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Exploring the unexpected formation of spirobibenzopyrans and benzopyrylium salts and effect of Lewis acids on the Claisen-Schmidt reaction

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ABSTRACT

Unexpected spiro cyclic products were obtained from the popular alkali catalyzed Claisen-Schmidt reaction of ketones with salicylaldehyde apart from the bis-chalcones. However, in the acid catalyzed reaction, we observe their transformation to a benzopyrylium salt. In the present study, we demonstrate this phenomenon for three ketones namely cyclopentanone, cylcohexanone and cylcoheptanone. Each of the corresponding products obained were characterized using UV-Vis, FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry. In addition, the structure of the spiro molecule synthesized from cylcoheptanone reactant has been determined by X-ray crystallography and further investigated for its thermal properties using TGA/DTA technique. To rationalize this phenomenon, we first studied the effect of solvents on the reaction, and further explored the effect of Lewis acid catalyst and various solvents for the same reaction conditions. Further, we have evaluated *in silico,* the impact of thermodynamic parameter like entropy. In this regard, we explored the relationship between the bis-chalcone, spiro molecule and the benzopyrylium salt. Consequently, the effect of the ring expansion from cyclopentanone to cycloheptanone was investigated. In conclusion, we have presented the anti-bacterial potential of the synthsized spirobibenzopyrans and the benzopyrylium salts.

1. Introduction

Claisen-Schmidt condensation is a popular organic reaction between an aldehyde lacking an alpha-hydrogen and a ketone having an alpha-hydrogen [1]. This reaction has been predominantly used to synthesize chalcones and bis-chalcone derivatives and has been extensively studied [2–7]. In an effort to explore the reaction between a cycloalkanone and 2-hydroxybenzaldehyde via the Claisen-Schmidt reaction, we discovered formation of unexpected spirobibenzopyrans along with the bis-chalcone in basic medium. Consequently, in order to avoid the spiro by-product, we attempted the Claisen-Schmidt reaction under acidic conditions where we obtained deeply coloured compounds called benzopyrylium salts instead of the corresponding bis-chalcone. This study is a repot of the unusual findings obtained during the course of our experiments. Interestingly, we observed that the reaction products were dependent on the ring size of the cycloalkanone reactant and form only with 2-hydroxybenzaldehyde reactant. Effect of solvents and catalyst also have been studied in order to understand the formation of these compounds. Additionally, the effect of Lewis acids also has been studied. To the best of our understanding, we herein report for the first time, the formation of spirobibenzopyrans from a $TiCl_4$ catalysed reaction. We have further explored the effect on structural stability with respect to the ring size of the ketone reactant by employing *in silico* techniques.

This phenomenon of obtaining spirobibenzopyrans, benzopyrylium salts and diarylidenecycloalkanone derivatives was studied using cyclopentanone, cyclohexanone and cycloheptanone as the ketone reactants in the Claisen-Schmidt reaction. The various schemes describing the synthetic procedures along with the

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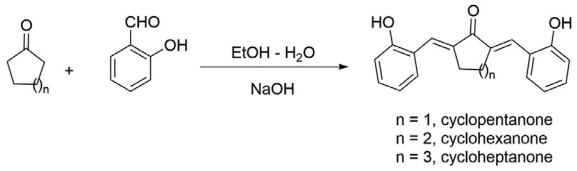


Scheme 1. The Claisen-Schmidt condensation reaction.

characterization data, the *in silico* techniques employed and the TGA/DTA technique used are provided in the supplementary section.

2. Materials and methods

2.1. General scheme for synthesis of the diarylidenecycloalkanone derivatives

The diarylidenecycloalkanone derivatives were synthesized as shown in Scheme 1 by the Claisen–Schmidt condensation procedure [8]. A solution of 15 ml of distilled ethanol and 15 ml of water was prepared in a 100 ml round bottom flask and nitrogen was flushed through the solution for 3–4 min. To the ethanol-water mixture, 7.5 g of sodium hydroxide was added and stirred continuously till it dissolved. Another mixture of 0.0625 moles of the aldehyde and 0.03125 moles of the ketone was prepared. Half of the aldehyde-ketone mixture was then added to the vigorously stirred ethanol-water mixture under nitrogen atmosphere. After the addition, a reddish coloration was obtained. 25 min later the rest of the aldehyde-ketone mixture was added to the reaction mixture and the nitrogen atmosphere was maintained. The reaction was monitored by TLC for completion. While the reaction was complete in less than a day for the cyclopentanone reactant, the cyclohexanone reactant took around 2 days. However, for the cycloheptanone reactant, the completion time was over 5 days.

At the end of the reaction, the whole reaction mixture was transferred into a beaker with ice cubes. Subsequently, acidification of the mixture was carried out using 1:1 HCl solution drop wise with vigorous stirring. After every drop, the pH was monitored using pH paper. When the pH became less than 5, a yellow precipitate was obtained. The precipitate was then filtered and washed with cold water until the washings were free of acid and dried.

Since the cyclopentanone reactant gave a single product, i.e., the bis-chalcone, the solid obtained was recrystallized from acetone.

In case of the cyclohexanone reactant, a mixture of both spiroketal and bis-chalcone was obtained. The two compounds were separated using column chromatography. The pure spiroketal was obtained directly from the fractions after the column chromatography. On the other hand, the bis-chalcone was purified by recrystallizing from methanol-water mixture.

The cyclopentanone product also gave a mixture of both spiroketal and bis-chalcone. In this case, the bis-chalcone was purified directly by recrystallizing from methanol.

