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Evaluatin The Effect of Primary Amine in Bitterness of Selected Cephalosporins

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Abstract

Cephalosporins, particularly Cephalexin and Cefaclor, are widely prescribed antibiotics known for their characteristic bitter taste, which poses challenges to their palatability and patient compliance. This bitterness is attributed to the specific chemical composition of these substances. The sensory experience of bitterness is influenced by distinct functional groups present in various foods and chemicals, along with the primary amine functional group being identified as a significant contributor to bitterness in compounds such as Cephalosporins. The prevalence of primary amine-related bitterness in both foods and medicines, including Cephalosporins, underscores the need to address this aversion factor. To overcome this taste challenge, researchers have developed diverse techniques and strategies to modify these antibiotics' chemical structure while preserving their therapeutic efficacy. In line with this objective, the current study endeavours to delve deeper into the role played by the primary amine functional group in generating the bitter taste associated with Cephalexin and Cefaclor. By doing so, this investigation aims to provide valuable insights that contribute to the optimization of future antibiotic formulations. The outcomes of this study have the potential to advance the development of antibiotics with enhanced palatability, fostering improved patient acceptance and adherence to treatment regimens. Results: The formation of imines on the primary amine functional group in both Cephalexin and Cefaclor was achieved through the creation of Schiff bases with aldehydes, specifically Citral, Vanillin, and benzaldehyde. This chemical transformation led to a slight alteration in the bitterness intensity measurements. From this, it can be deduced that the primary amine groups in Cephalexin and Cefaclor significantly conferred bitterness to these medications. Interestingly, a more pronounced reduction in bitterness was observed in the Cephalexin-Aldehyde complex compared to the same complex of Cefaclor. However, despite these changes, the modified complexes were still not transformed into palatable prodrugs of the respective cephalosporins. This observation suggests that while the primary amine groups are contributors to bitterness, they are not the sole determinants. Bitterness in Cephalexin and Cefaclor is likely influenced by multiple functional groups beyond primary amines. Therefore, the outcomes imply that addressing the primary amine alone, through chemical modifications, is insufficient to overcome the bitterness associated with these cephalosporins. In conclusion, the established bitterness in Cephalexin and Cefaclor involves more than just their primary amine functional groups. While efforts were made to mitigate bitterness through chemical modifications, focusing solely on masking the primary amine does not appear effective for overcoming the bitterness of these cephalosporins— at least in the case of Cephalexin and Cefaclor.

Keywords

Cephalexin, Cefaclor, bitterness, primary amine, Schiff base

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RESEARCH ARTICLE

Evaluating the Effect of Primary Amine in Bitterness of Selected Cephalosporins

Cephalexin and Cefaclor as Examples

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ABSTRACT

Background and objectives: Cephalexin and Cefaclor, are widely prescribed antibiotics known for their characteristic bitter taste. This bitterness is attributed to the specific chemical composition of these substances. The sensory experience of bitterness is influenced by distinct functional groups present in various foods and chemicals.
Researchers have developed diverse techniques and strategies to modify these antibiotics' chemical structure while preserving their therapeutic efficacy to overcome this taste challenge. This study examines the role of the primary amine functional group in generating the bitter taste associated with Cephalexin andCefaclor.
Methods: Schiff base reaction used to make Imines of Cephalexin and Cefaclor on their Primary amine functional group by reacting with Aldehydes (Citral, Vanillin, and Benzaldehyde). The Schiff bases bitternesselation evaluated through gustatory volunteers.
Results: Formation of imines on the primary amine functional group in both Cephalexin and Cefaclor was achieved through the creation of Schiff bases with aldehydes, specifically Citral, Vanillin, and b enzaldehyde. This chemical transformation led to a slight alteration in the bitterness intensity measurement.
Conclusions: *The bitterness of Cephalexin and Cefaclor could be attributed to their Primary amine to a considerable extent, but not solely to the primary amine. Since the bitterness would not be obliterated even after manipulating it. The bitterness of the Cephalosporins is multifactorial involving other functional groups happen to be found in their chemical structure. Further investigations should be carried out for each functional group of Cephalexin and Cefaclor evaluating the contribution of each of the.*
Keywords: Cephalexin, Cefaclor, bitterness, primary amine, Schiff base

