In silico design of enzyme $\alpha$-amylase and $\alpha$-glucosidase inhibitors using molecular docking, molecular dynamic, conceptual DFT investigation and pharmacophore modelling

Hadjer Chenaafa, Fouzia Mesli, Ismail Daoud, Radja Achir, Said Ghalem, and Abdelhak Neghrah

$^{a}$ Laboratory of Therapeutic Chemistry, Annaba, Algeria; $^{b}$ Department of Pharmacy, Faculty of Medicine, BADJI Mokhtar University of Annaba, Annaba, Algeria; $^{c}$ Department of Chemistry, Abu-Bakr Belk aid University of Tlemcen, Tlemcen, Algeria; $^{d}$ Laboratory of Naturals Products and Bioactive – Lasnabio, Tlemcen, Algeria; $^{e}$ Department of Matter Sciences, Mohamed Khider University of Biskra, Biskra, Algeria

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is characterized by elevated blood glucose levels and can lead to serious complications such as nephropathy, neuropathy, retinopathy and cardiovascular disease. The aim of this work is to identify and investigate the inhibition mechanism of natural flavonoids and phenolics acids against, the $\alpha$-amylase ($\alpha$A) and $\alpha$-glucosidase ($\alpha$G). Therefore, we used different approaches; such as conceptual DFT and pharmacophore mapping in addition to molecular mechanics, dynamics and docking simulations. Whereas, a close agreement was found out to decide that Linarin (Flavones) provides more optimized inhibition of $\alpha$A and $\alpha$G enzymes. Our results have shown that Linarin could be useful as preventative agent, and possibly therapeutic modality for the treatment of metabolic diseases.

1. Introduction

The incidence of type 2 diabetes is increasing at an alarming rate (Fleury-Milfort, 2008). Recent evidence suggests that high postprandial plasma glucose concentration is associated with an increased risk of developing type 2 diabetes and metabolic syndrome. Therefore, control of postprandial blood glucose level has been suggested as an important and effective way to mitigate and prevent the illnesses of hyperglycemia and diabetes.

$\alpha$-Amylase ($\alpha$A) is a key enzyme in the digestive system and catalyzes the initial step in the hydrolysis of starch (Vadivelan et al., 2012). It has been proposed that inhibition of $\alpha$A can effectively control the postprandial elevation of the blood glucose level (Yuan et al., 2014). $\alpha$-Glucosidase ($\alpha$G), which is located in the brush border of the enterocytes of the jejunum, is the most important enzyme in carbohydrates digestion (Proença et al., 2017). It catalyzes the hydrolysis of 1,4-$\alpha$ bonds of the unabsorbed oligo- and disaccharides, and converts them into monosaccharides, namely, glucose, which are absorbed in the upper jejunum, resulting in hyperglycemia. Inhibitors of $\alpha$G can retard the decomposition and absorption of dietary carbohydrates by restricting the breakdown of linear or branched oligosaccharide units like $\alpha$-limit dextrins, maltose and maltotriose to produce glucose, thereby preventing glucose absorption into blood stream and suppress the postprandial hyperglycemia (PPHG) (Proença et al., 2017). Among the therapeutic approach for treating type 2 diabetes is to decrease the (PPHG) by slowdown the intestinal absorption of glucose through the inhibition of the enzymes, $\alpha$A and $\alpha$G (Ali Asgar, 2013). Polyphenols are the important inhibitors of intestinal enzymes which are able to suppress carbohydrate digestion, delay glucose uptake and consequently, reduce blood sugar levels. Although drugs such as acarbose, voglibose and miglitol inhibit $\alpha$G and $\alpha$A in practice, they produce undesired side effects such as bloating, abdominal discomfort, diarrhea and flatulence (Alqahtani et al., 2019). So, it is vital to search for other compounds with acceptable $\alpha$A and $\alpha$G inhibitory activity but without serious side effects. Flavonoids are promising modulators of this enzyme’s activity (Alqahtani et al., 2019).

The study of natural products is one of the strategies for the discovery of new drugs that can be used in type 2 diabetes mellitus therapy. Many phenolic compounds such as gallic acid, tannic acid, morin, luteolin, kaempferol, myricetin, quercetin and apigenin have been shown to exhibit inhibitory effects against $\alpha$A and $\alpha$G (Abdelli et al., 2020; Meng et al., 2016).

The aim of this research was the bioinformatical study of $\alpha$A and $\alpha$G inhibition of PPs in all two main classes: by flavonoids and phenolics acids, compared to acarbose, voglibose and miglitol which are oral drugs inhibitors of those enzymes. The main interest was to develop new potential
inhibitors of the αA and αG interaction and finally discussed with the bioactivity scores, drug likeness, pharmacokinetics, molecular docking and molecular dynamic analysis of major components. The more we know about these interactions, the more we can do with that knowledge. Although many efforts have been made to produce the natural and reliable treatment during the first stage of type 2 diabetes mellitus (T2DM).

2. Material and methods

2.1. Selection of receptors and ligands

In this study, 29 compounds were mainly incorporated; the interaction of best flavonoids and phenolic acids obtained from the simulation of molecular docking with the lowest energy scores from compounds, as described in Table 1, were investigated. The structures of inhibitors (see Supplementary Table 1a) were downloaded from the PubChem server (https://pubchem.ncbi.nlm.nih.gov) (Wang et al., 2017). The PDB database (https://www.rcsb.org/) was used to obtain the complete structures of αA and αG enzymes 4GQR and 3L4Y, respectively (Figure 1). In general, the protein structure with a resolution between 1.5 and 2.5 Å have a good quality for further studies (Clément & Slenzka, 2006; Didierjean & Tete-Favier, 2016) whereas, the resolution value of αA and αG belongs to this interval.

2.2. Molecular docking

At first, we minimized the energy of the obtained 3D structures of the enzymes and the ligands and their geometries were conducted using Hamiltonian AM1 implanted in MOE software and then isolation of the active site of the targets was carried out. The most stable geometry of each ligand was minimized by the same method (AM1) and then, all simulations were run by using all explicit salvation models using TIP3P water.