2.2. General scheme for synthesis of acid catalyzed Claisen-Schmidt reaction

As shown in Scheme 2, a mixture of the ketone (0.01 mol) and salicylaldehyde (0.02 mol) was stirred in HCl saturated acetic acid (80 ml). The above reaction mixture was left standing overnight when crystals were observed settling down. The crystals were filtered, washed thoroughly with diethyl ether and dried.

2.3. Instrumentation

For **DA-CHX** and **DA-CP**, the ^{1}HNMR spectra were obtained on VARIAN 400 MHz, and ^{13}C NMR on VARIAN 100 MHz with DMSO-d_6 as the solvent. For **CHX-SK, DA-CHP** and **CHP-SK** the ^{1}HNMR spectra were obtained on Bruker Ascend 400 MHz, and ^{13}C NMR on Bruker Ascend 100 MHz with $CDCl_3$ as the solvent. TMS was used as the internal standard. For **CP-PS** and **CHX-PS**, the ^{1}HNMR spectra were obtained on Bruker Avance III 800 MHz, and ^{13}C NMR on Bruker Avance III 200 MHz at 10 °C using CD_3COOD as the solvent. AGILENT 6430 Triple Quad LC/MS was employed to obtain mass spectra. Only for **CP-PS** and **CHX-PS**, Bruker MALDI-TOF/TOF MS was employed to obtain their mass spectra. The FT-IR spectra were recorded between 400 and 4000 cm^{-1} using KBr pellets employing Thermo-Nicolet Avatar 370 spectrophotometer. UV-Vis spectra in methanol were recorded in the wavelength range 200–600 nm using Shimadzu 2450 spectrophotometer. The thermal analysis was carried out in TA SDT Q600 TGA/DTA thermal analyzer.

Single crystals of suitable dimensions were chosen carefully for X-ray diffraction studies. The X-ray intensity data were collected at a temperature of 293(2) K on a Bruker Proteum2 CCD diffractometer [9] equipped with an X-ray generator operating at 45 kV and 10 mA, using CuK_α radiation of wavelength 1.54178 Å. Data were collected for 24 frames per set with different settings of φ (0 and 90°), keeping the scan width of 0.5°, exposure time of 2 s, the sample to detector distance of 45.10 mm and 2θ value at 46.6°. The complete data sets were processed using *SAINT PLUS*. The structures were solved by direct methods and refined by full-matrix least squares method on F^2 using *SHELXS* and *SHELXL* programs [10]. The geometrical calculations were carried out using the program *PLATON* [11].

2.4. In silico *studies*

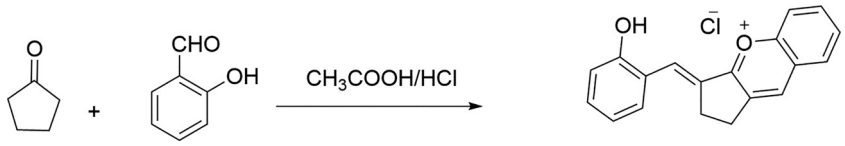
The spirobibenzopyrans, the benzopyrylium salts, and the diarylidenecyclocalkanone derivatives mentioned in the study were modelled using GaussView (5.0.8) [12]. The energy minimization of these molecules has been carried out using DFT (B3LYP/6–31G* basis set) theory in Gaussian 09 along with their frequency simulations [13].

2.5. In vitro *antibiotic susceptibility testing*

Antibiotic susceptibility testing was conducted according to the CLSI guidelines using the broth micro dilution-assay [14]. 10 mg/mL stock solutions of test compounds were prepared in DMSO. Bacterial cultures were inoculated in MHBII and optical density (OD) was measured at 600 nm, followed by dilution to achieve ~10^6 CFU/mL. The compounds were tested from 64–0.5 mg/L in two-fold serial diluted fashion with 2.5 μL of each concentration added to well of a 96-well round bottom microtiter plate. Later, 97.5 μL of bacterial suspension was added to each well containing either test compound or appropriate controls. The plates were incubated at 37 °C for 18–24 h following which the MIC was determined. The MIC is defined as the lowest concentration of the compound at which there is absence of visible growth. For each test compound, MIC determinations were carried out independently three times using duplicate samples.

3. Results and discussion

Due to the alkaline conditions involved in the reaction, the phenol group exists as phenoxide ion as shown in Fig. 1 that is extensively stabilized by the resonance.



Scheme 2. AcOH/HCl catalyzed benzopyrylium salt formation.

Table 1
Observations from the various Claisen-Schmidt reactions carried out with salicylaldehyde.

	Alkaline medium products			Acidic medium products		
Ketone	Bis-chalcone	Spirobibenzopyran	Pyrylium Salt	Bis-chalcone	Spirobibenzopyran	Pyrylium Salt
Cyclopentanone	Formed	Did not form	Did not form	Did not form	Did not form	Formed
Cyclohexanone	Formed	Formed	Did not form	Did not form	Did not form	Formed
Cycloheptanone	Formed	Formed	Did not form	Did not form	Formed	Did not form

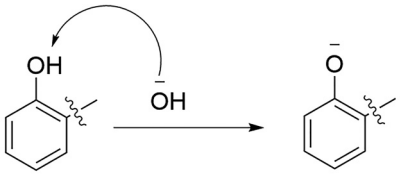


Fig. 1. Phenoxide ion in alkaline medium.

In order to obtain the neutral product at the end of the Claisen-Schmidt reaction, the pH of the reaction mixture was made acidic. We observed the precipitation of the desired bis-chalcone derivative. It was at the end of this step that we observed the presence of two different products by TLC. This particular phenomenon was crucially first observed in the reaction between cyclohexanone and salicylaldehyde. Upon purification and characterization, we could characterize the products as (a) and (b) shown below in Fig. 2.

