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INTRODUCTION Glimepiride (GMP) is often combined with metformin HCl (MET) as an oral antidiabetic in type II diabetes mellitus (T2DM), which provides complementary and synergistic effects with the dual goal of improving insulin secretion and insulin action in tissues1. Glimepiride includes in biopharmaceutics classification system (BCS) class II, which has low solubility but high permeability with practically insoluble solubility data in water, so that it will have an impact on the small bioavailability of the drug.

In contrast, MET includes in BCS class III, which has a high solubility in water, but has low permeability, which is about 50-60% absorbed in the gastrointestinal tract given orally2,3. Sanofi Aventis has produced GMP and MET in a fixed-dose combination (Amaryl M®) tablet dosage form, which is an innovator product4. However, some pharmaceutical manufacturers that make copy product of GMP and MET are constrained in producing tablet preparations that meet quality requirements so that efforts need to be made to increase the solubility of GMP as well as the permeability of MET by physically interacting GMP with MET through the cocrystallization method5,6.

Cocrystallization is a physical method based on the combination of active pharmaceutical ingredients acting as a host with co-formers acting as guests through hydrogen bonds or Van der Waals in the same crystal lattice7,8. Studies on the identification of the type of interaction between GMP and MET have not been previously reported. For this reason, it is necessary to identify the physical interactions that occur between GMP and MET using thermal analysis differential scanning calorimetry (DSC), the results of which are then constructed in the form of a phase diagram of the GMP-MET binary system9,10. Furthermore, the resulting physical interactions were confirmed by the computational approach using docking simulations methods, molecular dynamics simulations, and MM/PBSA binding-free energy calculations11,12.

MATERIALS AND METHODS Materials The material used were glimepiride (Glenmark, India) and metformin hydrochloride (Hildose, India). The instruments used include DSC-Thermogravimetric analysis (DSC-TGA STA PT1600, LINSEIS Thermal Analysis), analytical scales (Mettler Toledo AG204), vortex mixer (JEIO Tech) and microtube (Eppendorf). The in silico study was conducted with a computer with an Intel® Core i3-6100 CPU @ 2.30 GHz (4 CPUs) specification, 4096 MB RAM, 320 GB hard drive, and VGA Intel HD Graphics 520. The software used includes Quantum ESPRESSO v.6.6, PatchDock web server (https://bioinfo3d.cs.tau.ac.il/PatchDock/php.php), Gromacs 2016.3, VMD 1.9.4, and BIOVIA Discovery Studio Visualizer v16.1.0.15350.

Methods Molecular structure modeling and optimization The molecular structure of GMP and MET was modeled in two-dimensional using the BIOVIA Discovery Studio Visualizer v16.1.0.15350, which downloaded from the PubChem website in National

Center for Biotechnology Information (https://pubchem.ncbi.nlm.nih.gov/) as shown in Figure 1. Optimization of the molecular structure of the GMP and MET was performed using the Quantum ESPRESSO v.6.6 with density functional theory (DFT) B3LYP method based on the 3-21G set13. / a / b Figure 1.

The two-dimensional structure of (a) GMP and (b) MET Glimepiride-metformin complex formation simulations The optimized GMP and MET compounds were then simulated for complex formation. This complex formation simulation was accomplished using the PatchDock web server according to the procedure reported by Fakih et al15. Identification of glimepiride-metformin interactions The molecular interactions formed between GMP and MET molecules were then identified using the BIOVIA Discovery Studio Visualizer v16.1.0.15350 according to the procedure reported by Fakih et al15.

Glimepiride-metformin interaction dynamics Interaction dynamics simulations were performed using Gromacs 2016.3 to observe and identify the stability of GMP and MET. Electrostatic forces were selected using the Particle Mesh Ewald method. Neutralization of the system was carried out by adding Na+ and Cl- ions. Solvation was determined using the TIP3P water model. The simulation preparation stage includes minimization, heating to 310 K, temperature equilibration, pressure equilibration, and a 500 ns production run with a 2 fs timestep15,16.

MM/PBSA end-point binding-free energy calculations The Molecular Mechanics/Poisson-Boltzmann Surface Area (MM/PBSA) binding-free energy calculations were accomplished by the g_mmpbsa package integrated into the Gromacs 2016.3. The polar desolvation energy was calculated using the Poisson-Boltzmann equation with a grid size of 0.5 Å. The dielectric constant of the solvent was set to 80 to represent water as the solvent. The non-polar contribution was determined by calculating the surface area accessible to the solvent with a radius of 1.4 Å17-19.

