Using molecular docking to investigate the anti-breast cancer activity of low molecular weight compounds present on wild mushrooms

HUGO J.C. FROUFE, RUI M.V. ABREU, ISABEL C.F.R. FERREIRA*

CIMO/Escola Superior Agrária, Instituto Politécnico de Bragança, *Campus* de Santa Apolónia, Apartado 1172, 5301-855 Bragança, Portugal.

* Author to whom correspondence should be addressed (e-mail: iferreira@ipb.pt telephone +351-273-303219; fax +351-273-325405).

ABSTRACT

Mushrooms represent an unlimited source of compounds with antitumor and immunostimulating properties and mushroom intake as been shown to reduce the risk of breast cancer. A large number of LMW (low molecular weight) compounds present in mushrooms have been identified including: phenolic acids, flavonoids, tocopherols, carotenoids, sugars and fatty acids. In order to evaluate which wild mushroom LMW compounds may be involved in anti-breast cancer activity we selected a representative dataset of 43 LMW compounds and performed molecular docking against 3 known protein targets involved in breast cancer (Aromatase, Estrone Sulfatase and 17β-HSD-1) using AutoDock4 as docking software. The estimated inhibition constants for all LMW compounds were determined and the potential structure-activity relationships for the compounds with the best estimated inhibition constants are discussed for each compound family. 4-*O*-caffeoylquinic, naringin and lycopene stand out as the top ranked potential inhibitors for Aromatase, Estrone Sulfatase and 17β-HSD1, respectively, and the 3-D docked conformation for these compounds are discussed in detail. This information provides several interesting starting points for further development of Aromatase, Estrone Sulfatase and 17β-HSD1 inhibitors.

Keywords: Mushrooms; Nutraceuticals; Breast cancer; Molecular Docking; AutoDock4

1. Introduction

Mushrooms comprise a vast and yet largely untapped source of powerful new pharmaceutical products. In particular, and most importantly for modern medicine, they represent an unlimited source of compounds with antitumor and immunostimulating properties [1-3]. Mushrooms contain compounds known as long-chain, large-molecular weight polysaccharides which, when present in specific configurations or linkages (beta, 1-3 glucan and beta, 1-6 glucan), have strong effects on the immune system of humans [4-6]. Several phytochemicals have been isolated from medicinal mushrooms and three of these, which are carcinostatic polysaccharide drugs, have been developed from mushrooms in Japan. These are "Krestin" (PSK), from the cultured mycelium of Kawaratake (*Trametes versicolor*), "Lentinan" from the fruiting bodies of Shiitake (*Lentinus edodes*) and "Schizophyllan" (Sonifilan) from the culture fluid of Suehirotake (*Schizophyllum commune*) [7,8]. More importantly extracts from wild mushrooms species have been shown to reduce the risk of breast cancer in Chinese women [9] and in breast cancer cell lines [10]. Mushrooms are also rich sources of low molecular weight (LMW) antioxidant compounds mainly phenolic compounds (phenolic acids and flavonoids), followed by tocopherols, ascorbic acid and carotenoids as described by our research group [11]. In fact, in the last years tens of different mushroom species from Northeast of Portugal, one of the European regions with higher wild edible mushrooms diversity, were evaluated by us, for their composition on those LMW compounds [12-18]. Since the non-controlled production of free radicals has been related to more than one hundred diseases including several kinds of cancer, it was our goal to evaluate the potential properties of the LMW compounds found in mushrooms against some proteins identified as targets in breast cancer.

Most breast cancers (about 95%), whether in pre- or post-menopausal women, are initially hormone-dependent and it is well accepted that estradiol plays an important role in their development and progression. Estradiol in complex with their receptor can mediate the activation of proto-oncogenes or oncogenes (e.g. c-fos, c-myc), nuclear proteins, as well as other target genes. Consequently, processes that modulate the intracellular concentrations of active estrogens can have the ability to affect the etiology of this disease. It is known that that mammary cancer tissue contains all the enzymes responsible for the local biosynthesis of estradiol from circulating precursors [19]. Two principal pathways are implicated in the last steps of estradiol formation in breast cancer: the 'Aromatase pathway', with Aromatase enzyme (EC: 1.14.14.1) that converts androgens into estrogens and the 'Sulfatase pathway' which converts estrone sulfate into estrone by the action of Estrone Sulfatase enzyme (EC: 3.1.6.2). The final step of steroidogenesis is the conversion of the weak estrone to the potent biologically active estradiol by the action of a reductive 17-hydroxysteroid dehydrogenase type 1 enzyme (17β-HSD-1; EC: 1.1.1.62) [19].