In the end, positioning the ligands into the enzyme’s active site was done using the docking module implanted in MOE software and, the binding energy (score) between ligands and targets was calculated using molecular mechanics (Molecular Operating Environment (MOE), 2013).

2.3. Molecular dynamics simulation, DFT calculassions, ADMET, PASS cytochromes P450 and pharmacophore mapping

The best conformer of αA and αG enzyme with ligands was subjected to molecular dynamics simulations MD was performed for both the complex (4GQR–Ligand, 3L4Y–Ligand) using the MOE 2014.09 software (Molecular Operating Environment (MOE), 2013). Molecular dynamics (MD) aims to numerically simulate condensed phases of a molecular system in order to understand, predict and calculate the properties of a studying system (Adcock & McCammon, 2006). The stability of the targets with the best selected natural product compound was determined using MD simulations. The MD simulations were conducted by using Nose–Poincare–Andersen (NPA) equations in 20 ns (Bond et al., 1999; Sturgeon & Laird, 2000). The Berendsen thermostat is an algorithm to rescale the velocities of particles in molecular dynamics simulations to control the simulation temperature (Berendsen et al., 1984). The coordinates were stored every 0.2 ps to get an accurate view of molecular movement. In all simulations the van der Waals cut-out distance was set to 8 Å. Energy minimization process was applied by using MMFF94x force field (Parikesit et al., 2015). The default protocols and steps of the MD were used to optimize the system’s equilibrium for 100 ps and the production run in 600 ps. The MD simulations have been carried out by heating the complex system from 300 up to 330 K (Berendsen et al., 1984). During the production step, the root mean square deviation (RMSD) obtained was observed to determine the stability of the complex (Parikesit et al., 2015).

Here, we have shown the detailed analysis of MD simulation results of best compound LinarinL5 with target αA and αG enzyme were shown in (Figures 6 and 7) because these compounds show better binding affinity for both receptors. To identify the potential of these compounds, molecular dynamics studies were performed for canonical NVT units using MOE. In the end and according to the molecular dynamics simulation analysis among these two compounds, the most active compounds were L5 and L14 in αA and αG enzymes.

The molecular operating environment (MOE) software was used for our study because it has proven its performance in several recent studies; we can cite some example of work: Sadia Naz et al. (2021), Mourad Sitou et al. (2021), Daoud Ismail et al. (2018), Mehreen Ghufran et al. and Mesli Fouzia and Ghailem (2017). Furthermore, the stable conformation obtained in the MD simulation between the best two ligands and αA and αG enzyme was performed by iMDS. IMDS is a fast and easy server for determining and measuring the protein flexibility (Awan et al., 2017; Lopéz-Blanco et al., 2011). It can be used to investigate the values of deformability, eigenvalues, variance, co-variance map and elastic network. After that, The Gaussian 09 software (Frisch et al., 2009) is used for DFT calculation, Becke’s three-parameter exchange potential and Lee–Yang–Parr correlation functional (B3LYP) theory with 6-311 G(d,p) basis set, were used (Lopéz-Blanco et al., 2014; Lopéz-Blanco et al., 2011). The DFT calculation was done for the two best ligand molecules. The result of DFT calculation is summarized in Table 10 and Figure 11.

The molecular structures of the best ligands were analyzed using SWISSADME server (http://www.swissadme.ch/). The results of ADME/T for best ligand molecules are summarized in Table 8. In the drug-likeness property test, Lipinski’s rule of five or not, along with some other properties were predicted. The drug likeness properties of the selected compounds were scrutinized using SWISSADME server as well as the OSIRIS Property Explorer (Filimonov et al., 2014; Geronikaki et al., 1999). The results of drug likeness property analysis are depicted in Table 9. Lipinski’s rule of five (Lipinski et al., 1997), Veber’s rule (Veber et al., 2002), Egan’s rule and Polar Surface area (TPSA), number of rotatable were calculated using SwissADME properties calculation online.
Table 1. Various drugs used in the *in silico* docking studies.

| Drug    | Structure | IC50aG (μM) | IC50αA (μM) |
|---------|-----------|-------------|-------------|
| I- Flavonoids
| Flavones |
| L01 Baicalein | ![Baicalein](image) | 32 | 446.4 ± 7.3 |
| L05 Linarin | ![Linarin](image) | 0.13 | 3.8 |
| Flavonols |
| L08 Fisetin | ![Fisetin](image) | 0.13 | 19.6 ± 6.4 |
| L10 Morin | ![Morin](image) | 4.84 | 23 ± 1 % 200 |
| L12 Gallicatechin | ![Gallicatechin](image) | 119.34 | 328.06 |
| Flavanones |
| L14Dihydrin | ![Dihydrin](image) | 4.20 ± 0.60 | 31.62 ± 2.80 |
| L15Naringenin | ![Naringenin](image) | 0.50 | >0.10 μM |
| Isoflavones |
| L19Daidzein | ![Daidzein](image) | 0.71 | >0.50 μM |
| L20 Genistein | ![Genistein](image) | 0.71 | >0.50 μM |
| II-Phenolic acids
| Hydroxy benzoic acids |
| L21 Protocatechuic acid | ![Protocatechuic acid](image) | 4.11 ± 2.6 | 11.54 ± 0.45 |
| L24Syringic acid | ![Syringic acid](image) | 3.69 ± 2.57 | 44.81 ± 2.57 |
| L26 Sinapic acid | ![Sinapic acid](image) | 6.1 ± 0.8 | 12.3 ± 0.3 |
| Hydroxy cinnamic acids |
| L29 Rosmarinic acid | ![Rosmarinic acid](image) | 1.4 | 10 μM |

Adopted from online PubChem database (accessed on 07.01.2014). Adopted from online CHEMBL database (accessed on 13.11.2013).
The PASS approach has been previously reported in detail ((Filimonov et al., 2014), and there are also many publications where PASS predictions were approved by subsequent synthesis and biological testing (Filimonov et al., 2014; Singh et al., 2014). The PASS (Prediction of Activity Spectra for Substances) predictions of the two best selected ligands were conducted using PASS-Way2Drug server.