Preparation of glimepiride-metformin physical mixtures Preparation of the physical mixture of GMP-MET was carried out by weighing GMP and MET at various compositions based on the mole ratio between the two, which was carried out for three replications. It was known that the molecular weights of GMP and MET were 490.62 g/mol and 165.63 g/mol, respectively. Furthermore, thermal analysis was carried out using the DSC method to obtain the melting point of the endothermic peak of the DSC thermogram, which was constructed into a phase diagram of the GMP-MET binary system21.

RESULTS AND DISCUSSION Glimepiride-metformin binary mixtures Preparation of the GMP-MET binary mixture aims to identify the interactions between GMP and MET at

various compositions based on their molecular ratios, whether the cocrystal phase (molecular compound) or a simple eutectic mixture was formed as well as its molecular ratio, as shown in Table I. This binary mixture was thermally analyzed using the DSC method so that the melting point from the endothermic peak of the DSC thermogram was obtained, as presented in Table II.

Then, it was constructed into a binary system phase diagram by plotting the resulting melting points of the endothermic peak of the DSC GMP-MET thermogram at various compositions versus the mole ratio of the two, as presented in Figure 2. The results of the phase diagram analysis of the GMP-MET binary system show a congruent pattern that indicates the formation of cocrystal or molecular compounds. The physical mixture of GMP-MET showed this phenomenon at a mole ratio of 1 : 1 (GM 7), which had two endothermic peaks at a temperature of 196.6°C and 228°C.

228°C was the highest melting temperature between the melting temperatures of GMP and MET of pure forms were 205.8°C and 235.1°C, respectively22. If the two components form the compound of molecular, it would be flanked by two temperature melting compound called eutectic point (TE), TA and TB was the melting temperature of each pure components of GMP and MET, when the temperature was plotted based on the composition of the mixture of components would be obtained a TA-TE-TC-TE-TB track called the liquidus curve. Above the liquidus curve, GMP and MET were in the liquid phase, and the two components of the compound dissolve with each other22,23.

The highest melting point was TC of the liquidus curve, which was the point of formation of molecular compounds, while the lowest melting point is TE of the liquidus curve, which was the eutectic point. At TC point, two components, A and B, were melted together (congruent) without changing the composition of the two components at the same highest temperature and the liquid phase was in equilibrium with the solid phase. Under the liquidus curve, each component A and B was in a solid state and did not dissolve in one another25.

Whereas in the GMP-MET physical mixture, the mole ratio of 1 : 9 (GM 3), 2 : 8 (GM 4), and 3 : 7 (GM 5) also had two endothermic peaks, in which the melting temperature at the second endothermic peak was lower than the physical mixture GMP- MET mole ratio was 1 : 1 (GM 7). Therefore, it was not a point of formation of molecular or cocrystal compounds, but this phenomenon was only partial, meaning that the physical mixture of GMP-MET in these three ratios did not melt together26. Table I. Composition of the GMP-MET binary mixture Sample Code _Mole ratio (GMP : MET) _Weight (mg) _ _ _ _GMP _MET _GMP-MET _ _GM 1 _1 : 0 _490.62 _0 _490.62 _ _GM 2 _0 : 1 _0 _165.63 _165.63 _ _GM 3 _1 : 9 _490.62 _1490.67 _1981.29 _ _GM 4 _2 : 8 _981.24 _1325.04

_2306.28 _ _GM 5 _3 : 7 _1471.86 _1159.41 _2631.27 _ _GM 6 _4 : 6 _1962.48 _993.78 _2956.26 _ _GM 7 _5 : 5 _2453.1 _828.15 _3281.25 _ _GM 8 _6 : 4 _2943.72 _662.52 _3606.24 _ _GM 9 _7 : 3 _3434.34 _496.89 _3931.23 _ _GM 10 _8 : 2 _3924.96 _331.26 _4256.22 _ _GM 11 _9 : 1 _4415.58 _165.63 _4581.21 _ _ Table II. Melting point recapitulation of the endothermic peak of the DSC thermogram GMP-MET binary mixture Sample Code _Mole ratio (GMP : MET) _Melting point (°C) _ _ _ _1 _2 _ _GM 1 _1 : 0 _205.8