Intermolecular interactions between proteins and small ligands play essential roles in several life processes and understanding these interactions is critical for pharmaceutical and functional food industries [20]. Molecular docking is an *in silico* tool that predicts how a ligand (substrate or drug candidate) interacts with a receptor usually by predicting the ligand free energy of binding and the three-dimensional structure of the ligand-receptor complex. The use of molecular docking to search large databases of compounds for possible ligands of a protein receptor is usually termed virtual screening and has been successfully applied in several therapeutic programs at the lead discovery stage [21]. In this work we use AutoDock4 [22], acknowledged to be

one of the most reliable and broadly used molecular docking tool [23] with several examples of accurate docking predictions already published [24-26].

In the present study we performed molecular docking using 3-D structures of Aromatase, Estrone Sulfatase and 17β-HSD-1 as targets and phenolic acids, flavonoids, tocopherols, carotenoids, sugars and fatty acids as ligands. The main goal was to identify LMW wild mushrooms compounds with potential activity against human breast cancer by identifying the potential protein targets. Also the molecular basis of the interaction between the best LMW compounds identified and the selected protein targets is discussed.

2. Methodology

2.1. LMW compound dataset

The LMW compound dataset used was composed of 43 compounds representative of the chemical composition of wild mushrooms [11-18]. The 2-D structure of the dataset was constructed using the MDL Isis/Draw 2.5 software (http://www.symyx.com). The software VegaZZ 2.3.1 [27] was then used to: convert all compounds to 3-D, perform energy minimization and record files in pdb format. Next, AutoDockTools1.5.2 (ADT) [28] was used to: merge nonpolar hydrogens, add Gasteiger charges, and set up rotatable bonds through AutoTors [29]. Finally all compounds were recorded in pdbqt file format, a format needed for docking with AutoDock4.

2.2. Protein structures preparation

The X-ray 3-D structures of the protein targets used were extracted from the Protein Data Bank (PDB) (http://www.rcsb.org) including: Aromatase (PDB: 3EQM), Estrone Sulfatase (PDB: 1P49) and 17β-HSD1 (PDB: 1FDT).

For 3EQM and 1FDT the co-crystallized ligand (Androstenedione and Estrone respectively) was extracted from the PDB file (Table 1). This procedure was not done with 1P49 structure because this structure was determined without a co-crystallized ligand. ADT was then used to assign polar hydrogens and Gasteiger charges to the protein structures and the structures where also recorded in pdbqt format [22].

For each protein structure, AutoGrid4 [22] was used to create affinity grid maps for all the atoms types present on the protein and compounds. We used ADT to choose the correct parameters before using AutoGrid4. All affinity grid maps were centred on the active site and coordinates were selected in order to encompass all the active site for each protein. 3EQM affinity grids enclosed an area of 100 Å by 100 Å by 100 Å with 0.375 Å spacing, centred on the coordinates x=86.312 y=51.204 z=48.26, 1P49 affinity grids maps enclosed an area of 80 Å by 80 Å by 80 Å with 0.375 Å spacing, centred on the coordinates x=71.9 y=-5.072 z=30.368 and 1FDT affinity grids enclosed an area of 80 Å by 110 Å by 110 Å with 0.375 Å spacing, centred on the coordinates x=39.685 y=1.159 z=37.333.

2.3. Molecular docking using AutoDock4

AutoDock4 (version 4.0.1) with the Lamarckian genetic algorithm was used to simulate compound-protein molecular docking for the 3 selected protein structures [22]. Docking parameters selected for AutoDock4 runs were as follows: 50 docking runs, population size of 200, random starting position and conformation, translation step ranges of 2.0 Å, mutation rate of 0.02, crossover rate of 0.8, local search rate of 0.06,

and 2.5 million energy evaluations. Docked conformations were clustered using a tolerance of 2.0 Å RMSD (Root Mean Square Deviation). The entire virtual experiment was performed on a cluster of 8 Intel Dual-Core 2.8 GHz computers using a custom designed software called MOLA [30]. Estimated inhibition constant (Ki) values for all compounds were calculated by AutoDock4 as follows: Ki = exp((ΔG*1000)/(Rcal*TK)) where ΔG is the binding energy, Rcal is 1.98719 and TK is 298.15. The Michaelis-Menten constant (Km) values for natural ligands presented on table 1 were calculated by AutoDock4 using the same equation presented above and the pKm values were calculated using the following equation: pKm = log (1/Km). The 3-D compound-protein docking poses were analysed manually using ADT and the images presented on figures 1 and 3 were prepared using the software PyMOL 0.99r6 (http://pymol.sourceforge.net/).

3. Results and Discussion

3.1. Molecular Docking validation

In order to validate the molecular docking approach for the protein structures studied, the respective ligands (natural substrates) were docked to the active site of the proteins (from which the natural ligands were previously removed). Then the estimated binding energy (ΔG) and Km values obtained were compared with experimental ΔG and Km values (Table 1). Also the estimated 3-D binding pose was compared with the experimental (by X-ray crystallography) co-crystallized binding pose (Fig. 1).

