiol were -8.7, -6.5, -7.4, -7.8, -6.9, -8.5, -6.5, -7.0, -7.6 and -7.9Kcal/mol respectively. All the monosubstituted analogues of falcarindiol showed lower values than the non substituted falcarindiol. These lower values (more negative values), means that they show better antifungal activity than falcarindiol.

Conclusion: These results clearly indicated that the new substituents may be better antifungal agents. Synthesis and pre-clinical studies of these monosubstituted derivatives with *Aspergillus*

fumigatus chitinase is recommended in order to confirm their new potentials as better antifungal agent than the unsubstituted analogue.

Keywords: Docking; Falcarindiol; *Aspergillus fumigatus* chitinase; Pharmacokinetics; Lipophilicity.

Introduction

Aspergillosis is the name given to a wide variety of diseases caused by infection by fungi of the genus *Aspergillus*. Most of the cases occur in people with underlying illnesses such as tuberculosis [14] or chronic obstructive pulmonary disease (COPD), but with otherwise healthy immune systems [35]. Aspergillosis ofteh occurs in the form of chronic pulmonary aspergillosis (CPA), aspergilloma or allergic bronchopulmonary aspergillosis (ABPA) [19].

Drugs such as amphotericin B, caspofungin (in combination therapy only), flucytosine (in combination therapy only), or itraconazole [9, 21], are used to treat this fungal infection. However, a growing proportion of infections are resistant to the triazoles [13]. *A. fumigatus*, the most commonly infecting species, is intrinsically resistant to fluconazole [32].

Falcarindiol is the main compound responsible for bitterness in carrots and is reported to be part of the plant's defence against fungal infections [10, 16]. Falcarindiol and other falcarindiol-type polyacetylenes are also found in many other plants of the Apiaceae family, including some commonly used seasonings such as dill and parsley [6]. Falcarindiol also have been shown to be more abundant in the upper and outer parts of the root [3, 10, 17]. A variety of bioactivities have been reported so far for falcaridiol and the falcarindiol-type polyacetylenes [24, 38, 40], and because of potential health-promoting metabolic effects these compounds are studied as potential nutraceuticals [5].

Chitinases are enzymes that cleave the bond between the C1 and C4 of two consecutive N-acetylglucosamines of chitin. Chitinases have been found in microorganisms, plants and animal tissues [20]. These enzymes have been shown to have a variety of functions.

As one of the most ubiquitous of the airborne saprophytic fungi, *Aspergillus fumigatus* has been shown to be an opportunistic pathogen causing pneumonia and other fatal invasive infections in immunocompromised hosts, particularly among patients undergoing cytotoxin chemotherapy or bone marrow transplantation [4, 8, 27]. There has been a dramatic increase in severe and usually fatal invasive aspergillosis caused by *A. fumigatus* as this organism is also known to secret extracellular chitinases [15, 31].

In this study, the In-Silico Structure-Activity Relationship and molecular docking study was directed at investigating the inhibitory effect of falcarindiol and its monosubstituted analogues on

the structure and function of *Aspergillus fumigatus* chitinase, by predicting the binding energies and various pharmacokinetics parameters necessary for computational drug design.

Materials and Methods

Protein preparation

The crystal structure of *Aspergillus fumigatus* chitinase, was obtained from the Protein Data Bank, PDB 2A3B (Figure 23). The protein structure was subjected to a refinement protocol using the Pymol viewer [12].

Designing of 6-Gingerol structural analogues

The structure of Falcarindiol (Figure 1) was drawn with the Marvin Sketch software [36]. The structural analogues of falcarindiol were developed with structural modifications and different substituents [29]. The first OH functional group of falcarindiol was replaced with C=O, C2H5, CH3, CHO, COOH, C3H6O2, C4H8O2, NH2, OCH3 and C3H6O groups. The structures were built with the Marvin Sketch software and minimized using the Chimera software [33].

Molecular docking

Molecular docking was performed using the AutoDock Vina Software [37]. Physicochemical, lipophilicity, solubility, pharmacokinetics and Lipinski druglikeness of falcarindiol and its analogues were determined using SwissADME Server [11].