_- _ _GM 2 _0 : 1 _235.1 _- _ _GM 3 _1 : 9 _191.2 _223.6 _ _GM 4 _2 : 8 _197.9 _218.4 _ _GM 5 _3 : 7 _201.8 _216.2 _ _GM 6 _4 : 6 _194.4 _- _ _GM 7 _5 : 5 _196.6 _228 _ _GM 8 _6 : 4 _195.7 _- _ _GM 9 _7 : 3 _186.4 _- _ _GM 10 _8 : 2 _198.7 _- _ _GM 11 _9 : 1 _201.5 _- _ _/ Figure 2. Phase diagram of GMP-MET binary systems. TA: Melting point of GMP; TB: Melting point of MET; TC: Cocrystal point; TE: Eutectic point. Mole ratio: 0 = GMP : MET (1 : 0); 1 = GMP : MET (9 : 1); 2 = GMP : MET (8 : 2); 3 = GMP : MET (7 : 3); 4 = GMP : MET (6 : 4); 5 = GMP : MET (5 : 5); 6 = GMP : MET (4 : 6); 7 = GMP : MET (3 : 7); 8 = GMP : MET (2 : 8); 9 = GMP : MET (1 : 9); 10 = GMP : MET (0 : 1) Computational approach of glimepiride-metformin The computational approach was demonstrated to identify and confirm the physical interactions between GMP and MET. Figure 3 shows that the interaction between GMP and MET did not form new compounds.

However, the interaction that occurs was the formation of hydrogen bonds with heterosinton formation (Table III), as well as Van der Waals bonds were minimal, with a total energy of -0.00096 Å and a binding-free energy value of -415.35 kJ/mol. This binding-free energy produces a negative value which indicates a physical interaction between GMP and MET compounds that occurred spontaneously27. Overall poses of GMP and MET complexes changed during the simulation. However, based on the snapshots taken at 125, 250, 375, and 500 ns from the molecular dynamics simulation results, only slight conformational changes were observed (Figure 4).

It was predicted that this phenomenon would increase the ability of the GMP and MET to interact with the active site of the target receptor28. / a / b Figure 3. The three-dimensional (a) and two-dimensional (b) interaction of GMP and MET in docking simulations Table III. Interaction between GMP and MET from docking simulations Glimepiride atom _Metformin atom _Distance of interaction (Å) _Type of interation _ _Oxygen (O2) _Hydrogen (H11) _2.98128 _Hydrogen Bond _ _Oxygen (O2) _Hydrogen (H13) _1.89064 _Hydrogen Bond _ _ // // Figure 4. GMP (red) and MET (green) conformation snapshots at 125, 250, 375, and 500 ns The root-mean-square deviation (RMSD) values of GMP and MET were calculated to ensure the stability and rationality of the selected conformations.

Figure 5 shows that the complex formed fluctuates from 0 ns until 100 and 300 ns. Nevertheless, at the end of the complex simulation, the GMP and MET began to achieve stability29. The average RMSD value during the molecular dynamics simulation was in the range of 2.04 Å. / Figure 5. RMSD value during molecular dynamics simulation The MM/PBSA free-binding energy was calculated based on the trajectory from the beginning to the end of the molecular dynamics simulation.

Based on the MM/PBSA calculation results, it could be observed that the complex system had good binding-free energy, with a value of -107.74 kJ/mol (Table IV). The energies that contribute the most during the simulation were Van der Waals and electrostatic interactions. This was because the MM/PBSA approach allows observation of the influence of the contribution of Van der Waals and electrostatic and conformational changes that were influenced by the solvation process29. Table IV.

Binding-free energy calculation from MM/PBSA ?EVdW (kJ/mol) _?Eele (kJ/mol) _?GPB (kJ/mol) _?GNP (kJ/mol) _?GBind (kJ/mol) _ _-125.03 _-37.45 _66.78 _-12.04 _-107.74 _ _?EVdW: Van der Waals contribution; ?Eele: electrostatic contribution; ?GPB: polar contribution of desolvation; ?GNP: non-polar contribution of desolvation; ?GBind: ?EVdW + ?Eele + ?GPB + ?GNP CONCLUSION The identification results showed the presence of a co-crystal (molecular compound) interaction of glimepiride-metformin HCl at a 1 : 1 mole ratio and the formation of hydrogen bonds with heterosinton formation from docking simulations results which showed in binding-free energy of -415.35 kJ/mol.

Especially, the complex system is stable in molecular dynamics simulations with an average RMSD value of 2.04 Å and a calculated MM/PBSA value of -107.74 kJ/mol.

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