The authors of AutoDock4 used a large number of protein-ligand complexes to calibrate AutoDock4's algorithm and the binding energy model they used exhibited a residual standard error of 2.177 kcal/mol [22]. For Aromatase, Estrone Sulfatase and 17β-HSD1, when comparing estimated and experimental ΔG values, we observed

differences of 0.95, 1.60 and 0.42 Kcal/mol respectively (Table 1). These variations fall well within the residual standard error of 2.177 kcal/mol observed for AutoDock4. This is a strong indication that AutoDock4 is performing well with the selected protein structures thus validating them for docking with other LMW compounds.

When calculating Km (or Ki) values from ΔG values, the 2,177 kcal/mol standard error translates into an expected 1.6 orders of magnitude (pKm difference) accuracy for AutoDock4. In our study, the estimated Km calculated by AutoDock4 was 5 times lower than experimental Km for Aromatase (difference of 0.6 orders of magnitude), 20 times lower for Estrone Sulfatase (difference of 1.2 orders of magnitude) and 2 times higher for 17β-HSD1 (difference of 0.3 orders of magnitude) (Table 1). These values are well within the 1.6 orders of magnitude difference considered acceptable for AutoDock4.

Also the binding mode of the docked ligands for Aromatase and 17β-HSD1 corresponded well with the binding mode of the co-crystallized ligands, with RMSD values of 0.08 Å and 0.66 Å, respectively (Fig. 1 and Table 1). These values shows that the difference between the X-ray conformation and the predicted docked conformations of the compounds was very small thus validating further the protein structures for molecular docking with the LMW compound dataset. Particularly, for both Androstenedione (Fig. 1A) and Estrone (Fig. 1B), we can see that AutoDock4 accurately predicted the position of the atoms that form hydrogen bonds with residues of the catalytic sites.

This comparison is not possible for Estrone Sulfatase as there is no experimentally determined structure with a co-crystallized ligand, only the non-bounded protein structure. This probably explains the higher different between estimated and experimental Km for Estrone Sulfatase (1.60 Kcal/mol) although still well within the

expected standard error and thus the structure was validated for docking using AutoDock4.

3.2. Molecular Docking of the LMW Mushrooms compounds

We then performed molecular docking using AutoDock4 with the selected wild mushroom LMW compound dataset against the 3 target structures. The dataset used is not exhaustive but is a good representation of the different LMW families of compounds that can be found in wild mushrooms. The results will be discussed for each family of compounds: phenolic compounds (benzoic acid and cinnamic acid derivatives, and flavonoids), vitamins (tocopherols and ascorbic acid), carotenoids, sugars and fatty acids.

The results obtained using phenolic acids (Table 2) revealed that benzoic acid derivatives appear to have no significant inhibitory activity against the 3 enzymes studied with all values well above 1 μM. Cinnamic acid derivatives also had no significant inhibitory activity except for 4-*O*-caffeylquinic and 5-*O*-caffeylquinic which presented moderate inhibition activity for the enzymes with values in the hundreds of nM (Fig. 2 and Table 2). The presence of quinic acid seems to be an essential condition for phenolic acid inhibition. Also the fact that 4-*O*-caffeylquinic and 5-*O*-caffeylquinic present activity against all the 3 enzymes probably results from the fact that they have similar structures to the natural ligands. This simultaneous inhibition activity may result in a synergistic inhibition of overproduction of estrone in breast cancer by inhibiting both “Aromatase” and “Sulfatase” pathway as well as inhibiting estradiol to estrogen conversion by 17β-HSD1.

The results obtained using flavonoids (Table 3) showed that quercetin was the best compound to inhibit Aromatase, with naringin being the best for Estrone Sulfatase

and 17β-HSD1. Interestingly, *t*he substitution of the hydroxyl group for the disaccharide rutinose increases estimated Ki values for Aromatase and decreases the corresponding values for the other two enzymes (see in Table 3 and Fig. 2 rutin relative to quercetin and naringin relative to naringenin). The presence of rutinose in those compounds might increase the stereochemical hindrance of the molecules decreasing their binding capacity to Ar*o*matase, which contains a Heme group. In general, we observed that a good number of the flavonoids from different groups present inhibition activity. This is probably because, from all the LMW comp*o*unds used, flavonoids have more similar structures to the natural ligands.

The results obtained using vitamins and carotenoids are given in Table 4. Vitamin E (tocopherols) proved to have better binding capacity than vitamin C (ascorbic acid). The four isoforms of vitamin E (α, β, δ, and γ-tocopherol) revealed very good inhibition properties for 17β-HSD1 with estimated Ki in the nanomolar range. Furthermore, β-carotene and lycopene revealed excellent properties for inhibition of 17β-HSD1 with lycopene estimated Ki in the subnanomolar range (0.2 nM), the best result of all compounds. Also, α, δ, and γ-tocopherol showed moderate inhibition activity against Estrone Sulfatase.