Physiological–biochemical characterization

The Expasy Protparam server [22] was used for the physicochemical characterization and to know the molecular weight, theoretical isoelectric point (pI), total number of negative and positive residues, aliphatic index, extinction coefficient, instability index, and grand average hydropathicity (GRAVY) of this TIM protein [18].

Results

Structural formula

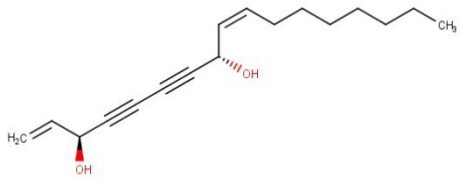

Figure 1: Falcarindiol structural formula.

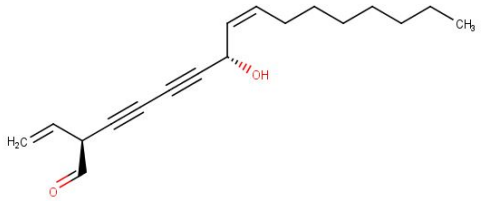

Figure 2: C=O analogue of falcarindiol

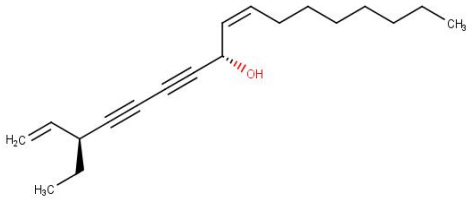

Figure 3: C2H5 analogue of falcarindiol

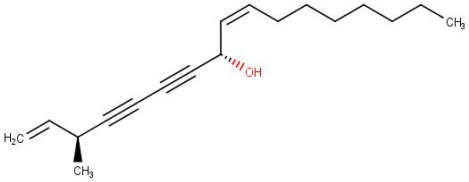

Figure 4: CH3 analogue of falcarindiol

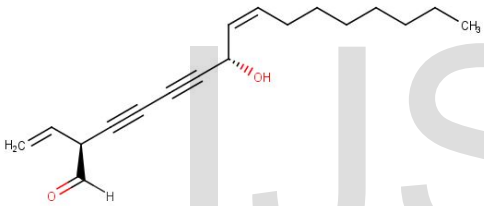

Figure 5: CHO analogue of falcarindiol

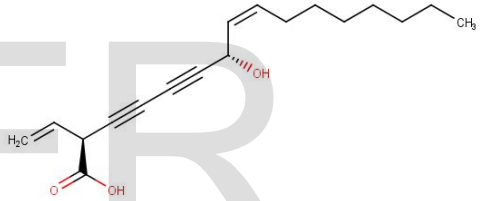

Figure 6: COOH analogue of falcarindiol

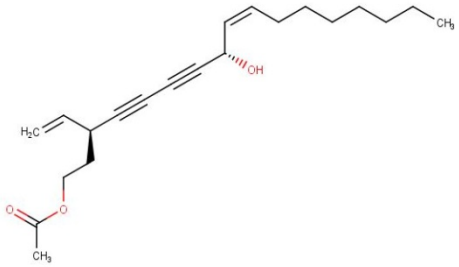