Spirobibenzopyrans and their synthesis have been reported in a few publications in the literature [15,16]. However, this is the first recorded formation of these molecules via the Claisen-Schmidt condensation route. We propose that due to the acidic conditions in the final step of the reaction to obtain the bis-chalcone derivative, an intramolecular ketal formation occurs by the following mechanism as shown in Fig. 3. At the end of the reaction, the obtained bis-chalcone was of only 60% yield, whereas the rest 40% yield was the spiro compound. Given this intriguing phenomenon, in the quest of higher yields of the bis-chalcone derivative while avoiding the formation of the spiro molecule, we pursued the Claisen-Schmidt condensation reaction using different reaction conditions.

The acid catalyzed reaction medium constituted glacial acetic acid saturated with dry HCl gas. One would expect the formation of bis-chalcone by this procedure without any by-products. Interestingly in this method, we obtained a different type of product that was not the desired bis-chalcone. The solution was deep violet in colour and so was the solid obtained. TLC showed presence of a single compound. Upon characterization, we concluded that the product belonged to a different class of compounds called the benzopyrylium salts. The structure of the compound isolated is captured in Fig. 4.

This type of reaction was first reported by Borsche and Geyer in 1913 along with the associated mechanism [17] and by other research groups [18,19]. Benzopyrylium salts are well known for various applications in color photography, fluorescent dyes and dye lasers [20]. These observations prompted us to verify the principle with the other ketone reactants under same reaction conditions. In this regard, we chose cyclopentanone and cycloheptanone to react with salicylaldehyde. The Table 1 below summarizes our findings upon carrying out the Claisen-Schmidt reaction with the cyclopentanone, cyclohexanone, and cycloheptanone with salicylaldehyde in both basic and acidic conditions. The tabulated observations (Table 1) highlight the different synthetic and structural aspects of organic chemistry. Primarily, from these unusual products observed, it is evident that these deviations occur due to the 2-hydroxybenzaldehyde/ salicylaldehyde reactant in the Claisen-Schmidt reaction.

It can be rationalized that the formation of the spiro molecule in basic medium depends on the structural aspect of the ketone reactant. The reduction in entropy due to ketal formation cannot be countered by conformational changes in the cyclopentane ring fragment due to the high ring strain in the cyclopentane ring itself. Hence, no spiro molecule was observed with the cyclopentanone reactant. On the other hand, both cyclohexane and cycloheptane ring fragments can alter their conformation in order to counter the entropy reduction suffering minimal conformational penalty, thus yielding the respective spiro molecules.

Unlike the structural aspect of the ketone reactant which drives the spiro molecule formation in the basic medium, the benzopyrylium salt formation in the acidic medium is due to the stability offered by aromaticity of the product. The formation of the benzopyrylium salt results in a highly conjugated aromatic system which offers stability even in case of the cyclopentanone reactant. Interestingly, cycloheptanone reactant did not yield either a benzopyrylium salt or a bis-chalcone in the acidic medium. Rather, the spiro molecule was obtained from the reaction. We propose that since the cycloheptane ring fragment can change its conformation to accommodate the entropy loss, it prefers the spiro structure.

We further explored the effect of the solvent on the reaction in both the acidic and basic media. In this regard, we chose cyclohexanone as the representative ketone reactant with salicylaldehyde, and studied the reaction using dichloromethane as moderately polar and toluene as the non-polar solvents. Triethylamine was used as the base catalyst whereas PTSA (p-toluenesulfonic acid) was used as the acid catalyst. We observed the formation of benzopyrylium salt in the acidic medium with both the solvents. The identification of the product was confirmed by co-TLC, UV and mass spectrometry by comparison with the authentic sample obtained previously. This confirms the influence of salicylaldehyde and acidic medium to generate the alternative product as benzopyrylium salt from the Claisen-Schmidt reaction irrespective of the solvent system. Intriguingly, we also observed the presence of the corresponding spiro molecule on the TLC. This could be attributed to the presence of the non-polar medium where the spiro molecule is soluble in the less polar solvents whereas the benzopyrylium salt precipitates. In the basic medium, we noted no progress in the reaction for ten days carried out in toluene, even with application of heat. However, we observed the progress of

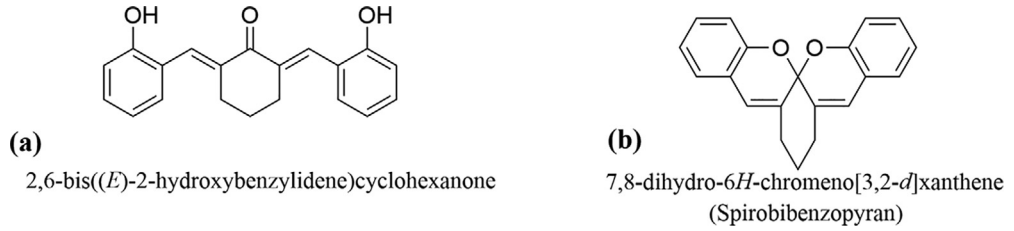


Fig. 2. Purified products obtained from the Claisen-Schmidt reaction.