In the PASS prediction study, both possible biological activities of the selected ligands were predicted. Table 1 lists the results of the PASS prediction studies. The P450 sites of metabolism (SOM) of the three selected ligand molecules were determined by online tool, RS-WebPredictor 1.0 (Release Maestro, version 11.8, 2018). The LD50 values of the best three ligands were determined from admetSAR server. The server predicts the acute oral toxicity class of a compound and the LD50 value can be derived from the predicted category (Lee et al., 1988). Table 13 lists the results of P450 SOM study.

The pharmacophore mapping study of the three best ligands was carried out by online server PharmMapper (Parr & Yang, 1989). The pharmacophore mapping experiment was done for the two best ligand molecules among the 29 selected ligands used for the creation of new drugs (Figures 14 and Table 13). The pharmacophore modelling of the two best ligands was performed using MOE software (Molecular Operating Environment (MOE), 2013). However, the PASS prediction, P450 SOM prediction, pharmacophore mapping and modelling, solubility prediction and DFT calculations were carried out to determine and compare the biological activities of the two best ligands.

3. Results and discussion

The enzyme’s active sites with co crystallization molecule are shown in Figure 1. The ligands’ flavonoids and phenolic acids minimized toxicity and energy obtained by MOE software is shown in Table 2 and for the properties of the other ligands see Supplementary Table 2a.

As stated in Table 2, we find that molecules L05 and L14 have a low value of log P and log S compared to other molecules and also the results obtained show that these ligands (L05 and L14) have a low value of torsion angle relative to other compounds. As well, we note that the growth of the torsion angle depends on the binding number of the molecule.

3.1. Affinity of compounds with two targets

Results of docking calculations and bonds between atoms of best compounds and residues of the active site are given in Table 3.

3.2. Molecular docking analysis

3.2.1. Interaction with αA

We note that the result obtained (Tables 3 and 4), out of the best compounds studied, Linarin (Ligand 5) (Figure 2) was predicted to be the strongest αA enzyme binder that forms a complex with the most stability with the lowest energy −8.386 kcal/mol and that interacted with a four amino acids (HIS 299, ASP300, GLU233 and THR163) strong or low, and

Table 2. Some properties of best compounds for anti-diabetic drug.

| Ligand Compounds | Toxic | LogP | Energies (kcal/mol) | LogS | Hdon + Hacc | TPSA |
|------------------|-------|------|---------------------|------|-------------|------|
| L01 Baicalein    | No    | 2.42 | 3.97320e + 001      | −3.46| don3; acc4  | 86.99|
| L05 Linarin      | No    | −0.95| 1.65973e + 002      | −3.89| don7; acc13 | 217.97|
| L08 Fisetin      | No    | 2.31 | 6.34194e + 001      | −3.14| don4; acc5  | 107.22|
| L10 Morin        | No    | 2.01 | 5.06763e + 001      | −2.77| don5; acc6  | 127.45|
| L12 Galloctechin | No    | 1.35 | 5.57867e + 001      | −1.37| don6; acc7  | 130.61|
| L14 Didymin      | No    | −0.77| 1.51010e + 002      | −2.88| don7; acc14 | 214.06|
| L15 Naringenin   | No    | 2.61 | 4.96034e + 001      | −2.45| don3; acc5  | 86.99 |
| L19 Daidzein     | No    | 2.71 | 5.19326e + 001      | −3.54| don2; acc3  | 66.76 |
| L20 Genistein    | No    | 2.42 | 4.48374e + 001      | −3.17| don3; acc4  | 86.99 |
| L21 Protocatechuic acid | No | 0.80 | 1.16516e + 001 | −0.63 | don3; acc4 | 77.76 |
| L22 Syringic acid| No    | 1.11 | 3.84830e + 001      | −1.09| don2; acc5  | 75.99 |
| L26 Sinapic acid | No    | 1.51 | 3.54787e + 001      | −1.60| don2; acc5  | 75.99 |
| L29 Rosmarinic acid | No | 1.76 | 4.24150e + 001 | −2.65 | don5; acc7 | 144.52 |

Figure 1. The active site of the isolated enzyme α-amylase and α-glucosidase.
the existence of two electric force (GLN63, ASP197), suggesting that Linarin can inhibit αA enzyme and interfere with HIS 299, ASP300, GLU233 and THR163. We note that the interactions between the residue of the active site of 4GQR and Linarin formed a stable complex with a strong interaction (H-donor). 

The second best binder was Didymin (Ligand 14) with the energy of −8.007 kcal/mol. The results of second best compounds bonds is shown in Tables 3 and 4 that interacted with a six amino acids (GLU233, ASP197, ARG195, HIS201, TRP59 and GLN63) at a distance of (2.92, 2.74, 4.18, 3.16, 2.69 and 2.88 Å) strong or low interaction, with the existence of four electric force (ASP542, ASP203, ARG526 and TRP539). Results of energy balance of best complexes formed with two targets are given in Table 4. For the Energy of the other compound see Supplementary Table 4a (see Figure 5).

The molecules that had the lowest binding energy of docking score were considered the best molecule and inhibiting the target receptor as the lower binding energy corresponds to higher binding affinity (Simon et al., 2017). We compared all the values score complexes; we found that complex formed by (4GQR–Linarin, 3L4Y–Linarin) L5 has the lowest value energy and gives the best docking score compared to Didymin L14 inhibitor and Genistein L20. Therefore, we can consider that the two complexes (4GQR–Linarin, 3L4Y–Linarin) and (4GQR–Didymin, 3L4Y–Didymin) are stable with higher binding affinity. According to these docking results, we can classify Linarin L5 as the good inhibitor of the enzymes, αA and αG compared to the all ligands studied.

### 3.2.2. Interaction with αG

We note that Linarin (Ligand 5) (Figure 4) was predicted to be the strongest αG binder that formed a complex with the most stability with the lowest energy −6.638 kcal/mol that interacted with a four amino acids (ASP327, HIS600 and ASP443 at a distance of 2.76, 3.02, 2.74 and 3.19 Å) strong or low interaction respectively, with the existence of four electric force (ASP203, ASP542, ARG526 and TRP539), suggesting that Linarin can inhibited αG and interfere with ASP327, HIS 600 and ASP443. We note that the interactions between the residue of the active site of 3L4Y and the ligand formed a stable complex with a strong interaction (H-acceptor and H-donor).