INTRODUCTION

Background

The oral route remains the predominant mode of drug delivery and the most common method of medication administration (Sastry et al., 2000). Notwithstanding the substantiation of the therapeutic efficacy of orally administered antibiotics (MacGregor and Graziani, 1997) and endeavours to elucidate the advantages inherent in transitioning to this route (Cunha, 1997), oral drug delivery stands as the preferred, pragmatic, and economically viable method (Anon, 2007). Nonetheless there are many problems encountered with orallyadministered medications (Verma et al., 2010, Kwan, 1997)A perceptible number of pharmaceutical agents exhibit a bitter taste (Mennella et al., 2013), rendering them unpalatable to both pediatric and adult populations for their bitter taste attributes (Karaman, 2014, Vetter et al., 2014), This unpalatable characteristic engenders patient aversion and consequently encumbers the process of drug administration palatability. (Beltrán et al., 2022, Behrens and Meyerhof, 2006). This sensory attribute engenders a palpable conundrum when it pertains to pediatric medicine administration, A main challenge with administering medicine to children is a "matter of taste" (Mennella et al., 2013) given the heightened gustatory sensitivity of children relative toadults (Vennerød et al., 2018). The resultant impact on patient adherence and compliance is profound, thus impeding therapeutic regimen adherence (Mennella et al., 2013, Karaman, 2014)). Consequently, the formulation of pharmaceutical agents possessing favourable taste profiles assumes primacy as it facilitates patient acceptance and adherence and augments commercial viability and corporate profitability (Sohi et al., 2004). The amelioration of the bitter taste of medications is widely acknowledged to ameliorate patient compliance, particularly when considering pediatric and geriatric populations (Beltrán et al., 2022).Cephalexin and Cefaclor, both extensively and widely used antibiotics for diverse clinical indications, are notable(2004), reflecting the inherent complexity inherent to thischemosensory response.

Chemistry and bitterness

The inherent human ability to perceive bitterness is ingrained and invokes instinctual aversive reactions (Behrens and Meyerhof, 2006). This perceptual phenomenon, rooted in an evolutionary context, holds profound significance in the realm of survival, as it functions as a sentinel mechanism against the inadvertent consumption of deleterious substances (Glendinning, 1994). Notably, the propensity of bitter compounds to effectively deter inadvertent pediatric intoxication underscores their intrinsic defensive utility (Rodgers and Tenenbein, 1994). Given the prevalence of toxic plant metabolites possessing bitter attributes, the receptor molecules orchestrating the gustatory experience of bitterness emerge as sentinel sentinels, warning organisms against potential hazards (Behrens and Meyerhof, 2006).

The distribution of bitterness receptors extends beyond the oropharyngeal tissues and encompasses the complex landscape of the gastrointestinal system. The spatial arrangement of these entities has led to conjecture, as proposed by Weiner and others, regarding their potential participation in facilitating digestive and metabolic functions (Wiener et al., 2011), thus beyond their traditional role in taste sensation, The diverse functions of these roles highlight the interconnectedness of chemosensory systems within physiological frameworks.

Of particular interest is the modulation of taste perceptions by saliva-soluble medications, wherein the interaction with taste receptors on the tongue manifests as a diverse array of sensations encompassing bitterness, sweetness, saltiness, sourness, and (Karaman, 2015). Bitterness, among these primary tastes, is renowned for its intricate orchestration (Behrens et al., 2004). The intricate conundrum posed by the structural diversity of compounds eliciting bitterness precludes a generalized molecular blueprint for the phenomenon. To this end, extensive exploration has been undertaken to elucidate fundamental principles governing bitterness perception (Wiener et al., 2011). While elucidation remains an ongoing endeavor, extant research underscores the necessity of a polar moiety coupled with a hydrophobic functional entity for the induction of bitterness (Karaman, 2014). Yet, despite these endeavors, the predictive characterization of molecules evoking bitterness remains an elusive pursuit, as aptly encapsulated by Pronin et al.

In sum, the intricate landscape of bitterness perception navigates intricate pathways interweaving evolutionary contexts, physiological functions, and chemical structures. Understanding the multidimensional facets of bitterness not only unveils fundamental insights into sensory physiology but also informs strategies for enhancing medication palatability and patient adherence.