In relation to the results obtained using sugars and fatty acids (Table 5) it was not observed any significant inhibition activity in any of the studied targets. Only maltose showed a very moderate activity against 17β-HSD1 but with a relatively high estimated Ki value (0.605 μM); interestingly maltose is the only reducing sugar which may be an important factor as 17β-HSD1 is a dehydrogenase enzyme. Nevertheless the studied sugars and fatty acids do not seem implicated in anti-breast cancer activity.

3.3 Binding mode analysis of the top ranked LMW Mushrooms compounds

The docked binding mode of all the compounds with good estimated Ki values was manually inspected in order to verify that they effectively bind to the catalytic site in a structurally viable conformation.

For Aromatase the top ranked compound was 4-*O*-caffeoylquinic and the docked structure occupies the space where the natural ligand Androstenedione binds (Fig. 3A). The aromatic rings from 4-*O*-caffeoylquinic occupies the space of the aromatic rings of Androstenedione and the quinic acid seems to be the key with the carboxylic acid stabilized by polar contacts with the Heme group of Aromatase. Also probably 4-*O*-caffeoylquinic mimics Androstenedione forming hydrogen bonds with ARG-115 and the peptide bond between VAL-373 and MET-374 of Aromatase (Fig. 1A). This is possible because one oxygen of 4-*O*-caffeoylquinic is positioned on the same space occupied by the oxygen of one of Androstenedione's carbonyl group (Fig. 3A). It's important to note that the estimated Ki values (315 nM for 4-*O*-caffeoylquinic) obtained with Aromatase as the protein target was at least one order of magnitude higher than the experimental Km value obtained with Androstenedione (20 nM). This fact indicates that Aromatase is probably not the most important target for LMW compounds in wild mushrooms.

For Estrone Sulfatase inhibition the top ranked compound was the flavanone naringin (Fig. 3B; Table 4). The disaccharide rutinose seems to play a pivotal role in naringin inhibition by promoting hydrogen bonds with the sulfate group present. The X-ray structure used for docking had no co-crystallized ligand. The estimated Ki value obtained for naringin (206 nM) was well below the experimental Km of the natural ligand estrone sulfate (6850 nM). Also several compounds have estimated Ki values in the order of naringin. These data indicates that several LMW compounds could be working synergistically and that probably the "Sulfatase" pathway is the most likely

target for LMW wild mushrooms compounds against breast cancer. This is even more interesting in view of recent findings in human breast cancer that point towards "Sulfatase" pathway as the most likely path for estradiol production with "Aromatase" pathway playing a secondary role [19].

Finally 17β-HSD1 was the most susceptible protein target with the lowest estimated Ki values of all the dataset. Lycopene (Fig. 3C; Table 4) presented the best estimated Ki value (0.2 nM) about three orders of magnitude lower than the experimental Km value for the natural ligand estrone (124 nM). Analysing the docked structure (Fig. 3C) we can see that lycopene "fits" exactly on the binding pocket occupied by the co-enzyme NADPH and the natural ligand estrone. Because lycopene is predominantly a hydrophobic compound, its structure is stabilized by van der Walls interactions and no so much by the hydrogen bonds that stabilize the natural ligand estrone (Fig. 1A). It is important to note that, although the estimated Ki value was very low, its inhibition ability is probably balanced by the difficulty of lycopene to reach the binding site of 17β-HSD1 due to low solubility in water.

4. Conclusions

In conclusion, this study highlights several LMW compounds from wild mushrooms that may act against breast cancer by inhibiting different proteins involved in overproduction of estrone and estradiol. From the phenolic acids studied the cinnamic acid derivatives esterified with quinic acid (4 and 5-*O*-caffeylquinic acid) were the only compounds with significant inhibition against the 3 protein targets studied, specially 4-*O*- caffeoylquinic acid that presented the best estimated Ki against Aromatase. Among flavonoids several compounds presented moderate to good inhibition ability with flavanones (naranigenin and naringin) and flavonols (quercetin and rutin) with the best

estimated Ki values. Naringin was the top ranked inhibitor against Estrone Sulfatase indicating that the presence of the disaccharide rutinose may be a key element for active compounds against breast cancer. Vitamins and carotenoids were target specific showing very good inhibition ability only against 17β-HSD1, with lycopene as the top ranked inhibitor. Sugar and fatty acids did not show any significant inhibition ability. This study suggests the LMW compounds to look for in wild mushrooms when searching for species with anti-breast cancer activity. Furthermore, the information provided shows several interesting starting points for further development of Aromatase, Estrone Sulfatase and 17β-HSD1 inhibitors.

