Computational efforts towards the analysis of synthetic preference of cis and trans isomers of the chalcones 4-chlorochalcone, 2-methoxychalcone and 3,4-methylenedioxychalcone.

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ABSTRACT

Thalidomide was introduced to pregnant woman in the late fifties, which caused tragic deformities of newborn children. This was caused by a lack of knowledge of potential differences of biological properties of different isomers. Determining the selection for isomers is important in development of pharmaceuticals because of a potential for unknown or dangerous biological properties when placed in the living media. Developing a better understanding of isomer structural differences can allow chemists to achieve the production of safer products. Computational chemistry is a helpful tool in simulating chemical reactions through computer programming. This allows for the potential analysis of reactions' outcomes without performing them in real laboratory time. The main advantage is the potential to eliminate laboratory experiments that may yield undesired outcomes and avoid waste of chemical materials as well as decrease production of hazardous waste. Overall, use of computational chemistry can allow one to achieve faster screening and undertake a more mechanistically oriented research, providing researchers with an ability of answering practical synthetic questions, such as the formation of isomers. This study focused on isomerism found in chalcones. Chalcones are small organic molecules originally isolated from plants that demonstrated a wide variety of biologically important properties. This study investigates the likelihood of forming cis-chalcone isomers versus trans-chalcone isomers, with trans-chalcones typically being the more favored synthesis. Programs such as GAMESS, Avogadro, and wxMacMolPlt aided in creating and analyzing the reactions. The data revealed that the trans isomer of various chalcones had a more negative change in Gibbs free energy value (ΔG) than the cis isomer, meaning that the formation of trans isomers is a more energetically favorable reaction. However, the difference in free energy values was minimal, so the possibility of forming a cis isomer should not be disregarded. Future studies are encouraged to determine what factors allow the formation of cis-chalcones to be more energetically favorable than trans-chalcones.

INTRODUCTION

Organic molecules containing double bonds can exhibit a phenomena known as cis-trans isomerism [1]. The cis and trans isomers have exact the same composition but differ in a particular arrangement of groups around the double bond (Chart 1). The two compounds are known as stereoisomers and will have different chemical and physical properties. The bigger difference in the substituents around the double bond the larger difference in properties is observed for the resulting compounds, this can have a tremendous impact on the overall bioactivity of organic molecules as the shape recognition and possibility of intermolecular interaction can be severely hindered by a geometric availability of atoms [1]. In addition, physical properties, such as solubility, melting point, boiling point, all have correlation to the internal molecular build [1].

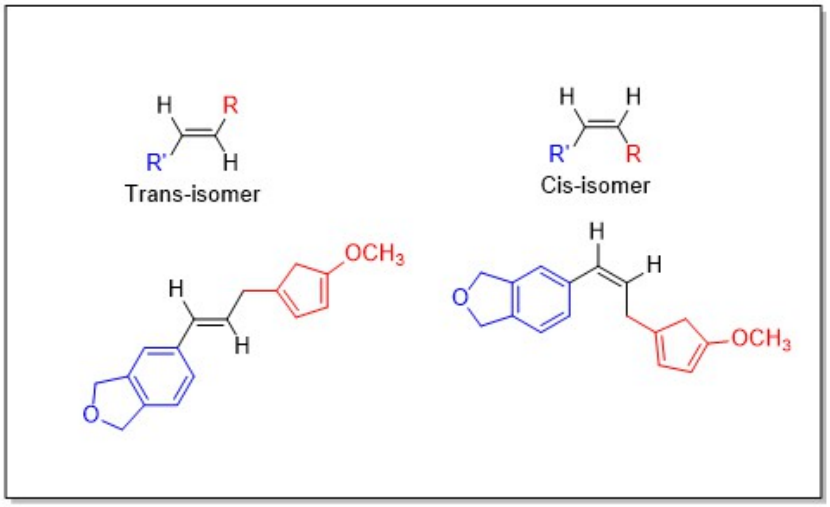
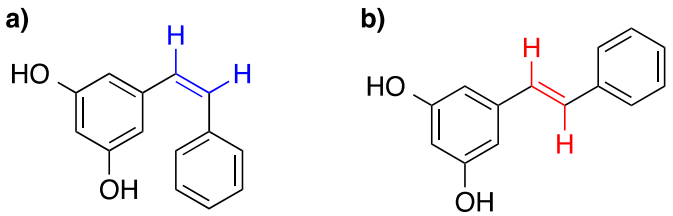