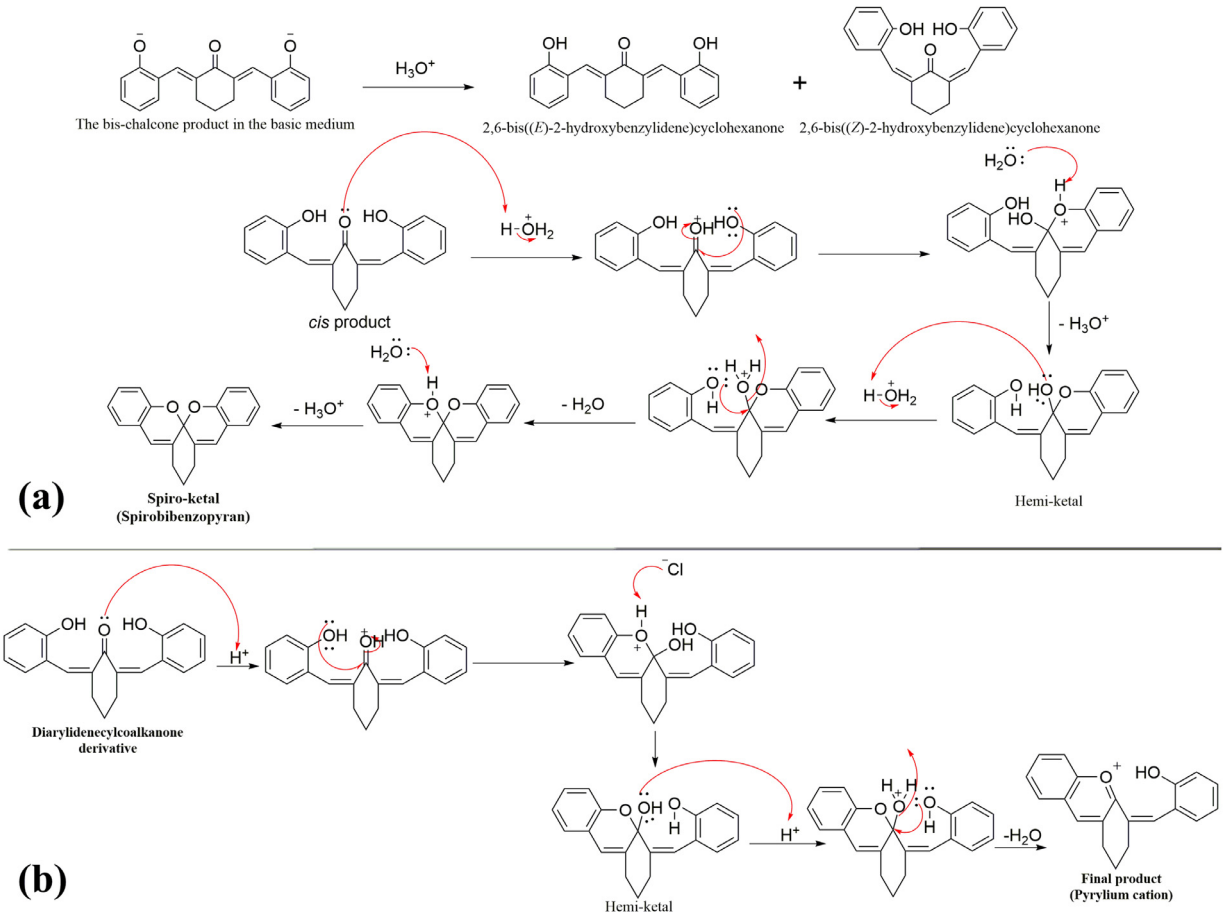


Fig. 3. Proposed mechanism of the intramolecular: (a) spiroketal formation and (b) benzopyrylium cation formation.

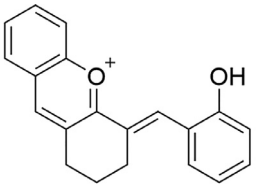


Fig 4. Benzopyrylium salt obtained from the Claisen-Schmidt reaction in acidic medium.

the reaction in dichloromethane when refluxed for seven days. We detected the presence of the bis-chalcone through TLC. The reaction was very slow but satisfyingly without the presence of by-products. This confirms the effect of the basic medium to enable the progress of the reaction only in polar solvents. The spiro by-product is observed only when aqueous medium is involved.

We consequently were able to grow the single crystals for the spiro molecule obtained from the cycloheptanone reactant that is provided in the following Fig. 5.

The UV-vis spectra of the spirobibenzopyrans showed one peak between 240–243 nm and the second between 278–321 nm. These absorptions can be attributed to the two substituted benzene rings. On the other hand, the benzopyrylium salts show three distinctive peaks, one between 250–260 nm, second in 290–320 nm and the third between 400–530 nm. The peak between 400–530 nm results from the benzopyrylium moiety and is responsible for the colour observed from the pyrylium salts. IR spectroscopy of all the compounds demonstrated a few key common features. The absence of C=O stretch in the range 1760–1660 cm-^{1} confirms the formation of the spiroketal and the benzopyrylium salt while the C=O stretch in the range 1640–1670 cm^{-1} was observed in the diarylidenecycloalkanones. The C=C double bond stretch was seen at around 1635–1600 cm^{-1}. The aromatic skeletal bands were noted in the range 1550–1400 cm^{-1} and C-H OOP bend in the region 900–650 cm^{-1}. Strong bands in the region 850–800 cm^{-1} affirm the tri-substituted benzene rings. The C-O stretch was observed in the range of 1380–1100 cm^{-1}.