### 3.3. Molecular dynamics analysis

#### 3.3.1. Thermodynamic properties

For the thermodynamic properties using the MD simulation approach, we have studied the evolution thermodynamic properties of ligand 5 and ligand 14 in NVT ensemble (see Table 5).
Table 4. Results of energy balance of best complexes formed with anti-diabetic drug molecules.

| Targets | Chemical Structure | Binding energy (kcal/mole) | Rmsd -refine | Energy-Conf | Energy-Place | Energy-Refine |
|---------|--------------------|-----------------------------|---------------|-------------|--------------|---------------|
| 4QGR    |                    | -4.660                      | 1.118         | -31.547     | -67.792      | -12.756       |
| 3L4Y    |                    | -4.750                      | 0.674         | 30.279      | -90.878      | -14.474       |
| Baicalein L01 | ![Baicalein](image) |                            |               |             |              |               |
| 4QGR    |                    | -8.386                      | 1.663         | 217.4777    | -101.815     | -35.474       |
| 3L4Y    |                    | -6.638                      | 3.572         | 234.822     | -68.026      | -10.996       |
| Linarin L05 | ![Linarin](image) |                            |               |             |              |               |
| 4QGR    |                    | -4.917                      | 1.893         | 40.060      | -93.245      | 12.622        |
| 3L4Y    |                    | -4.978                      | 1.123         | 39.057      | -80.057      | -13.020       |
| Fisetin L08 | ![Fisetin](image) |                            |               |             |              |               |
| 4QGR    |                    | -5.111                      | 5.117         | 31.272      | -102.046     | -19.133       |
| 3L4Y    |                    | -4.688                      | 2.055         | 35.000      | -103.729     | -7.355        |
| Morin L10 | ![Morin](image) |                            |               |             |              |               |
| 4QGR    |                    | -4.817                      | 2.595         | 17.979      | -88.556      | -17.873       |
| 3L4Y    |                    | -5.1024                     | 2.099         | 20.011      | -72.242      | -9.245        |
| Gallo catechin L12 | ![Gallocatechin](image) |                            |               |             |              |               |
| 4QGR    |                    | -8.007                      | 1.496         | 221.3972    | -125.053     | -38.649       |
| 3L4Y    |                    | -6.384                      | 3.318         | 224.164     | -85.915      | -14.478       |
| Didymin L14 | ![Didymin](image) |                            |               |             |              |               |
| 4QGR    |                    | -4.539                      | 3.293         | 9.716       | -94.066      | -15.304       |
| 3L4Y    |                    | -4.676                      | 1.463         | 9.5282      | -92.089      | -15.555       |
| Daidzein L19 | ![Daidzein](image) |                            |               |             |              |               |
| 4QGR    |                    | -4.657                      | 1.368         | 30.988      | -59.764      | 14.242        |
| 3L4Y    |                    | -4.344                      | 1.331         | 32.241093   | -76.514      | -8.892        |
| Genistein L20 | ![Genistein](image) |                            |               |             |              |               |
| 4QGR    |                    | -5.218                      | 6.093         | -4.921      | -74.805      | -16.506       |
| 3L4Y    |                    | -5.619                      | 1.928         | -9.069      | -77.703      | -17.148       |

(continued)
In contrast to the complex formed by L14 their energies and enthalpy obtained were low. By against on pressure fluctuations were significant for the complex formed by L5 is of order $0.0025 \rightarrow 0.0524$ which explains the instability of the system by its strong therefore the movement rotational and vibration energy is important oscillation. In regard to variation in the average temperature of translation is fixed as at the outset in considering isochors–isotherms ensemble. In addition, the equations of motion were solved with a constant of integration step $\Delta t = 5E^{-15}$. Therefore, L5 was predicted to be the most interactive system. These results are in total agreement with the docking prediction results (Tables 3 and 4) (see Figures 6 and 7).

### 3.3.2. Structural properties

For the structural dynamics properties using the MD simulation approach, we have studied the evolution structural dynamics of the best ligand molecule L5 (see Table 5) by IMODS.

The normal mode analysis (NMA) of the prepared $\alpha$A–Linarin and $\alpha$G–Linarin, respectively, complex was
illustrated in Figures 8(a) and 9(a). From the molecular dynamics study of the prepared Linarin–αA and αG docked complex, it was clear that the prepared enzyme–ligand complex had quite high eigenvalue of $2.436689 \times 10^{-4}$ and $1.646004 \times 10^{-4}$, respectively, the eigenvalue is illustrated in Figures 8(b) and 9(b), respectively. However, the variance map showed high degree of cumulative variances than individual variances (Figures 8(c) and 9(c)). The co-variance and elastic network map also produced quite satisfactory results (Figures 8(d, e) and 9(d, e, respectively). The two selected ligand molecules can be used as potential agents to treat (T2DM). Overall, in our study, Linarin emerged as the most potent anti-αA and αG agent. However, more in vitro and in vivo researches should be performed on the Linarin best ligands to finally confirm the findings of this study.

3.4. Prediction of the relative global softness

The relative interaction scores (docking results) of Lref, Lig5, and Lig14 (see Tables 3 and 4) in the inhibition were...
Figure 4. (c) The top scoring compound Linarin. (d) A novel inhibitor L-5 identified by molecular docking is shown in the active site.

Figure 5. 2D representations of the best pose interactions between the ligands and their receptor. L5 interaction between Linarinl and αG, L14. Interaction between Didymin and αG, L20. Interaction between Genistein and αG. The 2D representations of the best pose interactions between the ligands and their respective receptors were visualized using Molecular Operating Environment (MOE).
rationalized using the global softness S index. The numerical values of this quantity are given in Table 6.

Linarin (Flavones) (Ligand 5) was predicted to be characterized by a strong electrophilic power index (strong electron acceptor) and a high lipophilicity by providing Didymin (Ligand 14). The relative global reactivity of these systems was justified by means of electrophilic power index. The current study shows that the docking trends of the relative activities of these inhibitors are agree with their predicted electrophilic power. These results are in total synchrony with the docking prediction results (see Tables 3 and 5 and Figure 10).