Figure 7: C3H6O2 analogue of falcarindiol

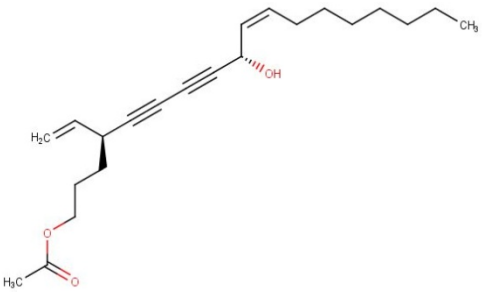

Figure 8: C4H8O2 analogue of falcarindiol

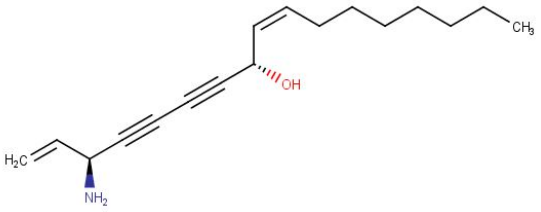

Figure 9: NH2 analogue of falcarindiol

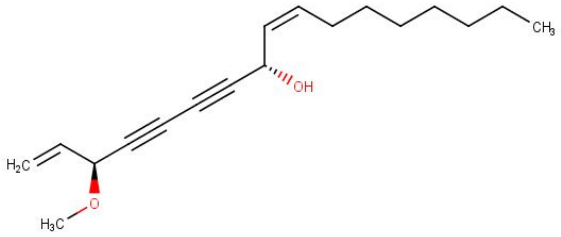

Figure 10: OCH3 analogue of falcarindiol

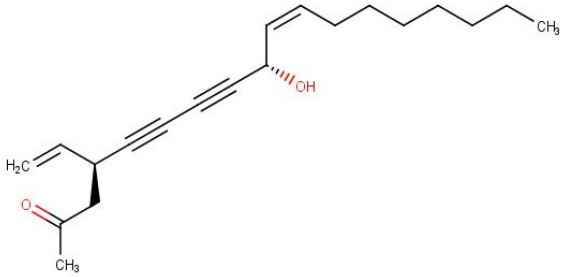

Figure 11: C3H6O analogue of falcarindiol

Docking results

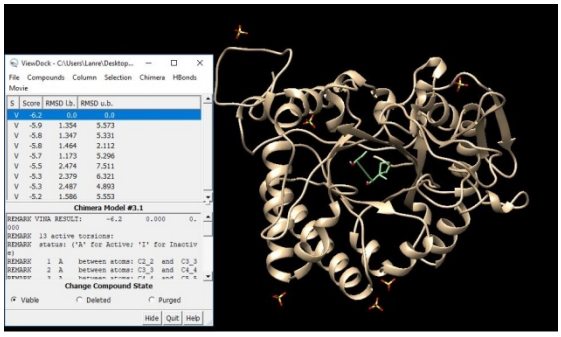

Figure 12: Falcarindiol in complex with *A. fumigatus* chitinase.

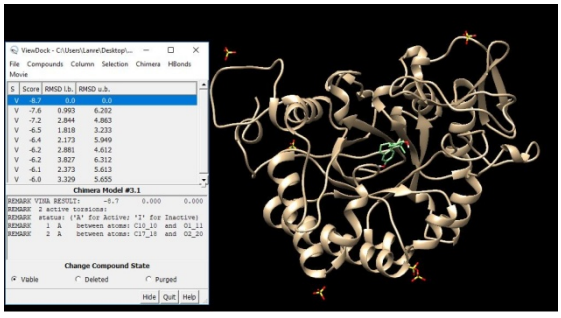

Figure 13: C=O analogue of falcarindiol in complex with *A. fumigatus* chitinase.

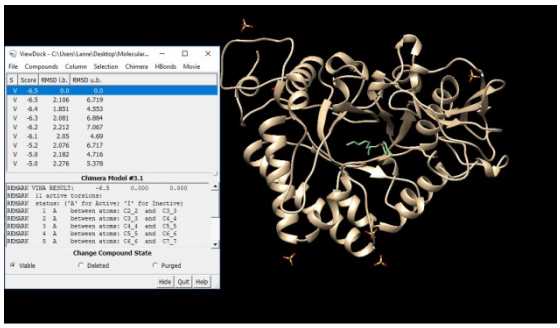

Figure 14: C2H5 analogue of falcarindiol in complex with *A. fumigatus* chitinase.

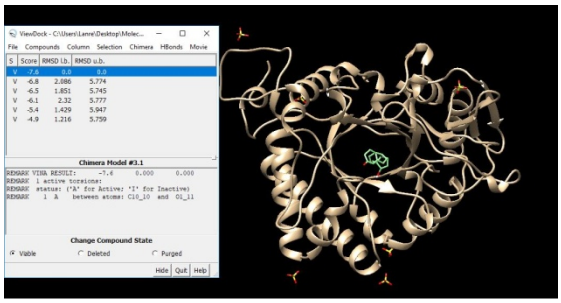

Figure 15: CH3 analogue of falcarindiol in complex with *A. fumigatus* chitinase.

Figure 16: CHO analogue of falcarindiol in complex with *A. fumigatus* chitinase.