Human ability to Bitterness perception is innate and evokes aversive reactions (Behrens and Meyerhof, 2006). From an evolutionary perspective, Bitterness is significant for the maintenance of life since it can act as a protective mechanism against consumption of poisonous substances (Glendinning, 1994). Bitter compounds effectively prevent pediatric intoxication (Rodgers and Tenenbein, 1994). As a result of the fact that numerous toxic plant metabolites have a bitter taste, the corresponding receptor molecules play an essential role as warning sensors (Behrens and Meyerhof, 2006). Bitterness receptors are expressed in extraoral tissues and the gastrointestinal tract, therefore Weiner and others suggested that they may play a role in digestive and metabolic processes (Wiener et al., 2011). Saliva-soluble medications which are capable of binding to taste receptors on the tongue give rise to a bitter, sweet, salty, sour, or umami sensation (Karaman, 2015). The bitter taste seems to be the most complex of the five primary flavours(Behrens et al., 2004). Due to the significant variation of structural features of bitter-tasting molecules, it is difficult to generalize the molecular requirements for bitterness. Several hundreds of compounds have been studied for their bitterness, and efforts have been made to investigate whether there are principles to rely on relating to bitterness (Wiener et al., 2011). Nevertheless, it was reported that a bitter tastant molecule requires a polar group and a hydrophobic moiety (Karaman, 2014). Till now, it is not applicable to predict theoretically which molecule will exhibit bitter taste, but indeed, it is a chemically based issue (Pronin et al., 2004).

The human tongue contains a group of around 25 bitter taste receptors, referred to as T2Rs. These receptors can identify a wide range of chemicals, including peptides, alkaloids, terpenoids, and amines (Hoon et al., 1999, Chandrashekar et al., 2000). A notable coincidence is seen wherein a significant fraction of drugs with a bitter taste have an amine group (Karaman, 2014). The aforementioned connection supports the deduction that the existence of an amine functional group can bestow bitterness, a perceptual characteristic that frequently evokes an aversive reaction (Normah et al., 2013, Karaman, 2015). Based on this premise, a logical proposal

emerges, suggesting that modifying the amine group can regulate the bitterness of therapeutic compounds. Given this assumption, our study focuses on conducting chemical modifications that specifically target the primary amine functional group. The objective of this undertaking is to determine its function as a factor that imparts bitterness. It is important to highlight that the absence of bitterness sensation observed when the amine group is occluded and afterwards exposed to saliva can be attributed to the limited interaction between the molecule and the receptors responsible for the experience of bitterness. This strategic methodology not only reveals the complex mechanisms involved in perceiving bitterness but also emphasizes the potential for strategically altering the chemical properties of medicinal drugs to reduce unwanted sensory characteristics. The utilization of the amine group as a factor influencing bitterness shows potential for enhancing the taste of pharmaceuticals and, consequently, encouraging patient adherence and therapeutic efficacy.

The strategic hindrance of the amine group in this particular scenario can effectively reduce bitterness, as suggested by Karaman (Karaman, 2014) and the reduction in intensity of taste perception is accomplished through the inhibition of oral solubility or the hindrance of interactions with bitter taste receptors. The resulting consequence is a decrease in the stimuli that elicit the sensation of bitterness (Karaman, 2013a, Karaman, 2014). Empirical research has substantiated the effectiveness of this methodology, particularly concerning the administration of atenolol and dopamine. The bitterness of these compounds can be efficiently eliminated by including them in a prodrug formulation, which prevents access to their free amine groups (Karaman and Hallak, 2010).

While Cephalosporins are widely used in treating various types of infections caused by a wide range of bacteria, and they are known to have an unpleasant bitter taste, many techniques have been developed to solve the aversive taste problem, numerous strategies have been developed to tackle this challenging flavour characteristic (Karaman, 2013b). Nevertheless, the current physical will not provide a comprehensive understanding of the specific functional group that underlies bitterness. The central focus of the majority of various tactics designed to improve the unpleasant taste of medications does not primarily rely on the chemical nature of bitterness. Instead, their ultimate goal is to mask the unpleasant taste. It is crucial to emphasize that the presence of bitterness in cephalosporins cannot be entirely attributed to the amine functional group. Instead, several separate functional groups contribute to this unpleasant characteristic. The objective is to modify the primary amine to investigate its function in relation to the bitter taste of orally administered cephalosporins. Cephalexin and Cefaclor are cephalosporines known to have a bitter taste and could be given orally. Both have a primary amine functional group and are candidates for testing.