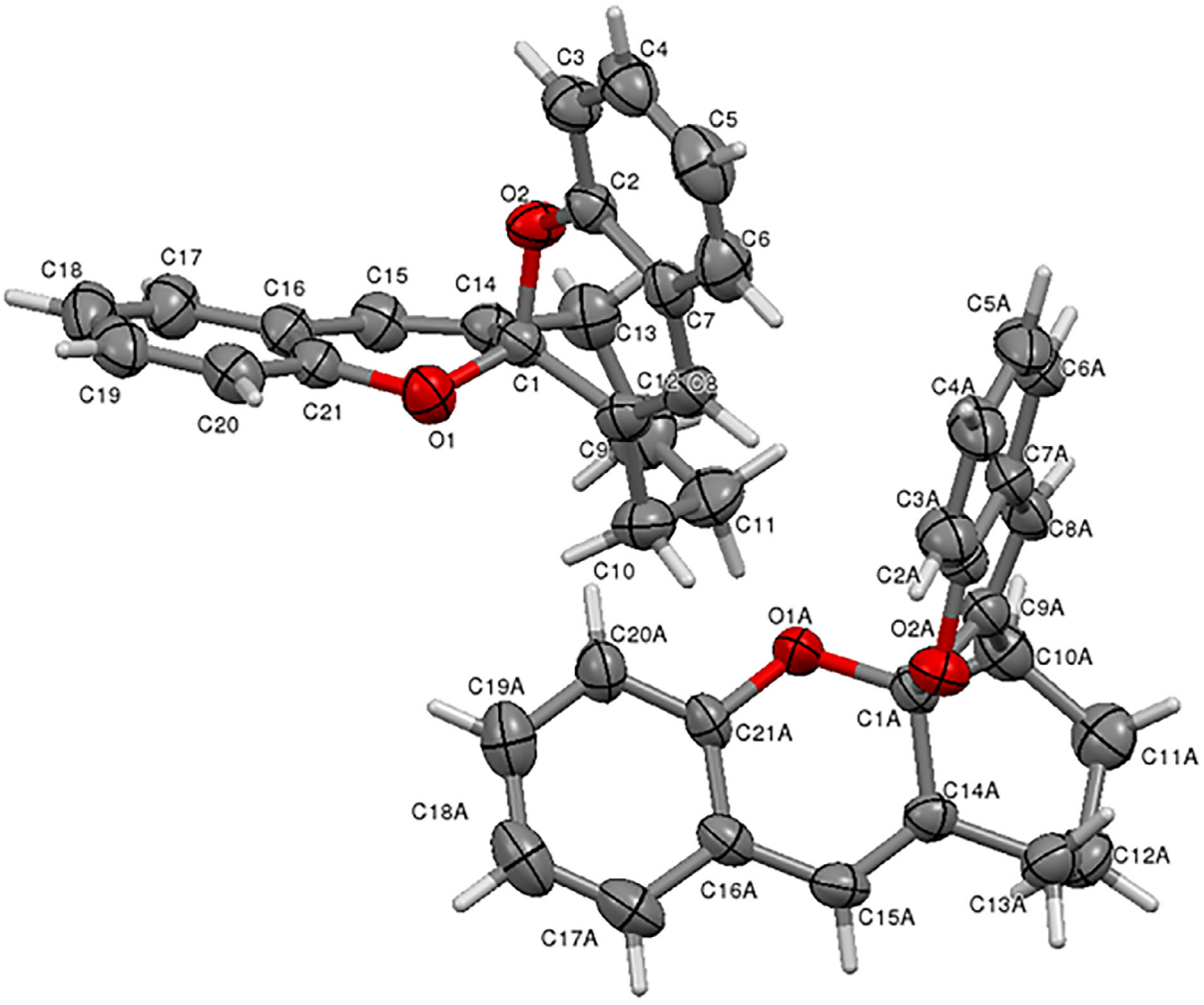


Fig. 5. ORTEP diagram of the Spiro molecule from the cycloheptanone reactant.

In ^{1}H NMR spectra, aromatic protons are observed at their expected values with various degrees of shielding (δ 6.60–7.40). The olefinic protons also appear in the same region as the aromatic protons. ^{13}C NMR clearly identifies the quaternary carbons by the weak signals. The spiro-carbon has a higher shift because it is attached to two oxygen atoms at around δ 100–95. In case of the benzopyrylium salts and the diarylidenecycloalkanones this peak is absent. The carbon attached to the oxonium ion shows a chemical shift value at δ 170–165 which is on the lower range of carbonyl carbon shifts as observed in the diarylidenecycloalkanones ~δ 190 ppm. Mass spectrometry for all samples gave the expected molecular ion peaks.

As revealed earlier, the synthesis of spirobibenzopyrans has been reported in literature [15,16]. The reactions conditions involve the application of Lewis acids, i.e., BF_3 [15] and $SiCl_4$ [16]. Following the procedures mentioned by Salama et. al., [16] we were able to synthesize novel spirobibenzopyrans and explore their biological properties [21,22]. But the formation of spirobibenzopyrans in similar reaction conditions as the Claisen-Schmidt condensation reaction by the use of Lewis acid is fascinating as the product formation occurs in a single step. In order to further investigate the reaction, we chose different Lewis acids as our catalyst and also chose different solvents.

The following Lewis acids were chosen: $SiCl_4$, $TiCl_4$, $SnCl_4$, $Ti\{OCH(CH_3)_2\}_4$, $InCl_3$, $AlCl_3$, $BF_3.OEt_2$, and $ZnCl_2$. The solvents chosen were absolute ethanol (abs. EtOH), dioxane, tetrahydrofuran (THF), acetonitrile (ACN), dichloromethane (DCM), and toluene. We chose the reactants to be cycloheptanone (ketone) and salicylaldehyde. The reaction conditions were kept similar to as described by Salama et. al. [16], i.e., ketone of 1 equivalent, aldehyde in 2 equivalents and the catalyst of 3 equivalents. Since the reported reaction completed in 2 h, we monitored all the reactions for 24 h.