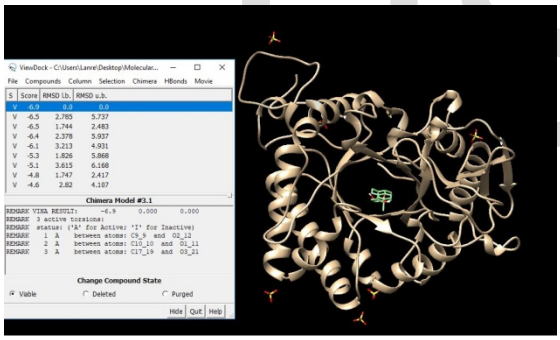

Figure 17: COOH analogue of falcarindiol in complex with *A. fumigatus* chitinase.

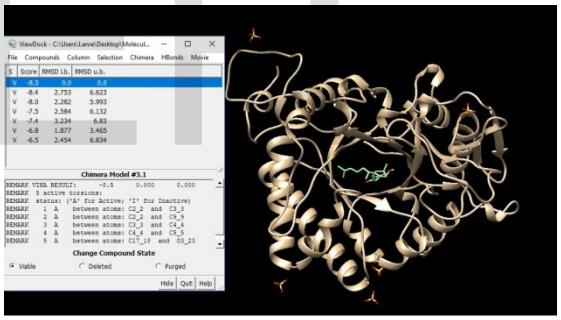

Figure 18: C3H6O2 analogue of falcarindiol in complex with *A. fumigatus* chitinase.

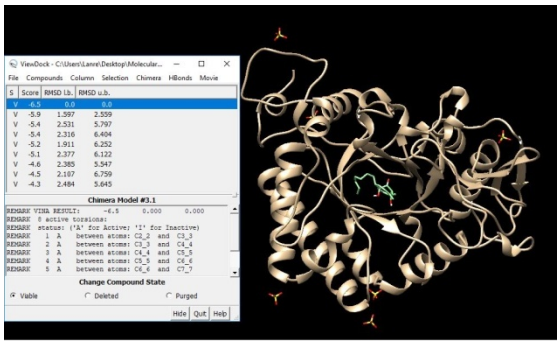

Figure 19: C4H8O2 analogue of falcarindiol in complex with *A. fumigatus* chitinase.

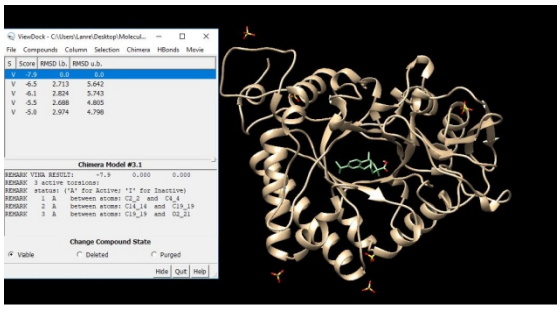

Figure 22: C3H6O analogue of falcarindiol in complex with *A. fumigatus* chitinase.

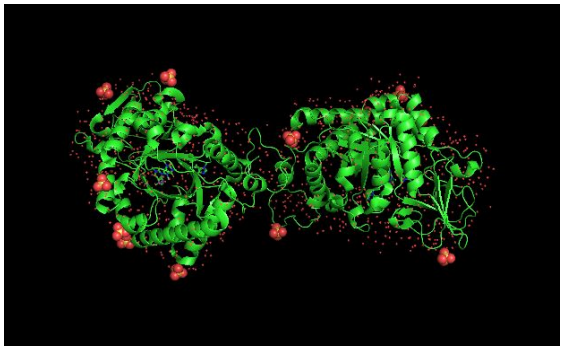

Figure 23: Crystal structure of the *A. fumigatus* chitinase PDB 2A3B

Table 1: Physicochemical, lipophilicity, solubility, pharmacokinetics and Lipinski druglikeness of falcarindiol and its monosubstituted analogues