Acknowledgements

The authors are grateful to Foundation for Science and Technology (Portugal) and COMPETE/QREN/UE for financial support through research project PTDC/AGR-ALI/110062/2009.

References

[1] S.P. Wasser, *Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides*, Appl. Microbiol. Biotechnol. 60 (2002), pp. 258-274.

[2] J.K. Zjawiony, *Biologically active compounds from aphyllophorales (polypore) fungi*, J. Nat. Prod. 67 (2004), pp. 300-310.

[3] B. Zaidman, M. Yassin, J. Mahajana and S.P. Wasser, *Medicinal mushrooms modulators of molecular targets as cancer therapeutics*, Appl. Microbiol. Biotechnol. 67 (2005), pp. 453-468.

[4] P. Mattila, K. Suonpaa and V. Piironen, *Functional properties of edible mushrooms*, Nutrition 16 (2000), pp. 694-696.

[5] A.T. Borchers, C.L. Keen and M.E. Gershwin, *Mushrooms, tumors, and immunity: An update*, Exp. Biol. Med. 229 (2004), pp. 393-406.

[6] M. Zhang, S.W. Cui, P.C.K. Cheung and Q. Wang, *Polysaccharides from mushrooms: a review on their isolation process, structural characteristics and antitumor activity*, Trends Food Sci. Technol. 18 (2006), pp. 4-19.

[7] T. Mizuno, *Food function and medicinal effect of mushroom fungi*, Foods Food Ingred. J. 158 (1993), pp. 55-70.

[8] D.H. Larone, *Medically Important Fungi, A Guide to Identification*, USA (2002).

[9] M. Zhang, J. Huang, X. Xie and C.D.J. Holman, *Dietary intakes of mushrooms and green tea combine to reduce the risk of breast cancer in Chinese women*, Int. J. Cancer 124 (2009), pp. 1404–1408.

[10] B.J. Grube, E.T. Eng, Y.-C. Kao, A. Kwon and S. Chen, *White Button Mushroom Phytochemicals Inhibit Aromatase Activity and Breast Cancer Cell Proliferation*, J. Nutr. 131 (2001), pp. 3288–3293.

[11] I.C.F.R. Ferreira, L. Barros and R.M.V. Abreu, *Antioxidants in Wild Mushrooms*, Curr. Med. Chem. 16 (2009), pp. 1543-1560.

[12] L. Barros, M.J. Ferreira, B. Queiros, I.C.F.R. Ferreira and P. Baptista, *Total phenols, ascorbic acid, beta-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities*, Food Chem. 103 (2007), pp. 413-419.

[13] L. Barros, D.M. Correia, I.C.F.R. Ferreira, P. Baptista and C. Santos-Buelga, *Optimization of the determination of tocopherols in Agaricus sp edible mushrooms by a normal phase liquid chromatographic method*, Food Chem. 110 (2008), pp. 1046-1050.

[14] L. Barros, T. Cruz, P. Baptista, L.M. Estevinho and I.C.F.R. Ferreira, *Wild and commercial mushrooms as source of nutrients and nutraceuticals*, Food Chem. Toxicol. 46 (2008), pp. 2742-2747.

[15] L. Barros, S. Falcao, P. Baptista, C. Freire and M. Vilas-Boas, I.C.F.R. Ferreira, *Antioxidant activity of Agaricus sp mushrooms by chemical, biochemical and electrochemical assays*, Food Chem. 111 (2008), pp. 61-66.

[16] L. Barros, B.A. Ventuizini, P. Baptista, L.M. Estevinho and I.C.F.R. Ferreira, *Chemical composition and biological properties of Portuguese wild mushrooms: A comprehensive study*, J. Agric. Food Chem. 56 (2008), pp. 3856-3862.

[17] L. Barros, M. Duenas, I.C.F.R. Ferreira, P. Baptista and C. Santos-Buelga, *Phenolic acids determination by HPLC-DAD-ESI/MS in sixteen different Portuguese wild mushrooms species*, Food Chem. Toxicol. 47 (2009), pp. 1076-1079.

[18] S.A. Heleno, L. Barros, M.J. Sousa, A. Martins and I.C.F.R. Ferreira, *Tocopherols composition of Portuguese wild mushrooms with antioxidant capacity*, Food Chem. 119 (2009), pp. 1443–1450.

[19] J.R. Pasqualini and G.S. Chetrite, *Recent insight on the control of enzymes involved in estrogen formation and transformation in human breast cancer*, J. Steroid Biochem. Mol. Biol. 93 (2005), pp. 221-236.

[20] G. Pujadas, M. Vaque, A. Ardrevol, C. Blade, M.J. Salvado and M. Blay, J. Fernandez-Larrea, L. Arola, *Protein-ligand docking: A review of recent advances and future perspectives*, Curr. Pharmaceut. Anal. 1 (2008), pp. 1-19.