### 3.5. In silico assessment of the ADME properties and drug-likeness

A computational study of the best compounds was performed for the assessment of ADME properties and is shown in Table 7 and ADME analysis of other compounds is shown in Supplementary Table 7a.

The results showed that compound L5 (Figure 11) and compound L14 have low absorptions. We note that the molecular weight MW of L5 and L14 is in the range of 592.55–594.56 (>500), HBA in the range of 14–15 (>10) and HBD in the range of 7–8 (>5), these large molecules can hardly be administered orally. But, inhaled. An improvement in the lives of millions of diabetics has been proven by inhaling insulin, which is found in the lungs in the form of millions of small particles and can reach the blood stream (https://www.doctissimo.fr/html/dossiers/diabete/articles/9043-diabete-insuline-spray-exubera.htm). So, inhaling our best molecule linarin (flavones) (Ligand 5) can easily reach the blood stream and represents high affinity with αA and αG.

So we can consider our natural Ligand L5 with its proven activity score $-8.386$, $-6.638$, respectively, for αA and αG, as a new inhaled ligand despite not obeying Lipinski’s rule,
because the Lipinski parameter is not a criterion for anchoring. ‘Lipinski rule of 5’ is an ADME filter for choosing the molecule for drug likeness. In addition, to study the binding interaction through docking, it is not necessary to see whether or not the molecule obeys the ‘Lipinski rule of 5’. It was predicted that Linarin (flavones) presents better interaction of $\alpha_A$ and $\alpha_G$ enzymes and consequently can be the best inhaled inhibitor candidate to be investigated in vivo and in vitro.

And in the meantime, we propose Ligand 20 present in (iso-flavones) with its proven activity score $-5.218$, $-5.619$, respectively, for $\alpha_A$ and $\alpha_G$ enzymes as a new oral ligand despite obeying Lipinski’s rule. It has a maximum of 3 H+ donors and 5 H+ acceptor atoms, as shown in Table 7. With respect to
pharmacokinetics, Ligand 20 present in isoflavones confirmed the safety of the compound for oral administration with good skin permeability, which allows applications in topical formulations. It was predicted that Genistein L20 present in (isoflavones) presents better interaction of $\alpha$A and $\alpha$G enzymes and consequently can be the best oral inhibitor candidate to be investigated in vivo and in vitro.

Regarding the absorption parameters, compounds L01, L04, L06, L20 and L19 present a promising oral availability, due to the optimal Caco-2 cell permeability and HIA (>0.9 and >90%, respectively, Table 8), and skin permeability (log $Kp < -2.5$, Table 8).

Minnow toxicity: < −0.3; high acute toxicity, VDSS: < −0.15 low, >0.45 high, BBB: >0.3 cross BBB, < −1 poorly
Table 7. Lipinski’s rule of five for ADME analysis of best inhibitors.

| No | Name          | Molecular weight (g/mol) | Lipophilicity (MLogP) | Hydrogen bond donors | Hydrogen bond acceptors | No. of rule violations | Drug-likeness Lipinski’s rule follows |
|----|---------------|--------------------------|-----------------------|----------------------|-------------------------|------------------------|-------------------------------------|
| 01 | Baicalein     | 270.24                   | 0.52                  | 5                    | 5                       | 0                      | Yes                                 |
| 05 | Linarin       | 592.55                   | -2.76                 | 7                    | 14                      | 3 violations: MW > 500, NorO > 10, NHorOH > 5 | No                                  |
| 08 | Fisetin       | 286.24                   | -0.03                 | 4                    | 6                       | 0                      | Yes                                 |
| 10 | Morin         | 302.24                   | -0.56                 | 5                    | 7                       | 0                      | Yes                                 |
| 12 | Gallicatechin | 306.27                   | -0.29                 | 6                    | 7                       | 1 violation: NHorOH > 5 | Yes                                  |
| 14 | Didymo       | 594.56                   | -2.57                 | 7                    | 14                      | 3 violations: MW > 500, NorO > 10, NHorOH > 5 | No                                  |
| 15 | Naringenin    | 272.25                   | 0.71                  | 3                    | 5                       | 0                      | Yes                                 |
| 19 | Daidzein      | 254.24                   | 1.08                  | 2                    | 4                       | 0                      | Yes                                 |
| 20 | Genistein     | 270.24                   | 0.52                  | 3                    | 5                       | 0                      | Yes                                 |
| 21 | Protocatechuic acid | 154.12 | 0.40 | 3 | 4 | 0 | Yes |
| 22 | Syringic acid | 198.17                   | 0.49                  | 2                    | 5                       | 0                      | Yes                                 |
| 26 | Sinapic acid  | 224.21                   | 0.73                  | 2                    | 5                       | 0                      | Yes                                 |
| 29 | Rosmarinic acid | 360.31 | 0.90 | 5 | 8 | 0 | Yes |

MW, molecular weight; MLogP, logarithm of partition coefficient of the compound between water and n-octanol, n-OH NH donors, number of hydrogen bond donors; n-ON acceptors, number of hydrogen bond acceptors; n-ROTB, number of rotatable bonds.

distributed to the BBB, CNS: > -2 penetrate CNS, < -3 unable to penetrate CNS, Low skin permeability: > -2.5, Caco-2 permeability: > 0.9, human intestinal absorption: > 90.

The overall lecture of Table 12 highlights that compounds L20 oral could be an excellent candidate as drugs or, however, lead to further studies and manipulations. All compounds are not substrates of the renal organic cation transporter 2 (OCT2). Compound L16 did not pass the AMES test, whereas all others do not present any particular toxicity problems. The volume of distribution (VDss) and fraction unbound are two of the most important pharmacokinetic drug parameters. Values of the VDss >0.45 indicate that the drug will be distributed in tissue whereas values < -0.15 indicate that the drug will be distributed in plasma. So, VDss describes the extent of drug distribution, and the fraction unbound describes the portion of free drug in plasma that may extravasate. Compounds 04, 05, 06, 10, 14 and 16 are entirely unable to penetrate the central nervous system (CNS). The absorption and distribution parameters, respectively, have been graphically represented by the extended and renewed version of the Edan–Egg model named Brain OfIntestinaEstimateD (BOILED) permeation predictive model (BOILED-Egg).