METHODS AND MATERIALS

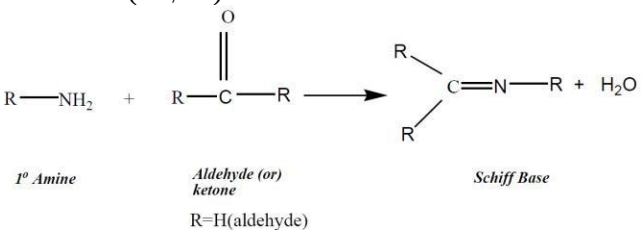
Method

Schiff's Reaction is used to alter the primary amine functional group of the selected cephalosporins to generate an imine to form Schiff base (Scheme 1.). Schiff's base reaction, also known as the imine formation reaction, is a chemical process involving the reaction between a primary amine and a carbonyl compound, typically an aldehyde or a ketone. This reaction results in the formation of a Schiff's base or imine linkage, which is a functional group containing a carbon-nitrogen double bond (C=N) (Cordes and Jencks, 1962).

The gustatory sensation test was handled with a sample within standard limitations (Anand et al., 2008), the tasters' panel (Anand et al., 2007) composed of 10 healthy humans chosen under the overarching known standards (Anand et al., 2008). The consent form for Tastants was adopted using Middlesex University London standards (MIDDLESEX, 2014).

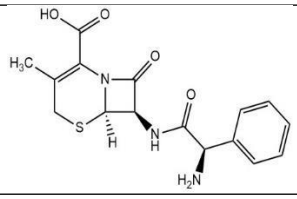
The reactants are two cephalosporins (Cephalexin and Cefaclor) (structures shown below in Table 1) which are known to have a bitter taste (Karaman, 2013a, Vetter et al., 2014, Paterson and Doi, 1987) and three aldehydes (citral, benzaldehyde and vanillin- structures shown below in Table 2) which are deemed to be available naturally.

R2C=NR' (R' ≠ H).



Scheme 1: Schiff Base Reaction (Xavier and Srividhya, 2014)

Table 1. Chemical Structure of Cephalexin and Cefaclor

Cephalexin	

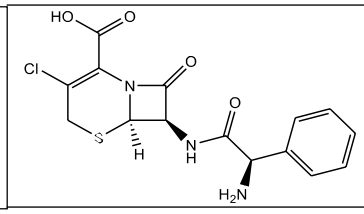
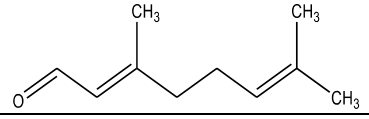
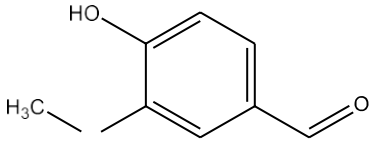
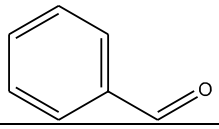
Cefaclor	

Table 2. Structure of Citral, Vanillin and Benzaldehyde

Citral	
Vanillin	
Benzaldehyde	

The experiment was carried out in the College of Pharmacy. Hawler Medical University. Distilled water was prepared in the laboratory.

Thin Layer Chromatography (TLC), FTIR spectroscopy, ^{13}C-NMR Spectroscopy and ^{1}H-NMR spectroscopy were carried out to identify and ascertain imine formation with laboratory experiments. All peaks are referenced according to the textbook Introduction to Spectroscopy by Pavia and others(Pavia et al., 2014). Sketches and chemical structures are done through ChemDraw software.

Materials

The cephalosporins and aldehydes are obtained from Sigma-Aldrich® Lab & Production Materials, and all chemicophysical properties of starting materials are deemed as mentioned by the manufacturer.

Generation Method of Schiff Bases:

Equimolecular weight (1 mmole) of cephalosporins (0.347 gm in case of Cephalexin and 0.3678 gm in case of Cefaclor) the same of corresponding aldehydes (0.171 ml citral, 0.1521 Vanillin, 0.1020 ml of Benzaldehyde) mixed in ethanol using round bottom flask. The catalyst is acetic acid

The procedure of formation of Schiff base complexes carried out through combining equimolar amounts (1 mmole) of Cephalosporins (0.347 gm Cephalexin or 0.3678 gm in case of Cefaclor) and aldehydes, correspondingly (0.171 mL Citral, 0.1521 gm Vanillin, 0.1020 mL Benzaldehyde) mixed in ethanol, using round bottom flask. The reactions are facilitated by the use of 2 or 3 drops of Glacial Acetic Acid as a catalyst.

The reaction mixture was refluxed for 5 hours at a temperature of 80 C° with continuous stirring. The solvent was evaporated under vacuum, and the precipitated product was filtered to collect the newly formed Schiff base ligand after Further purification by washing with hexane and ethanol successively.