[21] S. Ghosh, A.H. Nie, J. An and Z.W. Huang, *Structure-based virtual screening of chemical libraries for drug discovery*, Curr. Opin. Chem. Biol. 10 (2006), pp. 194-202.

[22] G.M. Morris, D.S. Goodsell, R.S. Halliday, R. Huey, W.E. Hart, R.K. Belew and A.J. Olson, *Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function*, J. Comp. Chem. 19 (1998), pp. 1639-1662.

[23] S.F. Sousa, P.A. Fernandes and M.J. Ramos, *Protein-ligand docking: Current status and future challenges*, Proteins 65 (2006), pp. 15-26.

[24] C.L. Li, L. Xu, D.W. Wolan, I.A. Wilson and A.J. Olson, *Virtual screening of human 5-aminoimidazole-4-carboxamide ribonucleotide transformylase against the NCI diversity set by use of AutoDock to identify novel nonfolate inhibitors*, J. Med. Chem. 47 (2004), pp. 6681-6690.

[25] K. Chen, A.F. Al Aowad, S.J. Adelstein and A.I. Kassis, *Molecular-docking-guided design, synthesis, and biologic evaluation of radioiodinated quinazolinone prodrugs*, J. Med. Chem. 50 (2007), pp. 663-673.

[26] K.H. Yan, Z.Y. Wang, H.L. Liu and H.X. Yu, Exploring the binding features of polybrominated diphenyl ethers as estrogen antagonists: docking studies, SAR QSAR Environ. Res. 21 (2010), pp. 351-367.

[27] A. Pedretti, L. Villa and G. Vistoli, *VEGA - An open platform to develop chemo-bio-informatics applications, using plug-in architecture and script programming*, J. Comput. Aided Mol. Des. 18 (2004), pp. 167-173.

[28] M.F. Sanner, *A component-based software environment for visualizing large macromolecular assemblies*, Structure 13 (2005), pp. 447-462.

[29] J. Gasteiger and M. Marsili, *Iterative Partial Equalization of Orbital Electronegativity - a Rapid Access to Atomic Charges*, Tetrahedron 36 (1980), pp. 3219-3228.

[30] R.M.V. Abreu, H. Froufe, M.J.R.P. Queiroz and I.C.F.R. Ferreira, *MOLA: a method for automation of parallel virtual screening using AutoDock 4/Vina in multiplatform non-dedicated computer clusters*, J. Cheminformatic (2010) Submitted.

[31] M. Numazawa, M. Oshibe, S. Yamaguchi and M. Tachibana, *Time-dependent inactivation of aromatase by 6 alkylandrosta-1,4-diene-3,17-diones, Effects of length and configuration of 6-alkyl group*, J. Med. Chem. 39 (1996), pp. 1033-1038.

[32] H. Ishida, N. Sato, J. Hosogi, H. Tanaka and T. Kuwarabara, *Inactivation of recombinant human steroid sulfatase by KW-2581*, J. Steroid Biochem. Mol. Biol. 108 (2008), pp. 17-22.

[33] G. Mendoza-Hernandez and M. Calcagno, H.R. Sanchez-Nuncio and J.C. Diaz-Zagoya, *Dehydroepiandrosterone is a substrate for estradiol 17beta-dehydrogenase from human placenta*, Biochem. Biophys. Res. Commun. 119 (1984), pp. 83-87.

Table 1

Estimated and experimental values of Km (nM) and ΔG (Kcal/mol) for the natural ligand of Aromatase, Estrone Sulfatase and 17β-HSD1.

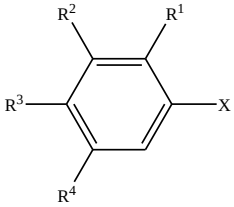
Enzyme	**Aromatase (PDB: 3EQM)**	**Estrone Sulfatase (PDB: 1P49)**	**17β-HSD1 (PDB: 1FDT)**
Ligand	Androstenedione	Estrone Sulfate	Estrone
Experimental ΔG (Kcal/mol)	-10,51	-7,04	-9,44
Estimated ΔG (Kcal/mol)	-11,46	-8,7	-9,02
ΔG difference	-0,95	-1,66	0,42
Experimental Km (nM)	20 [31]	6850 [32]	124 [33]
Estimated Km (nM)	4	416	238
pKm diference*	0,6	1,3	0,3
RMSD**	0,08 Å	-	0,66 Å

*pKm diference = (Estimated pKm – Experimental pKm).
**RMSD: Root Mean Square Deviation.