Parameters	**Falcarindiol**	**C=O analogue of Falcarindiol**	**C2H5 analogue of Falcarindiol**	**CH3 analogue of Falcarindiol**	**CHO analogue of Falcarindiol**	**COOH analogue of Falcarindiol**	**C3H6O2 analogue of Falcarindiol**	**C4H8O2 analogue of Falcarindiol**	**NH2 analogue of Falcarindiol**	**OCH3 analogue of Falcarindiol**	**C3H6O analogue of Falcarindiol**
Formula	C17H24O2	C18H24O2	C19H28O	C18H26O	C18H24O2	C18H24O3	C21H30O3	C22H32O3	C17H25NO	C18H26O2	C20H28O2
Molecular weight g/mol	260.37	272.38	272.43	258.40	272.38	288.38	330.46	344.49	259.39	274.40	300.44
Docking score Kcal/mol	-6.2	-8.7	-6.5	-7.4	-7.8	-6.9	-8.5	-6.5	-7.0	-7.6	-7.9
Num. H-Bond acceptors	2	2	1	1	2	3	3	3	2	2	2
Num. H-Bond	2	1	1	1	1	2	1	1	2	1	1

donors											
Molar Refractivity	81.53	85.38	89.98	85.18	85.38	86.95	100.88	105.69	83.08	86.26	94.99
Lipophilicity Consensus Log $P_{o/w}$	3.81	4.10	5.23	4.90	4.10	3.93	4.93	5.17	3.70	4.29	4.64
Water Solubility Class	Soluble	Moderately Soluble	Soluble	Soluble	Moderately Soluble	Moderately Soluble	Soluble	Moderately Soluble	Soluble	Moderately Soluble	Moderately Soluble
GI absorption	High	High	High	High	High	High	High	High	High	High	High
BBB permeant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
P-gp substrate	No	No	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes
CYP1A2 inhibitor	No	No	No	No	No	No	Yes	Yes	No	No	Yes
CYP2C19 inhibitor	No	No	No	No	No	No	Yes	Yes	No	No	No
CYP2C9 inhibitor	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
CYP2D6 inhibitor	No	No	No	No	No	No	Yes	Yes	No	Yes	Yes
CYP3A4 inhibitor	No	No	No	No	No	No	No	No	No	No	Yes
Lipinski Druglikeness	Yes; 0 Violation	Yes; 0 Violation	Yes; 1 Violation	Yes; 1 Violation	Yes; 0 Violation	Yes; 0 Violation	Yes; 0 Violation	Yes; 1 Violation	Yes; 0 Violation	Yes; 0 Violation	Yes; 0 Violation
Synthetic accessibility	4.65	4.76	4.99	4.72	4.76	4.76	4.81	4.90	4.58	4.76	4.98

Discussion

The pI of the *A. fumigatus* chitinase by the biochemical characterization analysis has predicted the protein to be slightly acidic with a value of 5.03 [34]. The hydrophobicity scale produced values that define relative hydrophobicity of amino acid residues. The more positive the value,

the more hydrophobic the amino acids located in that region of the protein [26]. The GRAVY calculator used in predicting the hydrophobicity assigned to the protein a value of -0.334.

The instability index provides an estimate of the stability of a protein in a test tube. A protein whose instability index is smaller than 40 is predicted as stable and a value above 40 predicts the protein may be unstable [26]. The *A. fumigatus* chitinase is therefore a stable protein with an instability value of 30.90.

A. fumigatus chitinase contains 378 amino acid residues. The docking structures of all the compounds showed that they bind in a very similar pattern with the active site of *A. fumigatus* chitinase, as is evident from the superposition of the falcarindiol and all its 10 analogues in Figures 12-22. The interaction between falcarindiol and the different monosubstituted analogues with *A. fumigatus* chitinase shows steric interactions with the amino acid residues. The calculated free energy of binding of the falcarindiol and its analogues were -6.2, -8.7, -6.5, -7.4, -7.8, -6.9, -8.5, -6.5, -7.0, -7.6 and -7.9Kcal/mol respectively (Table 1). This confirms that the structural modification implemented in this study is significantly related to their activity [25, 30]. Also, this proved the reliability of the docking results [39].

The solubility of a compound in water could improve its biotransformation and elimination as a drug [24]. Falcarindiol and all the substituted analogues were soluble in water (Table 1).