Procedures

Synthesis of Cephalexin – aldehyde Schiff bases (A_1, A_2, and A_3):

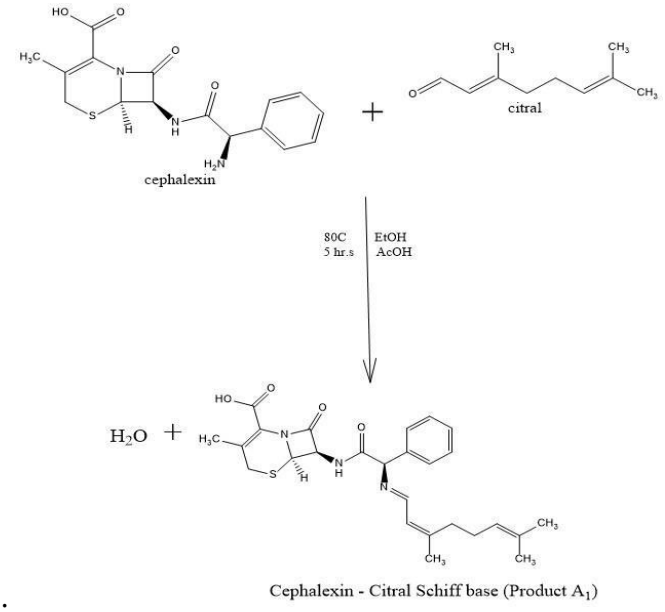
Through the implementation of above above-described method, Schiff Bases of Cephalexin are produced (Schemes: 2, 3 and 4). The physical properties of the products are written down in (Table 3).

Equimolecular weight (1 mmole, 0.347 gm) of Cephalexin added to the same amount of corresponding aldehydes (0.171 ml citral for A_1, 0.1521 Vanillin for A_2, and 0.1020 ml of Benzaldehyde for A_3), each accordingly in a separate procedure mixed in ethanol using round bottom flask and acetic acid as catalyst.

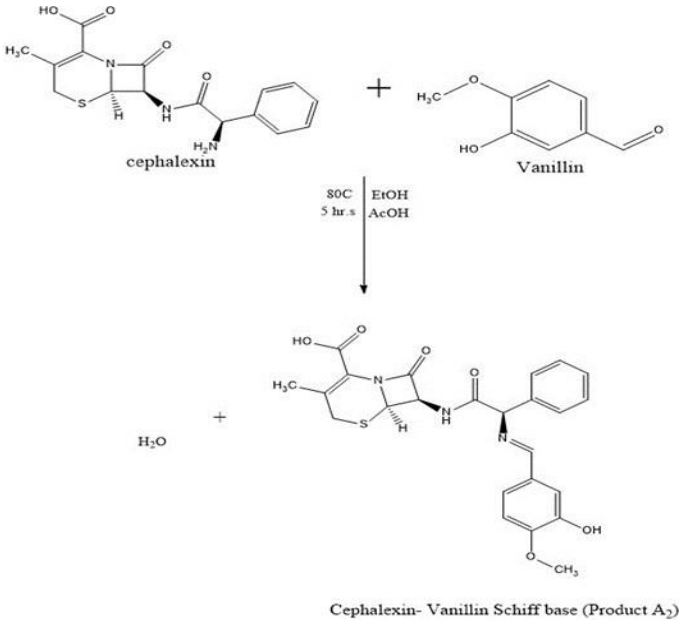
TLCs were well developed using a mobile phase of (ethyl acetate 1: 9 cyclohexane). The FT-IR spectrums asset to formation of Imine functional group (1642 cm^{-1} in A_1, 1641 cm^{-1} in A_2, and 1642 cm^{-1} in A_3), also absence of cephalexin primary amine double peaks(3419 cm^{-1} and 3198 cm-1) (Table 4, and Figures 1, 2, 3, and 4). ^{13}C-NMRs recorded in DMSO-^{d6} revealed the Characteristic peaks for imine carbon (151.04 ppm for C26 in A_1, 159.05 ppm for C27 in A_2, and 161.15 ppm for C25 in A_3) (see Table 5, Figures 5, 7, and 9), while 1H NMRs show characteristic imine Carbons (7.75 ppm in A_1, 8.50 ppm in A_2, and 8.75 ppm in A_3) (Table 5, Figures 6, 8, and 10).