The absorption and distribution parameters, respectively, have been graphically represented by the extended and renewed version of the Edan–Egg model named Brain Or IntestinaLEstimateD (BOILED) permeation predictive model (BOILED-Egg) is shown in Figure 12.

The ADMET properties and BOILED-Egg (Figure 12) plot validate compounds 05 and 14 to be unable to pass the brain barrier and have low absorption in the intestines with less bioavailability. All ligand discussed here in satisfy the Lipinski’s rule, except for 05, 14 and which significantly violates three parameters (MW >500, number of hydrogen bond donors >5 and number of hydrogen bond acceptors >10); furthermore, these latter ligands also violate the BOILED-Egg method.

Compound 5 has the highest binding affinity among all the inhibitors, it is not proposed as an orally active drug due to violation of the Lipinski’s rule.

Compound 14, the second best ligand, is not proposed as an orally active drug due to violation of the Lipinski’s rule. The graph showed that ligands 19 are absorbed by the brain. The ligands L1, L8, L10, L12, L15, L20, L21, L22, L26 showed gastrointestinal absorption within acceptable limits, except for ligands L5 and L14 (TPSA: 217.97, 214.06 Å²), respectively (see Table 2) (TPSA > 131.6). Analysis of the interactions with the protein–ligand interaction profiler between tree enzyme (αA and αG enzymes) and ligand 5 revealed the best binding affinity.

By analyzing the drug’s score (S-value), ligand 5 showed the lowest S-value (~ 8.386, ~ 6.638) respectively for (αA and α-G enzymes), resulting as the best ligand among our selected ligands to inhibit the activity of enzymes αA and αG. However, it violates three parameters (MW >500 D, H-bond donors >5 and H-bond acceptors >10) of the Lipinski’s rule, as well as Egan’s parameters (1 violation: TPSA > 131.6). Although ligand 5 is proposed to be a potential therapeutic inhibitor of αA and αG enzymes, it may fail as an orally active drug because it deviates from the Lipinski’s rule and from the Egan’s rule, these large molecules can hardly be administered orally. But, inhaled. Indeed, further analysis indicates that drugs well beyond the limits of the rule of five, including hydrophilic macromolecules, can be administered by inhalation (Attique et al., 2019). Lipinski explicitly warned in his paper that the Rule-of-Five does not apply to natural products (https://www.sciencedirect.com/topics/nursing-health-professions/lipinskis-rule-of-five). In recent years, however, a number of macromolecular drugs that flagrantly violate the Rule of Five have been efficiently propagated, via
the lung, such as insulin, due to the high permeability of alveolar epithelium (Petersson et al., 1988).

In this study, ligand 20 was predicted as the best targets inhibitor (with maximum binding affinity for two targets after L5) to be used as a potentially therapeutic orally active drug.

In addition to the Lipinski rule of five, other four drug-likeness rules named Ghose, Egan, Veber and Muegee, have been contemporarily satisfied by compound L20 with the exception of molecule L05 and L14. The results of drug likeness property analysis are summarized in Table 9.

Instead, the stringent lead-like criteria of Teague have been passed by compounds L20. Since lead-likeness tests are intended to provide leads with high affinity in high-throughput screens that allow for the discovery and exploitation of additional interactions in the lead-optimization phase, molecule L20 is excellent candidate to be investigated based on scaffold hopping approach. Finally, the outcome of the pan assay interference structures (PAINS) model, conceived to exclude small molecules that are likely to show false positives in biological assays, post no alert for the compound L20, concerning the presence of a molecule moiety.

On the other hand the two rules of Ghose et al. (1999), Egan et al. (2000), Muegee et al. (2001) are verified only for Genistein drug.

Two molecule is predicted not absorbed and not BBB permeant because outside of the range of the plot: L05 with a TPSA of 217.97 Å² and L14 with TPSA of 214.06 Å² (1 violation: TPSA > 131.6) (see Figure 12 and Table 2).

### 3.6. Pharmacokinetics and medicinal chemistry properties

The results of Medicinal Chemistry and Pharmacokinetics revealed that all compounds have high GI absorptions. We notice that there was a complement between our results for assessment of ADME properties (Table 9) and the predicted results in medicinal chemistry and pharmacokinetics (Table 10).

The results of Medicinal Chemistry and Pharmacokinetics showed that compound L5 and compound L14 have low GI absorptions. We notice that there is a correlation between our results found by B3LYP/6-311G(d, p) (Table 11) and the predicted results in medicinal chemistry and pharmacokinetics (Table 10).

Didymin (Flavanones) (Ligand 14) is predicted to be characterized by a high lipophilicity and by high coefficient of skin permeability log $K_p$ by providing Linarin from the Flavones (Ligand 5). We can conclude that the more negative the log $K_p$ (with $K_p$ in cm/s), the less the molecule is permeable to the skin (Kumbasar et al., 2015) which explains the reliability of our results. We quote, our previous research which confirms the stability of complexes and their affinities by MOE software (Fouzia & Salim, 2019; Mesli et al., 2019).

So, Ligand L5 represents high affinity with $\alpha$A and $\alpha$G enzymes. Synthetic accessibility (SA) is a major factor to take into account in this selection process an acceptable value between 6.27 and 6.43 for ligands L14 and L5, respectively, these are more promising molecules which can be synthesized or subjected to bioassays or other experiments. According to its pharmacokinetic properties (Figure 13), L20 present in Isoflavones showed a high level of gastrointestinal absorption which contributes to good oral bioavailability.

The pink area represents the optimal range for each properties (lipophilicity: XLOGP3 between $-0.7$ and $+5.0$, size: MW between 150 and 500 g/mol, polarity: TPSA between 20 and 130 Å², solubility: log S not higher than 6, saturation: fraction of carbons in the sp$^3$ hybridization not less than 0.25, and flexibility: no more than 9 rotatable bonds.