The molecular weight of all the substituted derivatives including falcarindiol were less than 500g/mol, showing that they can be considered as drug [2]. A compound can also be considered drug-like if it is characterized by high lipophilicity (less than 5) [1]. This is expressed as Log Po/w. The lipophilicity values of falcarindiol and all the other monosubstituted compounds except the C2H5 (5.23) and C4H8O2 (5.17) analogues are less than 5 and are most likely to be drugs.

Lipinski's rule of 5 [28] helps in distinguishing between drug-like and non drug-like molecules. It predicts high probability of success or failure due to drug likeness for molecules complying with 2 or more of the following rules: Molecular mass less than 500g/mol; High lipophilicity (expressed as Log Po/w less than 5); Less than 5 hydrogen bond donors; Less than 10 hydrogen bond acceptors; Molar refractivity should be between 40-130. These filters help in early preclinical development and could help avoid costly late-stage preclinical and clinical failures [23]. Falcarindiol and all the monosubstituted analogues complied with a minimum of two of the Lipinski's rule and therefore are likely to be drugs (Table 1).

High penetration is needed for most of the drugs targeting the central nervous system (CNS), whereas blood brain barrier (BBB) penetration should be minimized for non-CNS drugs to avoid undesired side-effects [7]. Pharmacokinetically, the gastrointestinal drug absorption of falcarindiol and all the substituents was high and could cross the blood brain barrier (BBB). This calls for caution in the administration of the drugs to minimize side effects due to the penetration of the blood brain barrier.

For synthetic accessibility, values of 5 to10 means that the drug could be synthesized [23]. Falcarindiol and all it analogues showed values less than 5. This means that the compounds can easily be synthesized in the laboratory. Synthetic studies followed by pre- clinical studies are further recommended.

Conclusion

We carried out an In-Silico Structure Activity Relationship and molecular docking study on *Aspergillus fumigatus* chitinase, using falcarindiol and ten of its structurally diverse analogues as the experimental compounds. The results obtained indicated that all the analogues may have a better functional activity having shown a higher binding energy value and exhibited a higher level of specificity and affinity with the target enzyme. The C2H5 and C4H8O2 analogues did not satisfy the conditions of lipophilicity required for a drug-like compound and as such, should be excluded from this list.

Falcariondiol and all its analogues could pose a threat to the Central Nervous System (CNS) as they can penetrate the blood brain barrier. Caution should therefore be taken in the administration of these drugs in order to avoid undesirable side effects.

Synthesis and pre-clinical studies of these monosubstituted derivatives of falcarindiol with *Aspergillus fumigatus* chitinase is recommended.

Abbreviations: PDB: Protein Data Bank; BBB: Blood Brain Barrier; CNS: Central Nervous System; COPD: Chronic Obstructive Pulmonary Disease; CPA: Chronic Pulmonary Aspergillosis; ABPA: Allergic Bronchopulmonary Aspergillosis; pI: Isoelectric Point.

References

1. ARNOTT, J. A. & PLANEY, S. L. (2012). The influence of lipophilicity in drug discovery and design. Expert Opinion on Drug Discovery 7, 863–875.
2. ARTURSSON, P. & KARLSSON, J. (1991). Correlation between oral-drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells. Biochemical and Biophysical Research Communications 175, 880–885.
3. Baranska, M.; Schulz, H. Spatial tissue distribution of polyacetylenes in carrot root. Analyst **2005**, 130, 855–859.
4. Barnes, A.J. & Denning, D.W. (1993) Aspergilli – significance as pathogens. Rev. Microbiol. 4, 176–180.
5. Christensen, L. P. (2011). "Aliphatic C17-Polyacetylenes of the Falcarinol Type as Potential Health Promoting Compounds in Food Plants of the Apiaceae Family". *Recent*

Patents on Food, Nutrition and Agriculture. **3** (1): 64–77. doi:10.2174/2212798411103010064. PMID 2111446.