Table 3: Physical properties of cephalexin and its Schiff bases

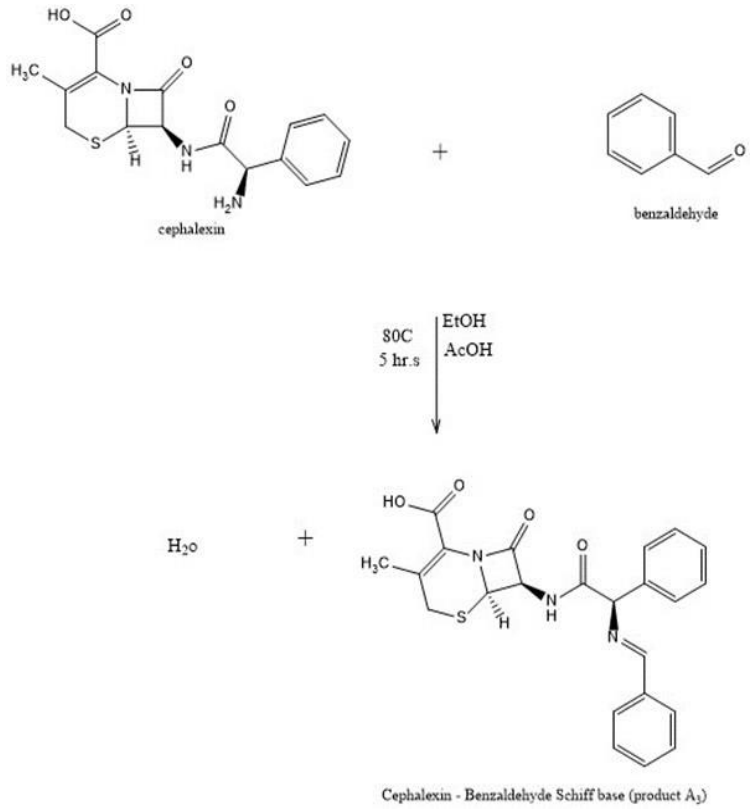
item	Reactants	Colour	m.p. ºC	Yeild %
Cephalexin	none	White	197	
A_1	Cephalexin + Citral	Dark yellow	221	92
A_2	Cephalexin + Vanillin	Light brown yellow	225	88
A_3	Cephalexin + Benzaldehyde	Light brown yellow	231	80



Scheme 2: Cephalexin and Citral reaction (Product A_1)



Scheme 3: Cephalexin and Vanillin reaction (Product A_2)



Scheme 4: Cephalexin and Benzaldehyde reaction (Product A_3)

Table 4: FT-IR Spectra characterization of Cephalexin, A_1, A_1, and A_3

Item	ν Carboxylic O-H (cm^{-1})	ν Carboxylic C=O (cm^{-1})	ν Imine C=N (cm^{-1})	ν Primary amine -NH_2 (cm^{-1})	ν Carbonyl C=O (cm^{-1})	ν Citral Aliphatic C=C (cm^{-1})	ν C-S-C (cm^{-1})	ν C-O Methoxy (cm^{-1})
Cephalexin	3038	1760	NA	3419, 3198	1688	NA	1160	NA
A_1	3096	1732	1642	NA	1735	1672	1180	NA
A_2	3108	1751	1641	NA	1684	NA	1183	1036
A_3	3122	1762	1655	NA	1742	NA	1180	NA