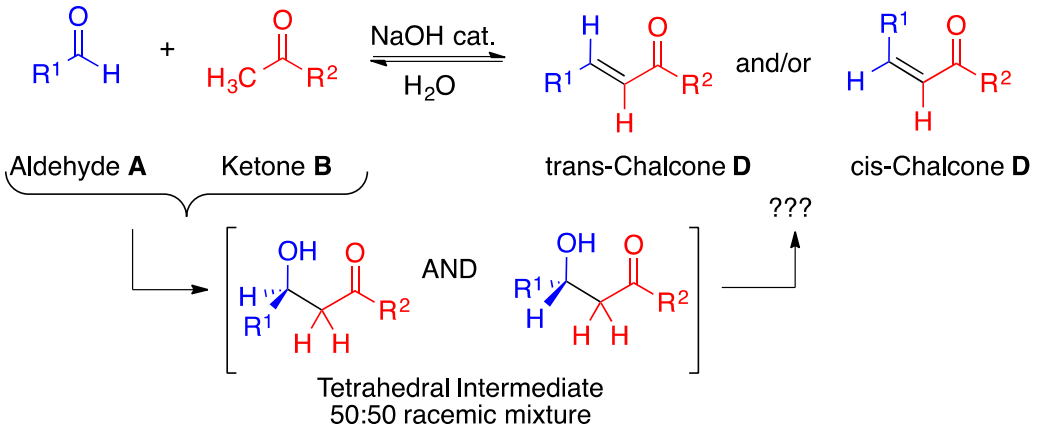
Chart 1. Cis and trans isomerism diagram illustrating 3D differences of resulting compounds [1].

The significant difference between these two stereoisomers raises from the combination of the type of the bridge and its position. The geometry of a double bond places a planar requirement on atoms involved and this is translated to the groups attached. Since the bridging atoms are in the middle of the molecule this has a drastic effect on the overall shape of the molecule. In biological systems or cells, the biologically active organic molecules often interact with 3D enzymes – biological catalysts, and therefore, a significant shift in geometry can alter the enzyme coordination sites and contribute to the differences in bioactivities of certain isomers [1]. One of the few examples of study using a cis-isomer was reported by Song et al. They were able to observe that the inhibitory capacity of 3,5-dihydroxystilbene, an inhibitor in the biosynthesis of melanin the primary pigment in skin, was significantly different for the cis and trans stereoisomer (Scheme 1). In particular, the cis form of the molecule had demonstrated higher bioactivity by a factor of 0.1 mM. This connection to bioactivity is interesting for us given that the general structure of the two compounds is somewhat similar to the chalcones analyzed in this study.