At the set conditions, toluene and ACN as solvents did not show any progress in the reaction, even upon reflux. With the catalysts, $ZnCl_2$, $Ti\{OCH(CH_3)_2\}_4$, $InCl_3$, and $AlCl_3$ did not show any progress in the reaction. The following Table 2 summarizes the yield from various reaction conditions:

From the point of view of yield, both $SiCl_4$ and $TiCl_4$ have given the best results. Accordingly, $SnCl_4$ is less effective for the formation of the spirobibenzopyran. From time taken for the reaction to complete, $SiCl_4$ and $TiCl_4$ showed completion between 4 to 6 h. In this regard, although $BF_3.OEt_2$ yielded comparable spirobibenzopyran in abs. EtOH, it took 24 h to completion. Hence, as effective catalysts, we shortlisted $SiCl_4$ and $TiCl_4$ as ideal for further analysis.

Among the solvents, it is clear that both abs. EtOH and dioxane proved optimum and hence were chosen for further studies. In the next step of the study, we chose to optimize the quantity of the catalyst. It is known that a catalyst must be able to carry out the reaction at a minimum quantity.

Here we chose to compare the yields at 3 equivalents, 2 equivalents, 1 equivalent, 0.1 equivalents and 0.01 equivalents of both

Table 2
Reaction conditions for the formation of the spirobibenzopyran CHP-SK at 3 equivalents of the catalyst.

Formation of CHP-SK (24 hrs)	$SiCl_4$	$SnCl_4$	$TiCl_4$	$BF_3.OEt_2$	$InCl_3$	$AlCl_3$	$ZnCl_2$	$Ti\{OCH(CH_3)_2\}_4$
Abs. EtOH	72.60%	25.60%	71.18%	69.00 %	-	-	-	-
Dioxane	68.00%	10.00%	69.50%	5% (incomplete)	-	-	-	-
THF	52.70%	5% (incomplete)	47.90%	-	-	-	-	-
ACN	-	-	-	-	-	-	-	-
DCM	28%	10% (incomplete)	29% (incomplete)	-	-	-	-	-
Toluene	-	-	-	-	-	-	-	-

Table 3
Reaction conditions for the formation of the spirobibenzopyran CHP-SK at varying catalyst quantities.

CHP-SAL (24 hrs)	**$SiCl_4$**					**$TiCl_4$**				
	3 eq	**2 eq**	**1 eq**	**0.1 eq**	**0.01 eq**	**3 eq**	**2 eq**	**1 eq**	**0.1 eq**	**0.01 eq**
Abs. EtOH	72.60%	76.23%	79.50%	83.22%	Incomplete	71.18%	79.63%	87.45%	incomplete	incomplete
Dioxane	68.00%	70.12%	-	-	-	69.50%	incomplete	incomplete	incomplete	incomplete

Table 4
Optimized conditions for the formation of the spirobibenzopyran CHX-SK and CP-SK.

Other Ketones (24 hrs)	0.1 eq $SiCl_4$		1eq $TiCl_4$	
	CHX-SK	CP-SK	CHX-SK	CP-SK
Abs. EtOH	56%	61%	65.97%	73%
Prior yields (studied above)	43%	30%	43%	30%

$SiCl_4$ and $TiCl_4$ in the best solvents identified, i.e., abs. EtOH and dioxane for the formation of CHP-SK. The observations are tabulated below in Table 3.

Interestingly, we observed that the yield of the CHP-SK increased as the quantity of the Lewis acid catalyst reduced. It can be attributed to the lower amount of catalyst that increases its efficiency in the reaction. Among the solvents, we observe that abs. EtOH is better reflected in the higher yields. $SiCl_4$ gave the best yield at 0.1 equivalents of the catalyst whereas $TiCl_4$ gave the best yield at 1 equivalent of the catalyst. Here we would like to draw attention to the work-up procedure. In order to quench the catalyst and obtain the desired product, the contents of the reaction mixture are poured in ice-cold water and then the solid obtained is filtered. We have observed that by-products were formed in case of $SiCl_4$ which from the literature was found to be $Si(OEt)_4$ along with the solid spiro compound. In order to obtain the spirobibenzopyran we have to further extract the desired product using DCM or diethyl ether. This process leads to the loss of the spiro compound thus reducing the yield. On the other hand, $TiCl_4$ forms water soluble TiO_2 which makes the work-up procedure very easy and we obtain the spiro product directly as a solid hence providing a higher yield than $SiCl_4$. We also observed that the reaction catalyzed by $SiCl_4$ completed faster, within 4 h, as compared to $TiCl_4$ catalyzed reactions which took around 6 h.

After optimizing the reaction conditions, we must apply the same with the other ketones. Hence, we applied the same for the cyclohexanone and cyclopentanone ketones giving the spirobibenzopyrans CHX-SK and CP-SK, respectively. The results are summarized below in Table 4.

We observe that the yields of the respective spirobibenzopyrans have improved in the optimized conditions indicating that our study of the catalyst and solvent effects have been successful. We also for the first time conclude that the application of Lewis acid in the Claisen-Schmidt condensation reaction yields spirobibenzopyrans rather than the expected bis-chalcones when the aldehyde chosen is salicylaldehyde. The final conclusions from the study are as follows:

- A good yield of spirobibenzopyrans are obtained when a Lewis acid catalyst is used instead of an alkali or acid in the Claisen-Schmidt condensation reaction between a ketone and salicylaldehyde as a reactant.
- Among the solvents, absolute ethanol is the best.
- Among the Lewis acids, $SiCl_4$ gives faster yields at 0.1 equivalent whereas $TiCl_4$ gives higher yields though at 1 equivalent.