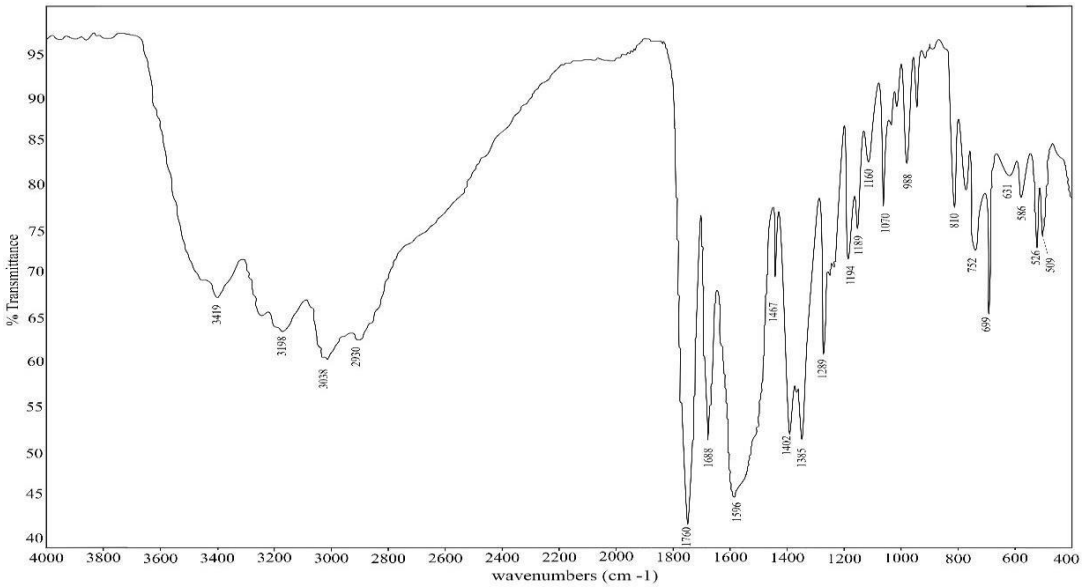


Figure 1: FT-IR spectrum of Cephalexin

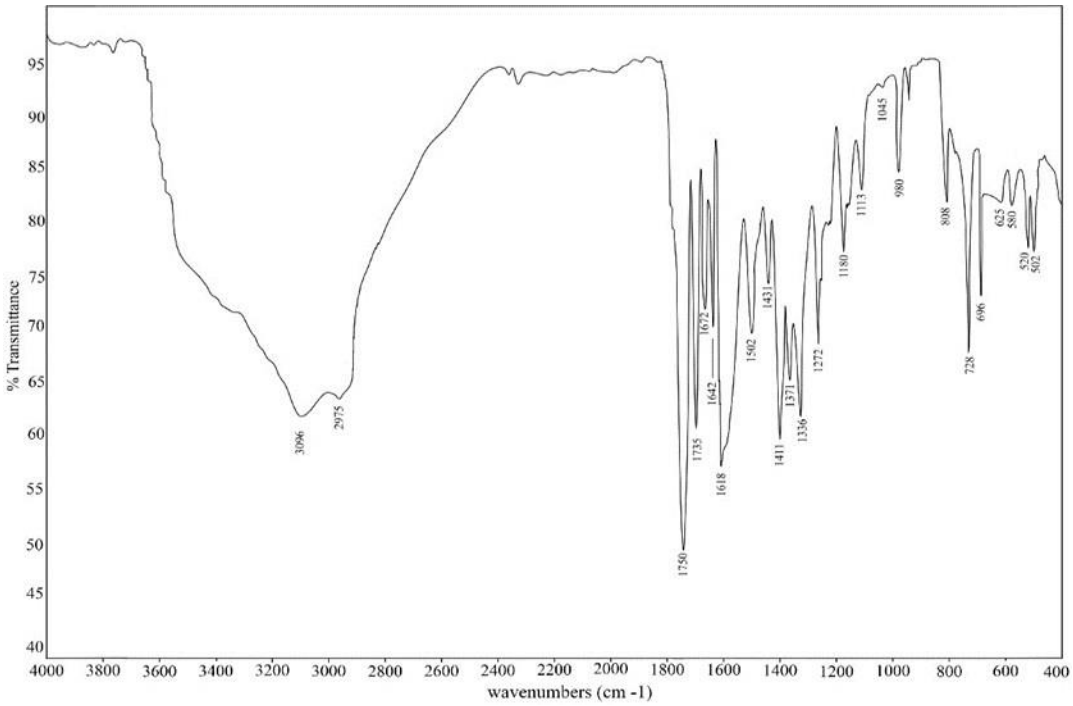


Figure 2: FT-IR spectrum of A_1