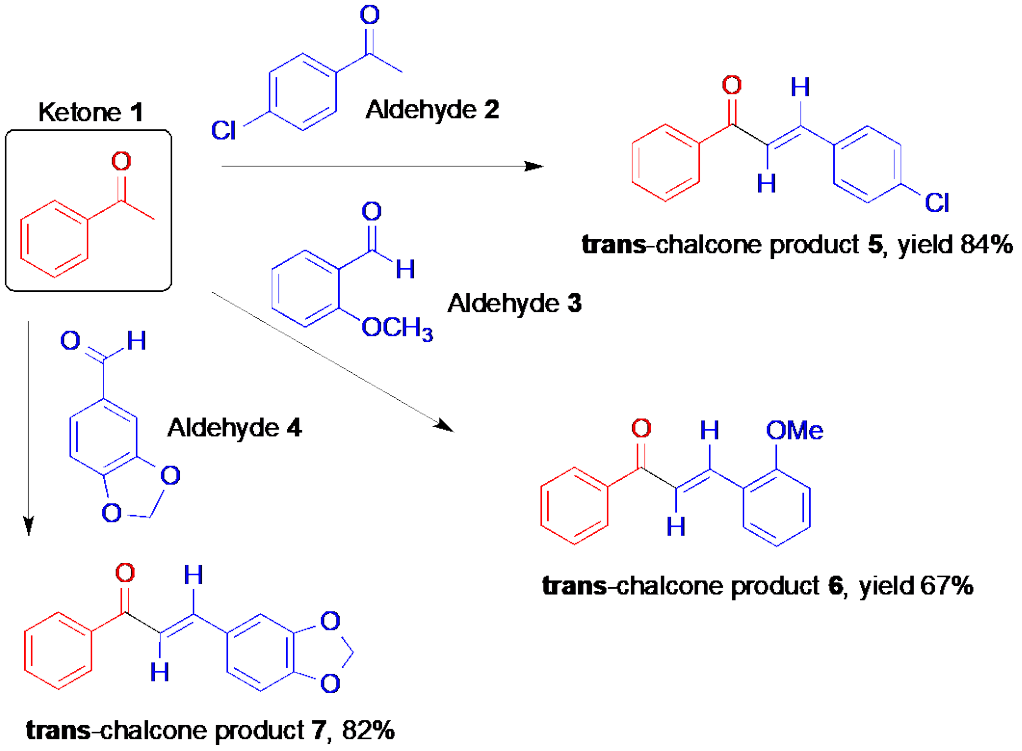
Scheme 1. Structure of a) cis and b) trans 3,5-dihydroxystilbene (Song et al.)

Chalcones are a group of bicyclic compounds that are defined by the presence of two aromatic rings linked by a rigid double bond bridge [2]. Natural chalcones are responsible for the active natural defense mechanism in some plants [3]. Chalcones have been identified as an important structure for medicinal chemistry based on their numerous biological activities which includes anti-cancer and anti-diabetic properties [4, 5]. The syntheses of chalcones are primarily conducted via Claisen-Schmidt condensation (Scheme 2), involving a cross aldol condensation of capable ketones and aldehydes [6]. In a typical reaction an aldehyde is combined with a ketone in a one-pot synthesis along with the base catalyst and solvent. A general reaction is depicted using a general aldehyde compound **A** and a general ketone compound **B** is shown on Scheme 2. The translation of each reagent into the final product composition is emphasized using different color scheme. Prior to the formation of the product the condensation of two starting reagents results in a tetrahedral intermediate **C** which contain a chiral center but will be formed as a racemic mixture since both starting materials are achiral compounds. Although formation of **C** is reversible sequential elimination of water locks the geometry at a double bond either in a trans or a cis arrangement (product **D**) and is not easily changed. In other words, the interchange of trans and cis isomers is not usually observed during the reaction.



Scheme 2. Illustration of a typical synthesis of a chalcone via Claisen-Schmidt condensation reaction.

Although there is a clear geometric difference between chalcone **D** in its trans or cis forms (Scheme 2) studies similar to the one of Song et al. described above are limited as commercial availability of cis-chalcones is limited (Millipore Sigma-Aldrich site was accessed on May 2021). In addition, even if the cis-isomer could demonstrate higher efficacy, there is no potential pathway to synthesize and isolate exclusively the cis product at a cost-effective rate due to similarity in physical and chemical properties the two isomers (Scheme 2). For example, our own previous synthetic efforts towards several target chalcones **5**–**7** using a ketone **1** and three aldehydes **2**–**4** led to successful isolation of only trans-isomers (Scheme 3). No presence of cis-isomers was detected using a variety of analytical techniques, including sample nuclear magnetic resonance, spectroscopical analysis of reaction mixtures, and thin-layer chromatography. All reactions were conducted under same experimental conditions and products **5** – **7** were isolated in moderate to good yields exclusively as trans-isomers.



Scheme 3. Previously collected experimental results using the target compounds, yields represent averages.