We have thus illustrated the effect of Lewis acids and solvents on the Claisen-Schmidt reaction yielding spirobibenzopyrans.

Returning to the unexpected formation of the spirobibenzopyrans and benzopyrylium salts in the original reaction conditions, we aim to understand the role of entropy. In order to understand that, we carried out *in silico* studies for each of these molecules. Fig. 6 given below shows the structures along with their labels used in the study. The global reactivity and thermal parameters [23] have been tabulated in **SD Table I** in the supplementary section.

In Table 5 given below, the entropy values of each of these molecules have been tabulated.

The *in silico* findings confirm our hypothesis regarding the loss of entropy in all the intramolecular cyclizations observed. The entropy loss is highest for the cyclopentanone reactant from the bis-chalcone to the spiro structure accounting for the lack of formation of the respective spiro molecule. Further, we observe that the entropy loss between the spiro form and the benzopyrylium salt form is almost half in case of both cyclopentanone and cyclohexanone reactants indicating that benzopyrylium salt forms readily in their case. Interestingly, in the case of the cycloheptanone reactant, the entropy loss for both spiro and benzopyrylium form is comparable which reinforces our earlier observation that cycloheptanone reactant favours the spiro product.

We have compared the bond angles and bond lengths obtained from the single crystal XRD experiment and the Gaussian calculations in order to establish the soundness of the chosen theory and basis set for the calculations. The two are summarized in **SD Table IIa** and **b** in the supplementary section. The regression analysis of the comparison between the experimental XRD data and the simulated Gaussian data returned a significant R^2 of 0.9724 for the crystal structure (1) and 0.9671 for the associated crystal (1A) in case of the bond lengths. Similarly, we recorded, an R^2 of 0.9319 and 0.8371 for 1 and 1A, respectively, in case of the bond angles. These high coefficients indicate the minimized confirmation of the Gaussian structure to align with the synthesised XRD structure. Further, the theoretical and the experimental IR values comparison returned a R^2 of 0.8099. This demonstrates the accuracy of the chosen level of theory for computation and the soundness of our computed values.

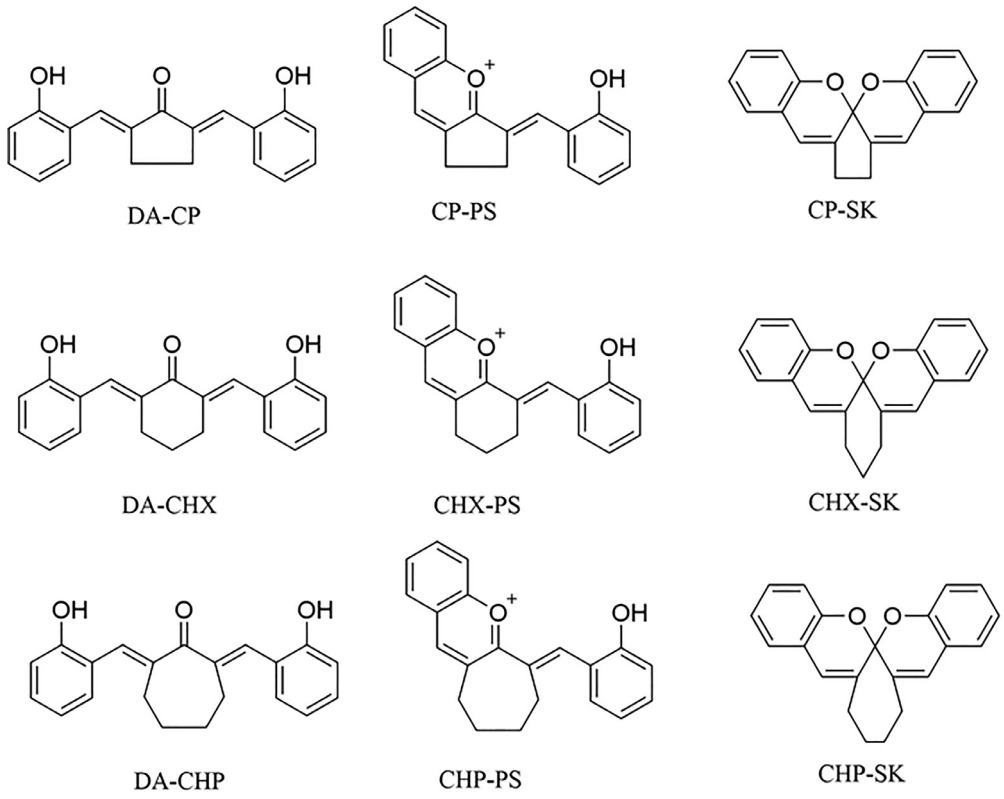


Fig. 6. Molecules used for the *in silico* studies.

Table 5
Simulated entropy values obtained from the energy minimization studies.

Molecule	S (cal/mol-kelvin)	Entropy loss ΔS (cal/mol-kelvin) w.r.t. to the bis-chalcone
CP-SK	118.39	25.06
CP-PS	130.81	12.64
DA-CP	143.45	–
CHX-SK	124.60	24.58
CHX-PS	133.76	15.42
DA-CHX	149.18	–
CHP-SK	133.49	19.77
CHP-PS	139.46	13.80
DA-CHP	153.26	–

Thermal analysis of a material provides useful information regarding the thermal stability of that material [24]. Dried crystals of the sample were selected for this purpose and the analysis was carried out under nitrogen flow of 100 mL/min from 30 to 600 °C at a heating rate of 10 °C/min in the SDT Q600 TGA/DTA analyzer. The **SD** Fig. 1 in the supplementary section shows the TGA/DTA curve for CHP-SK since we have reported the single crystal XRD of the same. The DTA curve implies that the material undergoes an irreversible endothermic transition at 214 °C, where melting begins. The endothermic peak represents the temperature at which the melting terminates, which corresponds to its melting point at 215 °C. Further, it indicates that there is no phase transition before melting. The sharpness of the peak shows the good degree of crystallinity and purity of the sample. The TGA curve of this sample indicates that the sample is stable up to 245 °C beyond which the weight loss is not due to self-degradation of CHP-SK, but merely due to evaporation after its melting. The exothermic peak at 297 °C indicates that the sample undergoes decomposition at this temperature.