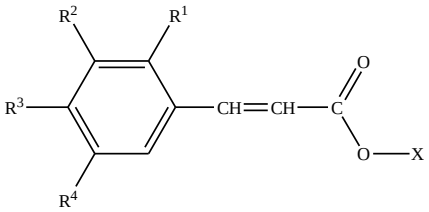
Table 2

Docking studies with phenolic acids found in wild mushrooms.

Benzoic acids



Cinnamic acids

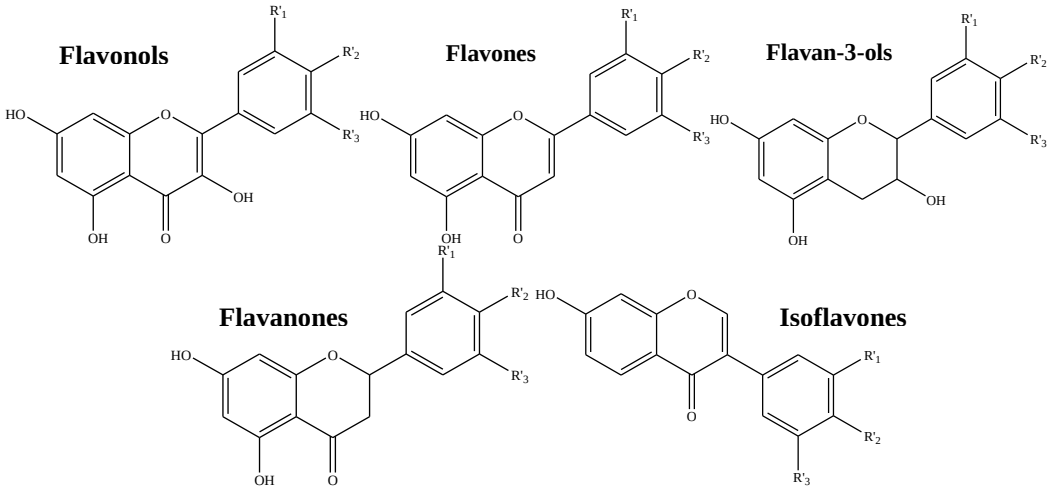


Benzoic acid derivatives	Substitution					Estimated Ki (μM)		
	X	**R^1**	**R^2**	**R^3**	**R^4**	**Aromatase**	**Estrone Sulfatase**	**17β-HSD1**
p-Hydroxibenzoic	COOH	H	H	H	OH	607.4	278.3	101.6
Protocatechuic	COOH	H	H	OH	OH	365.9	155.0	102.2
Gallic	COOH	H	OH	OH	OH	358.3	129.2	100.6
Gentisic	COOH	OH	H	H	OH	546.1	599.0	578.3
Homogentisic	CH_2COOH	OH	H	H	OH	939.4	583.9	116.4
Vanillic	COOH	H	OCH_3	OH	H	227.0	98.08	89.70
5-Sulphosalicylic	COOH	OH	H	H	HSO_3	219.7	381.8	365.5
Syringic	COOH	H	OCH_3	OH	OCH_3	239.9	212.4	93.68
Veratric	COOH	H	OCH_3	OCH_3	H	309.4	98.04	98.60
Vanillin	CHO*	H	OCH_3	OH	H	213.5	299.4	>1000
Cinnamic acid derivatives	**Substitutions**					**Estimated Ki (μM)**		
	X	**R^1**	**R^2**	**R^3**	**R^4**	**Aromatase**	**Estrone Sulfatase**	**17β-HSD1**
p-Coumaric	H	H	H	OH	H	80.91	28.81	124.6
o-Coumaric	H	OH	H	H	H	78.17	27.27	118.2
Caffeic	H	H	OH	OH	H	57.28	19.48	73.97
Ferulic	H	H	CH_3O	OH	H	26.63	105.5	91.29
Sinapic	CH_3O	H	CH_3O	OH	CH_3O	8.260	122.5	27.10
4-*O*-caffeoylquinic	*	H	OH	OH	H	0.315	0.474	0.289
5-*O*-caffeoylquinic	*	H	OH	OH	H	0.760	3.990	0.255

* The carboxylic group is esterified with quinic acid.

Table 3

Docking studies with flavonoids found in wild mushrooms.