We hypothesize that the reason for the observed reactivity lies in the considerable steric hindrance of a tetrahedral intermediate **C** (Scheme 2) that would occur prior to elimination of water and formation of an alkene moiety. Therefore, our group analyzed the thermodynamic possibility of synthesizing cis chalcones with a variety of steric influences. Steric hindrance occurs when a reaction is slowed or does not occur due to the physical spacing of the structure. *Our hypothesis was that with the increase of steric hindrance, i.e., larger substituents on the aromatic rings, the Gibbs free energy would approach more positive values thus preventing spontaneous formation of the corresponding product.* We anticipate that results of our study could support the experimental observations from our previous syntheses of chalcones that led to isolation exclusively trans-isomers. To the best of our knowledge, modeling of starting materials and products that are subjects of this study have not been reported before.

METHODS

To investigate our research question, our group analyzed the Gibbs free energy of the reaction of the two isomers and compared them to one another. Gibbs free energy is a way to quantitatively analyze the spontaneity of a reaction, i.e., the effect of the entropic forces driving the reaction forward [8]. In general, the more negative the change in Gibbs free energy the more likely the reaction will occur at a given temperature and vice versa for a greater positive Gibbs free energy. It should be quickly noted that Gibbs energy is a state function, only examines the initial and final energy values, and cannot be used to glean information on the favorability of the kinetics, the pathway, the reaction may have.

EXPERIMENTAL PROCEDURE

To model the desired molecules, we used the building software Avogadro to construct the cis and trans products as well as the respective ketones and aldehydes as reactants [9, 10]. Once built in Avogadro, we allowed the software to optimize the geometry of the molecule and then optimize the field energies using the MMF94s setting. These optimized molecules were exported as GAMESS input files with the below header for calculations [11].

```
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$CONTRL SCFTYP=RHF RUNTYP=OPTIMIZE ICHARG=0 MULT=1 MAXIT=200 $END
$SCF DIRSCF=.t. DAMP=.t. $END
$FORCE METHOD=ANALYTIC NVIB=1 $END
$STATPT NSTEP=200 HSSEND=.t. OPTTOL=0.0005 $END
$SYSTEM MWORDS=25 $END
```

Figure 1. Header used for calculations of all compounds in this study.

All structures were modeled at the HF/3-21G level of theory using the computational chemistry suite GAMESS (5 Dec 2014 R1) [12, 13]. All structures were verified as true minima as indicated by the absence of any imaginary vibrational frequencies. Structures were pre-optimized using Avogadro and visualizations were produced using wxMacMolPlt [14]. The outputs of the GAMESS calculations were visualized and confirmed using wxMacMolPlt.

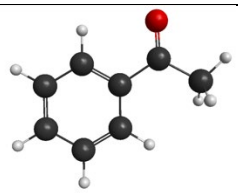
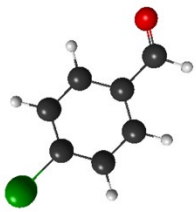
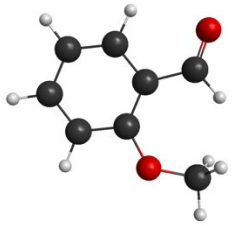
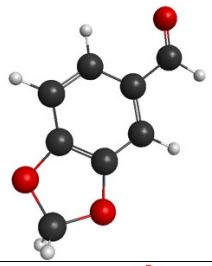
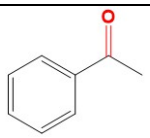
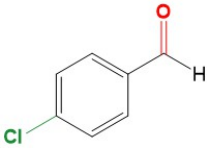
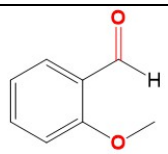
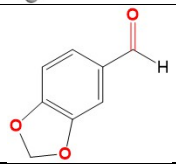
RESULTS AND DISCUSSION

Although multiple combinations of chalcones can be obtained using various combinations of commercially available aldehydes and ketones (Scheme 2) we focused our study on a combination of three different aldehydes **2**–**4** (Scheme 3) and acetophenone as ketone **1** (Scheme 3). The aldehydes were chosen to achieve a substantial difference in structural build of selected aldehydes, steric hindrance, and electronic effects perspective. We anticipated that the impact of the conformation for the resulting tetrahedral intermediate **C** (Scheme 2) will determine the likelihood of forming a cis chalcone compared to a trans chalcone. The aldehydes observed included 4-chlorobenzaldehyde as aldehyde **2**, 2-methoxybenzaldehyde as aldehyde **3**, and piperonal as aldehyde **4**.