L5 was found less suitable to be proposed as oral drug because they violated at least two criteria, polarity and size in case of Linarin (L5) (MW >500 g/mol, polarity: TPSA >130 Å²).

The compound Genistein showed violation of only one criterion, that is, insaturation (L20), saturation: fraction of carbons
in the sp³ hybridization less than 0.25, as observed in oral bioavailability radar (Figure 13), and its skin permeation coefficient (6.05 cm/s) was also noticeably more than two top scored compounds L5 and L14. The order of skin permeation ability (log Kp value) of the ligands was as allows: L20 (6.05 cm/s) > L5 (9.56 cm/s) > L14 (10.40 cm/s), and a small log Kp value represents less cutaneous permeability. Ligand 5 has a maximum of 7H⁺ donors and 14H⁺ acceptor atoms, as shown in Table 7. According to its pharmacokinetic properties, ligand 5 showed a low level of absorption, skin permeability, and distribution.

All results have been obtained from the pkCSM web server. Green = good, yellow = tolerable, red = bad.
gastrointestinal absorption which contributes to bad oral bioavailability. But, inhaled.

Ligand 5 according to pharmacokinetic parameters evaluated in silico showed no inhibition of cytochrome P450 iso- 
ers 1A2.

Ligand 20 has a maximum of 3H+ donors and 5H+ acceptor atoms, as shown in Table 7. According to its pharmacokinetic properties, ligand 20 showed a high level of gastrointestinal absorption which contributes to good oral bioavailability.

Ligand 20 can inhibit CYP1A2, inhibition of these isoenzymes is certainly one major cause of pharmacokinetics- 
related drug–drug interactions leading to toxic or other unwanted adverse effects due to the lower clearance and accumulation of the drug or its metabolites. However, it should be noted that inhibition of CYP1A2 activity in vitro

Table 9. Drug-likeness, lead-likeness and PAINS parameters of best compounds.

| Ligands | L05 | L14 | L06 | L20 | L12 | C08 | C02 | C22 | C24 | C03 | L10 | L19 | L15 | L9 |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Drug-likeness | Lipinski violations | 3 | 3 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Ghose violations | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Veber violations | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Egan violations | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Muegge violations | 3 | 3 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Lead-likeness violations | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| PAINS alerts | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |

Table 10. Pharmacokinetics and medicinal chemistry properties for tree top-scoring lead compounds.

| Molecules | Pharmacokinetics | Medicinal chemistry |
|-----------|------------------|---------------------|
| GI absorption | Log \( K_p \) (skin permeation) (cm/s) | Lead-likeness | Synthetic accessibility |
| L5: Linarin | Low | –9.56 | No; 1 violation: MW > 350 | 6.43 |
| L14: Didymin | Low | –10.40 | No; 1 violation: MW > 350 | 6.27 |
| L20: Genistein | High | –6.05 | Yes; 0 violation: MW ≤ 350 | 2.87 |

Table 11. The PASS prediction results showing the biological activities of the best two molecules.

| Sl. no. | Biological activities | Linarin L5 | Genistein L20 |
|---------|-----------------------|------------|---------------|
|         | Predicted LD50: 5000 mg/kg | Predicted LD50: 2500 mg/kg |
|         | Toxicity class: 5 | Toxicity class: 5 |
| 01 | Vasoprotector | 0.987 | 0.000 | 0.822 | 0.004 |
| 02 | CYP1A1 inhibitor | 0.970 | 0.001 | 0.893 | 0.004 |
| 03 | Free radical scavenger | 0.976 | 0.001 | 0.458 | 0.013 |
| 04 | Membrane integrity agonist | 0.970 | 0.002 | 0.913 | 0.008 |
| 05 | Anticarcinogenic | 0.954 | 0.001 | 0.715 | 0.008 |
| 06 | Chemopreventive | 0.952 | 0.002 | 0.648 | 0.004 |
| 07 | Hepatoprotectant | 0.932 | 0.002 | 0.680 | 0.008 |
| 08 | TP53 expression enhancer | 0.856 | 0.007 | 0.824 | 0.009 |
| 09 | Antioxidant | 0.815 | 0.003 | 0.765 | 0.004 |
| 10 | Antiviral | 0.746 | 0.004 | 0.430 | 0.024 |

Figure 13. Oral bioavailability radars of ligands L5, and L20. Colored zone is the suitable physicochemical space for oral bioavailability.
does not necessarily imply drug interaction in vivo. Further studies will be needed to determine if this L20 can influence the CYP enzyme in vivo.

### 3.7. PASS and P450 site of metabolism (SOM) prediction

The PASS prediction study for best ligands was conducted to predict 10 intended biological activities. To carry out the PASS prediction experiment, *P* > 0.7 was kept, since this threshold give highly reliable prediction (Release, 2018). The PASS prediction results of all the best selected ligands are listed in Table 11. However, at *P* > 0.7, the LD50 and toxicity class was predicted using ProTox-II server (Pearson, 1986). When analyzed by the admetSAR server, both Linarin and Genistein were predicted to have the acute oral toxicity class of 5, for this reason, they had the same predicted LD50 value (greater than 2000 mg/kg but less than 5000 mg/kg) (Becke, 1988). Prediction tests were also carried out for 10 biological activities, the results of which are in Table 11. We observe that all the compounds show some biological activities among the 10 tested.

### 3.8. Pharmacophore mapping

The possible sites of metabolism by CYPs 1A2, 2A6, 2B6, 2C19, 2C8, 2C9, 2D6, 2E1 and 3A4 of two best ligands...
Linarin L5 and Genistein L20 are summarized in Table 12. The possible sites of a chemical compound, where the metabolism by the isoforms of CYP450 enzymes may be taken place, are illustrated by circles on the chemical structure of the molecule (Zaretzki et al., 2013). So the Linarin L5 and Genistein L20 can be metabolized by these enzymes.

The P450 SOM predictions showed that Linarin L5 had six sites of metabolism (SOMs) for the CYP450 2B6, CYP450 2C8, CYP450 2C9, CYP450 2D6, CYP450 2E1, CYP450 3A4 and five sites for CYP450 1A2, CYP450 2C19.