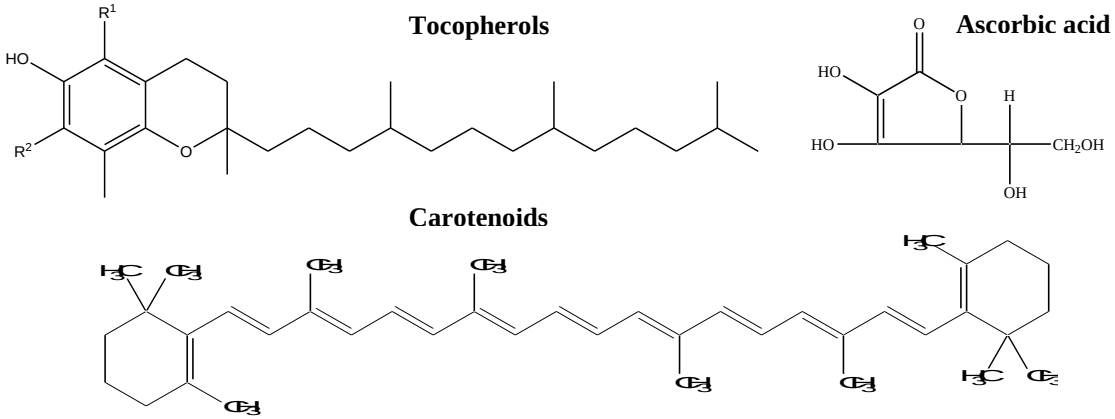


	Substitution			Estimated Ki (μM)		
Flavonols	**R'$_1$**	**R'$_2$**	**R'$_3$**	**Aromatase**	**Estrone Sulfatase**	**17β-HSD1**
Quercetin	OH	OH	H	0.316	4.560	0.092
Rutin*	OH	OH	H	29.80	0.488	0.094
Kaempferol	H	OH	H	1.090	8.960	0.249
Myricetin	OH	OH	OH	0.790	5.620	0.091
Flavones Chrysin	H	H	H	0.610	15.03	0.467
Flavan-3-ols Catechin	H	OH	OH	11.76	11.54	3.210
Flavanones	**R'$_1$**	**R'$_2$**	**R'$_3$**	**Aromatase**	**Estrone Sulfatase**	**17β-HSD1**
Hesperetin	OH	H	H	5.410	7.970	2.310
Naringenin	H	OH	H	0.342	10.67	0.413
Naringin *	H	OH	H	0.743	0.206	0.001
Isoflavones	**R'$_1$**	**R'$_2$**	**R'$_3$**	**Aromatase**	**Estrone Sulfatase**	**17β-HSD1**
Formonetim	H	OCH_3	H	590	17.40	0.571
Biochanin**	H	OCH_3	H	0.710	11.14	0.771

* OH in position-3 is substituted with the disaccharide rutinose; ** OH in position-5.

Table 4

Docking studies with vitamins and carotenoids found in wild mushrooms.



	Substitution		Estimated Ki (μM)		
Tocopherols	**R_1**	**R_2**	**Aromatase**	**Estrone Sulfatase**	**17β-HSD1**
α-tocopherol	CH_3	CH_3	41.23	0.672	0.002
β-tocopherol	CH_3	H	35.38	1.510	0.009
γ-tocopherol	H	CH_3	61.36	0.505	0.010
δ-tocopherol	H	H	59.68	0.882	0.012
Ascorbic acid	-		277.7	85.87	268.8
Carotenoids					
β-Carotene	-		16.27	> 1 000	0.016
Lycopene*	-		> 1 000	5.100	0.0002

*The rings are opened.

Table 5

Docking studies with sugars and fatty acids found in wild mushrooms.

	Estimated Ki (µM)		
Sugars	**Aromatase**	**Estrone Sulfatase**	**17β-HSD1**
Maltose	1.520	3.340	0.605
Trehalose	9.080	5.650	12.23
Melezitose	> 1 000	> 1 000	> 1 000
Fatty acids			
Myristic acid (C14:0)	10.59	283.8	56.01
Palmitic acid (C16:0)	6.940	157.0	12.04
Stearic acid (C18:0)	2.770	108.6	7.810
Oleic acid (C18:1n9c)	6.070	84.64	5.070
Linoleic acid (C18:2n6c)	1.450	28.18	3.730

Fig. 1. Superimposition of X-ray (sticks and balls representation) and docked conformations (wire representation, black) for: (A) Androstenedione in Aromatase and (B) Estrone in 17β-HSD1. The 10 best docked configurations are represented. The catalytic residues that form hydrogen bonds (traced lines) with the ligands are shown.

Fig. 2. Color coded representation of the best results obtained by molecular docking of the LMW compounds dataset against the 3 protein targets. Colours used are light grey for good inhibition activity (< 0.1 μM), white for moderate inhibition activity (0.1 μM > Ki > 1 μM) and dark grey for weak or no inhibition activity (Ki > 1 μM). Compounds with good or moderate inhibition activity are ordered from best to worst estimated Ki value.

Fig. 3. Docking results of the top ranked inhibitor for each of the studied protein targets. Figure shows (A) Aromatase, (B) Estrone Sulfatase and (C) 17β-HSD1 docked with 4-*O*-caffeoylquinic, naringin and lycopene, respectively. Protein target are represented in cartoon format, docked inhibitor in sticks and balls format (red) and natural X-ray ligands in wire format (blue). Relevant interactions are shown (traced lines).