The optimized geometries of one starting ketone and three used aldehydes are presented in Table 1. Although computational modeling provides all thermodynamic parameters, such as enthalpy, entropy, and Gibbs free energy we only report here the latter. Considering our research question focused on a study of the correlation between steric hindrance of chalcone products and the preference for the isomer formation values of enthalpy and entropy would

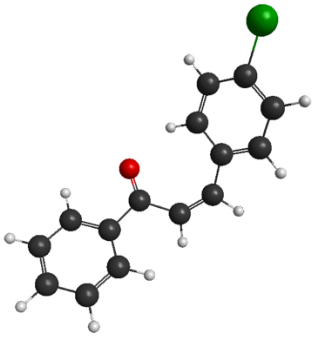
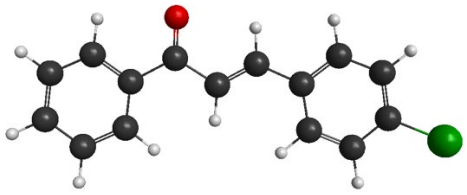
not be necessary to be analyzed. We estimated that the values of Gibbs free energy, G, for all starting materials were relatively similar. This is mostly due to the similar atom count between the compounds. Aldehyde **2** containing a deactivating chloro-group on the aromatic ring and aldehyde **4** containing an activating cyclic diether group both showed lower value of Gibbs free energy then one found for aldehyde **3** that contains a strong activating methoxy-group on the aromatic ring. In terms of steric hindrance, out of the three aldehydes studied aldehyde **4** has the most strain due to a mismatch of geometric angles required by the joining of the sp^2-hybridized carbons building the aromatic ring and the sp^3-hybridized atoms building the diether ring attached. One can speculate that a close positioning of hydrogen atoms on methoxy group in aldehyde **3** can result in a generation of a through space repulsion with a nearby carbonyl group as a potential explanation for the higher value of G.

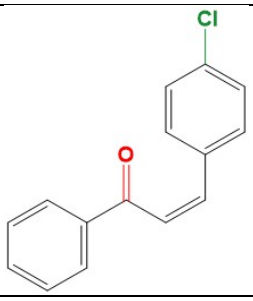
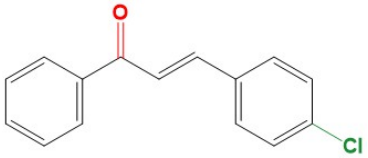
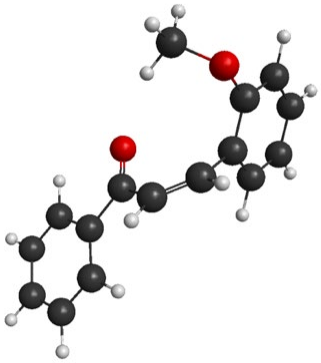
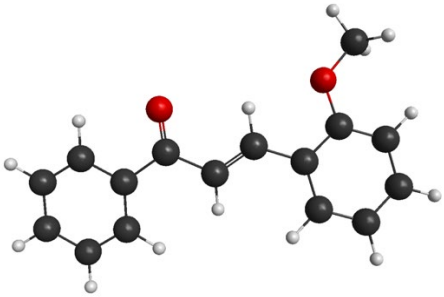
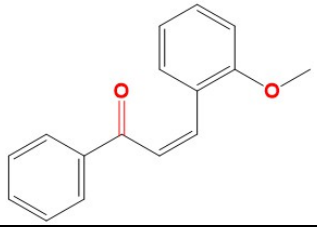
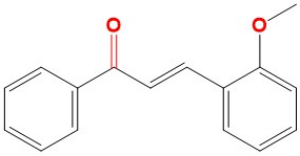
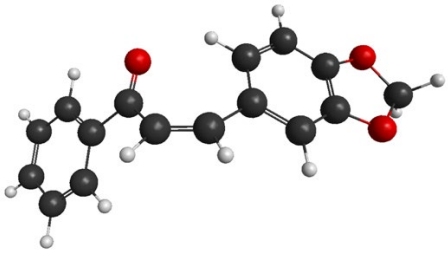
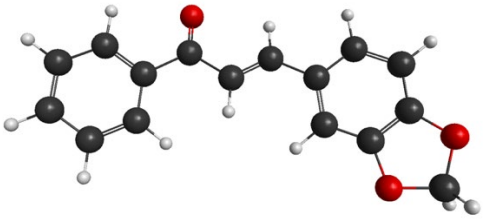
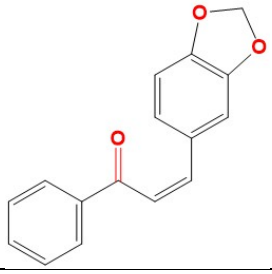
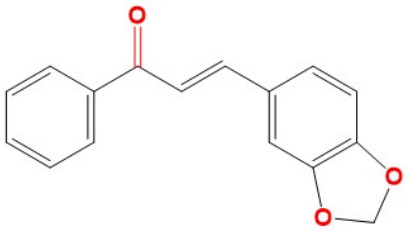
Table 1. Optimized geometries and Gibbs free energy of starting materials.