6. Christensen, L. P.; Brandt, K. (2006). "Bioactive polyacetylenes in food plants of the Apiaceae family: Occurrence, bioactivity and analysis". *Journal of Pharmaceutical and Biomedical Analysis*. **41** (3): 683–693. doi:10.1016/j.jpba.2006.01.057. PMID 16520011.
7. CLARK, D. E. (2003). In silico prediction of blood-brain barrier permeation. Drug Discovery Today 8, 927–933.
8. Cohen, J. (1991) Clinical manifestations and management of aspergillosis in the compromised patient. In Fungal Infection in the Compromised Patient (Warnock, D.W. & Richardson, M.D., eds), pp. 118–152. John Wiley and Sons, Chichester, UK.
9. Cornely OA, Maertens J, Bresnik M, et al. (May 2007). "Liposomal amphotericin B as initial therapy for invasive mold infection: a randomized trial comparing a high-loading dose regimen with standard dosing (AmBiLoad trial)". *Clin. Infect. Dis*. **44** (10): 1289–97. doi:10.1086/514341. PMID 1744346.
10. Czepa, A.; Hofmann, T. (2003). "Structural and sensory characterization of compounds contributing to the bitter off-taste of carrots (*Daucus carota* L.) and carrot puree". *Journal of Agricultural and Food Chemistry*. **51** (13): 3865–3873. doi:10.1021/jf034085+. PMID 127977.
11. Daina A, Michielin O, Zoete V (2017) A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep 7: 42717.
12. DeLano, W. L. (2002). Pymol: An open-source molecular graphics tool. CCP4 Newsletter On Protein Crystallography, 40, 82-92.
13. Denning DW, Park S, Lass-Florl C, Fraczek MG, Kirwan M, Gore R, Smith J, Bueid A, Bowyer P, Perlin DS (2011). "High-frequency Triazole Resistance Found In Nonculturable Aspergillus fumigatus from Lungs of Patients with Chronic Fungal Disease". *Clin Infect Dis*. **52** (9): 1123–9. doi:10.1093/cid/cir179. PMC 3106268. PMID 21467016.
14. Denning, D. W.; Pleuvry, A.; Cole, D. C. (March 2013). "Global burden of chronic pulmonary aspergillosis complicating sarcoidosis". *European Respiratory Journal*. **41** (3): 621–6. doi:10.1183/09031936.00226911. PMID 22743676.
15. Escott, G.M., Hearn, V.M. & Adams, D.J. (1998) Inducible chitinolytic system of Aspergillus fumigatus. Microbiology 144, 1575–1581.
16. Garrod, B.; Lea, P.; Lewis, B.G. Studies on the mechanism of action of the antifungal compound falcarindiol. New Phytol. **1979**, 83, 463–471.
17. Garrod, B.; Lewis, B.G. Location of the antifungal compound falcarindiol in carrot root tissue. Trans. Br. Mycol. Soc. **1979**, 72, 515–517.