3.1. In vitro *antibacterial activity*

The spirobibenzopyrans have previously not been explored for any biological properties. Our group was the first to report their anti-cancer properties [21,22]. In this study, for the first time we report the anti-bacterial properties of the spirobibenzopyrans. The spirobibenzopyrans and the benzopyrylium salts were screened for their antibacterial potency against ESKAPE pathogen panel. The results are tabulated below in Table 6. Both the spirobibenzopyrans and benzopyrylium salts were found to be inactive against *E. coli*

Table 6
Anti-bacterial susceptibilities of the synthesized spirobibenzopyrans and the benzopyrylium salts.

Sample Code	MIC (μg/ml)				
	E. coli ATCC 25,922	*S. aureus* ATCC 29,213	*K. pneumoniae* BAA 1705	*A. baumannii* BAA 1605	*P. aeruginosa* ATCC 27,853
CHP-SK	>64	>64	>64	>64	>64
CHX-SK	>64	>64	>64	>64	>64
TH-SK	>64	>64	>64	>64	>64
CP-SK	>64	**32**	>64	>64	>64
3P-SK	>64	>64	>64	>64	>64
CP-PS	>64	**64**	>64	>64	>64
CHX-PS	>64	>64	>64	>64	>64
Levofloxacin	***0.0156***	***0.25***	***64***	***8***	***1***

(ATCC 25,922), *K. pneumoniae* (BAA 1705), *A.baumannii* (BAA 1605), and *P.aeruginosa* (ATCC 27,853) strains. However, they were found to have some activity against *S. aureus* (ATCC 29,213). The active compounds CP-SK has a MIC of 32 µg/mL and CP-PS has a MIC of 64 µg/mL. This study was limited to the parent spirobibenzopyrans and benzopyrylium salts. The results indicate that the derivatives might possess better activity than the parent molecules. Further studies are under progress in this regard.

4. Conclusions

In summary, this study reported unusual products formed from the well-known Claisen-Schmidt reaction between a cycloalkanone and salicylaldehyde. Using the standard reaction conditions, in alkaline medium we obtained spiro molecule along with the expected bis-chalcone. This study is the first report of isolating spirobibenzopyrans using the Claisen-Schmidt reaction. In acidic medium, we demonstrated the formation of benzopyrylium salt. Firstly, we concluded that the 2-hydroxybenzaldehyde reactant is essential to observe these deviations. Further, we evaluated the effect of polarity of solvent and ring size on the cycloalkanone reactant. For the cyclohexanone, we confirmed the influence of salicylaldehyde and acidic medium to generate the alternative product as benzopyrylium salt from the Claisen-Schmidt reaction irrespective of the solvent system, while the basic medium enabled the progress of the reaction only in polar solvents. For the spiro molecules, we concluded that the conformational changes in the ring system must aid the entropy loss in the intramolecular cyclization reaction, which in case of cyclopentanone is not possible due to high ring strain. In case of the benzopyrylium salts, we observed that the stability offered by aromaticity is the driving factor. The effect of Lewis acid instead of alkali and acid was evaluated. We observed that $SiCl_4$ and $TiCl_4$ yielded spirobibenzopyrans with absolute ethanol as the solvent. This is the first report of formation of spirobibenzopyrans from $TiCl_4$. The reaction conditions were optimized to get the best yield of spirobibenzopyrans.

By employing *in silico* techniques we understood the entropy loss factor. We also observed the comparable entropy loss between the spiro molecule and the corresponding benzopyrylium salt of the cycloheptanone reactant indicating that it favors the spiro product formation. The resonance between the various structural parameters obtained from the *in silico* simulations and the experimental observations verified the accuracy of the chosen Gaussian basis set, level of theory and the soundness of our computed values. This high degree of correlations between the experimental and simulated values confirms the reliability of the undertaken *in silico* techniques to understand the structural aspects of a chemical system and predict its thermodynamics. Finally, we have reported for the first time the anti-bacterial potential of the synthesized spirobibenzopyrans and benzopyrylium salts. The results indicate that with further optimization, these molecules could be explored as potential anti-bacterial leads.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Swayamsiddha Kar: Conceptualization, Methodology, Validation, Investigation, Writing - original draft, Writing - review & editing. **Prashant Rai:** Investigation, Formal analysis. **Sai Manohar Chelli:** Conceptualization, Methodology, Validation, Investigation, Formal analysis, Writing - review & editing. **Abdul Akhir:** Investigation, Formal analysis, Validation. **Naveen Shivalingegowda:** Investigation, Formal analysis, Validation, Project administration. **Sidharth Chopra:** Formal analysis, Validation, Supervision, Project administration. **Lokanath Neratur Krishnappagowda:** Formal analysis, Validation, Supervision. **Siva Kumar Belliraj:** Formal analysis, Validation, Supervision, Project administration. **Nageswara Rao Golakoti:** Formal analysis, Validation, Supervision, Project administration.

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Supplementary materials

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