	Ketone **1**	Aldehyde **2**	Aldehyde **3**	Aldehyde **4**
Optimized geometry				
Structure drawing				
ΔG, kJ/mol	308.191	201.598	320.417	270.248

The following table 2 contains calculated parameters for both forms of corresponding chalcone products. Similarly, to the starting materials we report here the Gibbs free energy only as values of enthalpy and entropy will not allow for a conclusive comparison of chalcone pairings. Since chalcones molecules contain quite a few atoms to illustrate the difference between two stereoisomers the side-by-side comparison of optimized geometry is provided along with the structural drawings of the same compounds. Color coding in structural drawings is of exact match to the colors of atoms generated in Avogadro to further facilitate the comparison.

Table 2. Optimized geometries and Gibbs free energy of chalcone products.

Name	cis-Chalcone **5**	trans-Chalcone **5**
Optimized geometry		

Structure drawing		
G	502.696, kJ/mol	497.374, kJ/mol
Name	cis-Chalcone **6**	trans-Chalcone **6**
Optimized geometry		
Structure drawing		
G	616.455, kJ/mol	615.766, kJ/mol
Name	cis-Chalcone **7**	trans-Chalcone **7**
Optimized geometry		
Structure drawing		
G	570.858, kJ/mol	569.148, kJ/mol

Our data demonstrate that for all chalcone products trans-isomer has lower value of Gibbs free energy, G. The most difference of 5.322 kJ/mol is observed for the cis- and trans-isomers of chalcone **5**. The value drops to 1.71 kJ/mol for the isomers of chalcone **7**. Stereoisomers of chalcone **6** are only differentiate in 0.689 kJ/mol. All condensation reactions leading to chalcones produce stochiometric quantities of water as byproduct. Therefore, the value is not included in the tables as it is formed in all reaction but the energy value of 9.124 kJ/mol is included in all following calculations of the changes of Gibbs free energy, ΔG, for all reactions as shown later in Table 3.

Table 3. Change in Gibbs free energy value (ΔG) and vibrational frequency of each chalcone isomer.

Chalcones	cis-**5**	trans-**5**	cis-**6**	trans-**6**	cis-**7**	trans-**7**
ΔG, kJ/mol	2.031	-3.291	-3.029	-3.718	1.543	-0.167
C=O frequency, cm^{-1}	1880.1	1888.4	1885.20	1885.16	1880.3	1886.9

To calculate changes in Gibbs free energy for the formation of each chalcone isomer the Gibbs free energies of corresponding starting materials (Table 1) were combined and the Gibbs free energies of products (Table 2) including water. For exact reactions and compound coding please see Scheme 3. The equations are organized to demonstrate exact starting materials used as well as the geometry and name of the chalcone produced.