18. Gasteiger E., Hoogland C., Gattiker A., Duvaud S., Wilkins M.R., Appel R.D., Bairoch A.; *Protein Identification and Analysis Tools on the ExPASy Server;* (In) John M. Walker (ed): The Proteomics Protocols Handbook, Humana Press (2005). pp. 571-607.
19. Goel, Ayush. "Pulmonary aspergillosis". *Mediconotebook*. Retrieved 29 May 2015.
20. Gooday, G.W. (1990) Physiology of microbial degradation of chitin and chitosan. Biodegradation 1, 177–190.
21. Herbrecht R, Denning D, Patterson T, Bennett J, Greene R, Oestmann J, Kern W, Marr K, Ribaud P, Lortholary O, Sylvester R, Rubin R, Wingard J, Stark P, Durand C, Caillot D, Thiel E, Chandrasekar P, Hodges M, Schlamm H, Troke P, de Pauw B (8 August 2002). Invasive Fungal Infections Group of the European Organisation for Research and Treatment of Cancer and the Global Aspergillus Study Group.. "Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis". *N Engl J Med*. **347** (6): 408–15. doi:10.1056/NEJMoa020191. PMID 1216768.
22. http://us.expasy.org/tools/protparam.
23. Ikpeazu OV, Otuokere IE, Igwe KK (2017) In Silico Structure-Activity Relationship and Virtual Screening of Monosubstituted Doxycycline with Pseudomonas Aeruginosa Lipase. J Anal Pharm Res 5(3): 00139. DOI: 10.15406/japlr.2017.05.00139.
24. Jin, H. R.; Zhao, J.; Zhang, Z.; Liao, Y.; Wang, C. Z.; Huang, W. H.; Li, S. P.; He, T. C.; Yuan, C. S.; Du, W. (2012). "The antitumor natural compound falcarindiol promotes cancer cell death by inducing endoplasmic reticulum stress". *Cell Death and Disease*. **3** (8): e376. doi:10.1038/cddis.2012.122. PMC 3434669. PMID 22914324.
25. Kitchen DB, Decornez H, Furr JR, Bajorath J. Docking and scoring in virtual screening for drug discovery: methods and applications. Nat Rev Drug Discov. 2004;3(11):935–949.
26. Kyte, J. and Doolittle, R.F. (1982) A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. 157, 105-132. [PubMed: 7108955]
27. Latge´, J.-P. (1999) Aspergillus fumigatus and aspergillosis. Clin. Microbiol. Rev. 12, 310–350.
28. Lipinski CA (2004) Lead- and drug-like compounds: the rule of-five revolution. Drug Discovery Today: Technologies 1(4): 337-341.
29. McBride, Ryan (1 Oct 2012). "ChemAxon opens shop in 'heart' of Boston biotech hub". Retrieved 11 May 2014.
30. Moitessier N, Englebienne P, Lee D, Lawandi J, Corbeil CR. Towards the development of universal, fast and highly accurate docking/scoring methods: a long way to go. Br J Pharmacol. 2008; 153(Suppl 1):S7–26.
31. Monreal, J. & Reese, E. (1969) The chitinase of Serratia marcescens. Can. J. Microbiol. 15, 689–696.
32. Perea, S; Patterson, TF (1 November 2002). "Antifungal resistance in pathogenic fungi". *Clinical Infectious Diseases*. **35** (9): 1073–80. doi:10.1086/344058. PMID 12384841. Retrieved 14 January 2015.

33. Pettersen, EF; Goddard, TD; Huang, CC; Couch, GS; Greenblatt, DM; Meng, EC; Ferrin, TE (2004). "UCSF Chimera--a visualization system for exploratory research and analysis". *J Comput Chem*. **25** (13): 1605–12. doi:10.1002/jcc.20084.
34. Shi Q, Zhou Y, Sun Y. Influence of pH and ionic strength on the steric mass-action model parameters around the isoelectric point of protein. Biotechnol Prog. 2005;21:516–23.
35. Smith, N; Denning, D.W. (1 April 2011). "Underlying conditions in chronic pulmonary aspergillosis including simple aspergilloma". *European Respiratory Journal*. **37** (4): 865–872. doi:10.1183/09031936.00054810. PMID 20595150.
36. Toure, O.; Dussap, C.-G; Lebert, A. (2013). "Comparison of Predicted pKa Values for Some Amino-Acids, Dipeptides and Tripeptides,Using COSMO-RS, ChemAxon and ACD/Labs Methods". *Oil & Gas Science and Technology – Rev. IFP Energies nouvelles*. **68** (2): 281–291. doi:10.2516/ogst/2012094.
37. Trott, O.; Olson, A.J. (2010), "AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading", *Journal of Computational Chemistry*, **31** (2): 455–461, doi:10.1002/jcc.21334.
38. Wang L, Palme V, Schilcher N, Ladurner A, Heiss EH, Stangl H, Bauer R, Dirsch VM, Atanasov AG. The Dietary Constituent Falcarindiol Promotes Cholesterol Efflux from THP-1 Macrophages by Increasing ABCA1 Gene Transcription and Protein Stability. Front Pharmacol. 2017 Sep 1;8:596. doi: 10.3389/fphar.2017.00596.
39. Wei BQ. "Testing a flexible-receptor docking algorithm in a model binding site". Journal of Molecular Biology 337.5 (2004): 1161-1182.
40. Wyrembek, P.; Negri, R.; Kaczor, P.; Czyżewska, M.; Appendino, G.; Mozrzymas, J. W. (2012). "Falcarindiol allosterically modulates GABAergic currents in cultured rat hippocampal neurons". *Journal of Natural Products*. **75** (4): 610–616. doi:10.1021/np2008522. PMID 22432736.