The P450 SOMs predictions showed that Genistein L20 had five sites of metabolism (SOMs) for the CYP450 1A2, 2A6, 2B6, 2C19, 2C8, 2C9, 2D6, 2E1 and 3A4.

The pharmacophore mapping is conveyed for the Linarin L5 and Genistein L20 best inhaled ligand and oral, respectively, of the flavonoids showed for Linarin 2 hydrogen acceptor bonds, six hydrophobic groups and three aromatic rings and for Genistein 2 hydrogen acceptor bonds, eight hydrophobic groups and three aromatic rings. It also generated a good number of good contacts with the pharmacophore of αA and αG.

Figures 14 and 15. The pharmacophore of Linarin generates a hypothesis which can be applied successfully in biological screening for further experiments (Dixon et al., 2006).

Validation of our results, for flavonoids and phenolics acids different Immunotherapy (Clinic) is mentioned in Table 13.

Our molecular docking results with αA and αG coincide with clinical results; the flavonoids compounds were the most dominant.

These three molecules (Acarbose, Miglitol and Voglibose) are pseudo-carbohydrates that competitively inhibit enzymes, αG and αA.

Our ligands natural Linarin L5 better stabilizes the system with its energy of αA and αG $-8.3867$ kcal mol$^{-1}$ and $-6.638$, respectively, we compare with the components of Clinical Drugs (see Table 13).

Linarin (LigandS) could be excellent candidate as drugs because represents better energy (see Table 8) for all ligand molecules and the volume of distribution (VDss) suggest that the drug will be distributed in tissue. And again from the Table 5, Linarin L5 was characterized by a high lipophilicity and high coefficient of skin permeability log $Kp$. 

Figure 14. Pharmacophore mapping of Linarin L5. Here, cyan color – hydrogen bond acceptor, orange – aromatic, green color – hydrophobic.

Figure 15. Pharmacophore mapping of Genistein L20. Here, cyan color – hydrogen bond acceptor, orange – aromatic, green color – hydrophobic.
Therefore, we propose ligand 5 as the best ligand which allows the inhibition of $\alpha$A and $\alpha$G. And in the meantime, we suggest ligand L5 Linarin present in (flavonoid) with its validated activity score ($/C_0 8.386$, $/C_0 6.638$) respectively for $\alpha$A and $\alpha$G as a new inhaled ligand.

At the same time, we suggest ligand L20 Genistein present in Isoflavones as an oral inhibitor despite obeying Lipinski’s rule without side effects. The latter has the same activity score $-5.61986256$ as Acarbose with $\alpha$G. The present bioinformatic analysis molecular dynamics simulations used to scrutinize novel (flavonoids). Linarin inhibitor of enzyme ($\alpha$A and $\alpha$G). Preceding studies has indicated that Linarin has been shown to have antidiabetic activity.

In vitro, many studies were focused on the inhibitory effect of the phenolic substances in two black legumes of different genera and the Mesocarp Tissue Extracts of Sugar Date Palm, on key enzymes linked to diabetic therapy, $\alpha$A and $\alpha$G.
The researches of Tan et al. (2017) proved that Myricetin exhibited the most potent αG inhibitory activity with IC50 (0.87 mg/mL = 2.73 mM), and had the highest inhibition for αA activity with IC50 values (1.19 mM = 0.38 mg/mL) lower than the commercial αA inhibitor (3.23 mg/mL).

The study of Das et al. (2017) showed the high inhibitory activities with IC50 values of IC50 = 1.42 μM for αA and IC50 = 1.8 μM for αG, IC50 = 0.23 μM for αA and IC50 = 4.95 μM for αG, IC50 = 0.52 μM, and 4-hydroxycinnamic namic amylase and IC50 = 2.58 μM for αG) and IC50 = 4.91 μM for α-Gacid (IC50 = 203 μM) inhibited only αG for caffeic acid, 3,4-dihydroxy benzoic acid, Ferulic acid, 4-hydroxycinnamic and quinic acid, respectively (see Figure 16).

Dabur et al. (2018) reported that 5,6,7-trihydroxyflavone (baicalein), isolated from Scutellaria baicalensis acts as inhibitor of rat intestinal αG (IC50 = 32 μM).

Jeong et al. (2012) reported that γ-aminobutyric acid and ferulic acid, isolated from Tricitum aestivum. L. Sprouts as inhibitor activities with IC50 values of 5.4 ± 0.2 and 9.5 ± 0.1 mM, respectively, against αA, and 1.4 ± 0.4 and 4.9 ± 0.3 mM against αG.

In our research the software package (MOE) does not detect any mark of the hydrophobic interactions between Linarin and both the αA and αG; what may be connected to the large size of this compound and the high number of torsion angles.

The results are identified to have inhibitory activities against novel αA and αG. Of these ligands, Linarin (ligand 5) has a stronger bond and high absorption in the intestines with good bioavailability. Therefore, the study carried out in this research reveals many secrets conveyed by the use of magic plants. Currently, herbal medicine offers solutions to heal with plants. It is a solution that is both alternative and complementary to the treatments of classical medicine, which are more and more popular and whose effectiveness is increasingly recognized.

4. Conclusion

In this investigation, the inhibition of αA and αG was theoretically examined by molecular docking analyses MD simulations, in silico assessment of the ADME and DFT calculation. Our calculations showed that the natural inhibitor Linarin of (flavones) provide more optimized inhibition of α-A and α-G for type-2 diabetic treatment. These interactions between αA and αG and those inhibitors are undergoing different interactions between (H-donor, H-acceptor and Pi-H) of natural ones. This model shows a considerable decrease of the complex energy and there by an increase of the inhibition activity. However, the docking simulation results are optimized under dynamic conditions by MD simulations to prove the stability of the interaction between both proteins and each ligand. Although compounds L5 and L14 have binding affinity with α-A and α-G in the docking simulation, the ligand-protein interactions mentioned in docking simulation are almost stable in dynamic conditions. Linarin inhibitor L5 has a stronger bond and high affinity with α-A and α-G. We can conclude that Linarin may be considered as effective αA and αG anti-diabetic drugs.

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Disclosure statement

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