Eq 1: Free energy (G) of reactants (ketone **1** and aldehyde **2**) = 308.191 kJ/mol + 201.598 kJ/mol = 509.789 kJ/mol

Eq 2: ΔG for trans-chalcone **5** = 497.374 kJ/mol – (518.913 + 9.124) kJ/mol = −3.291 kJ/mol

Eq 3: ΔG for cis−chalcone **5** = 502.696 kJ/mol – (518.913 + 9.124) kJ/mol = +2.031 kJ/mol

Eq 4: Free energy (G) of reactants (ketone **1** and aldehyde **3**) = 308.191kJ/mol + 320.417 kJ/mol= 628. 608 kJ/mol

Eq 5: ΔG of trans−chalcone **6** = 615.766 kJ/mol− (628.608 + 9.124) kJ/mol = − 3.718 kJ/mol

Eq 6: ΔG of cis−chalcone **6** = 616.455 kJ/mol – (628.608 + 9.124) kJ/mol= −3.029 kJ/mol

Eq 7: Free energy (G) of reactants (ketone **1** and aldehyde **4**) = 308.191 kJ/mol + 270.248 kJ/mol = 578.439 kJ/mol

Eq 8: ΔG of trans−chalcone **7** = 569.148 kJ/mol – (578.439 + 9.124) kJ/mol = −0.167 kJ/mol

Eq 9: ΔG of cis−chalcone **7** = 570.858 kJ/mol – (578.439 + 9.124) kJ/mol = +1.543 kJ/mol

Our data demonstrate that the change of Gibbs free energy, ΔG, for all trans-chalcones was found to be negative. This implies that the formation of trans-isomer for all chalcones **5**–**7** is a thermodynamically favorable and spontaneous process. Our data also demonstrate that the change of Gibbs free energy, ΔG, for two out of three cis-chalcones was found to be positive. This implies that the formation of cis-isomer for chalcones **5** and **7** is a not thermodynamically favorable and is not a spontaneous process. However, cis-isomer of chalcone **6** does have a negative change of Gibbs free energy which implies that the formation of this isomer can occur as a sponteneous process and considering a very small difference in a value observed for trans-isomer our data indicate possibility of the partial formation of cis-isomer from a thermodynamic perspective.

LIMITATIONS

One of the main limitations that we can assess in our study that we only considered the thermodynamic aspects of the chalcone formation. Given that experimentally only trans-isomers are isolated, despite very similar energies calculated for cis- and trans-isomers, especially for chalcone **6**, there is a high likelihood that the product selection is kinetically controlled. Further investigations into a mechanism using transition state modeling may be required to ascertain the most important factors determining the product outcomes. Another limitation is a scope of the study, which only included six target chalcones. Considering each of those chalcones has unique steric and electronic features a comparison solely of steric effects may be not as clear within a small group sample. Inclusion of another ketone would drastically expand the library of possible isomers to compare as well as introduction of an aldehyde that contain either electronic or steric features similar to those used in this study. However, since this project was intended as a proof-of-concept study our data are sufficient for deriving preliminary conclusions and trends.

CONCLUSION

The changes in Gibbs free energy (ΔG) of the cis and trans isomers of three chalcones **5**–**7** formed using combinations of acetophenone as a ketone **1** component and three aldehydes (4-chlorobenzaldehyde **2**, 2-methoxybenzaldehyde **3**, and piperonal **4**) were successfully determined. Our data showed that the trans isomer of each chalcone had a more negative ΔG value and thus is more likely to be the major product of each reaction. Two of the three cis-isomers have demonstrated positive changes in Gibbs free energy. For chalcone **6** both isomers have negative changes, meaning that the formation of both isomers is a spontaneous process. The data showed that the ΔG values for the cis- and trans-isomers of chalcone **7**, formed by a combination of acetophenone **1** and piperonal **4**, differed more than those for chalcone **6**, but the most difference was observed for isomers of chalcone **5**. Considering chalcone **5** is formed from acetophenone **1** and 4-chlorobenzaldehyde **2** our original hypothesis of correlation between steric strain and product selectivity was not supported. However, considering that this may correlate to electronic effects of a deactivating chloro-substituent and comparing only chalcones **6** and **7** steric hindrance does correlate with product preference. Both of chalcones **6** and **7** contain electron-donating groups while chalcone **7** is more sterically hindered than chalcone **6** which correlates to a bigger difference in changes of Gibbs free energies between cis- and trans-isomers for chalcone **6** which does support our original hypothesis.

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