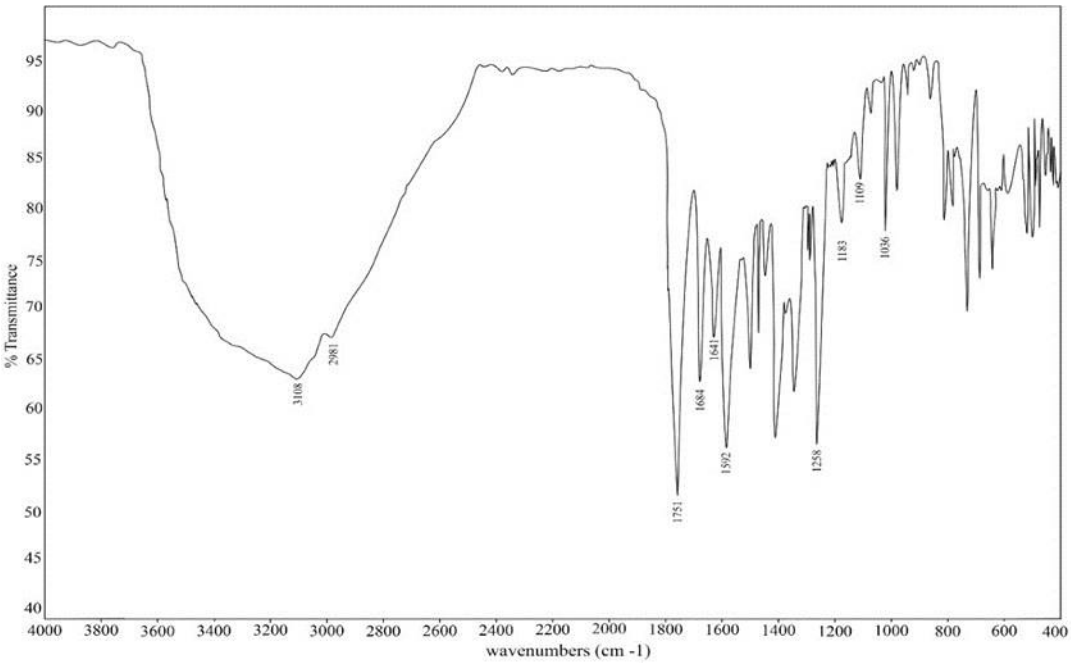


Figure 3: FT-IR spectrum of A_2

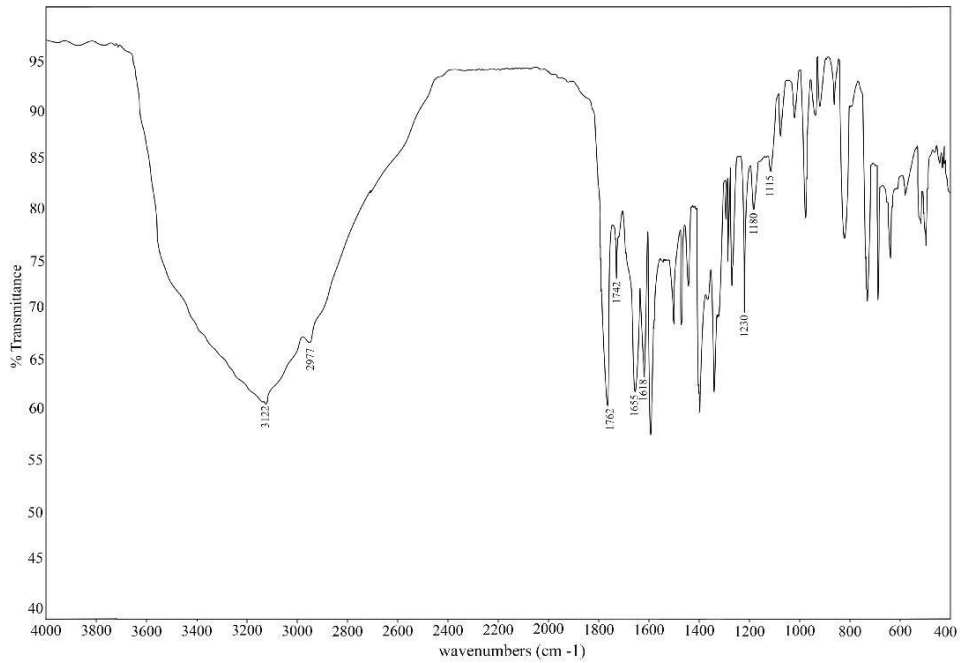
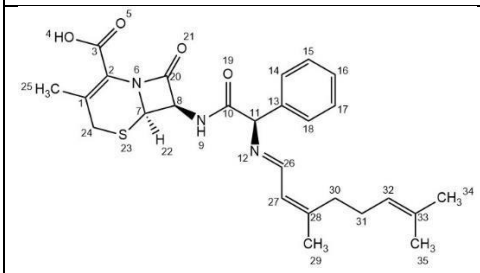
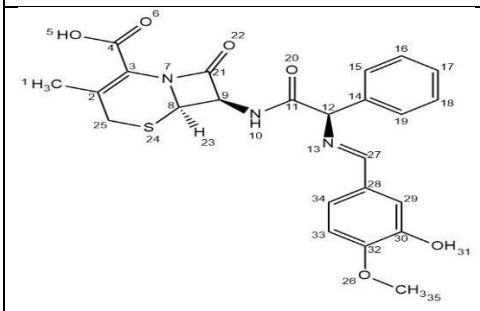
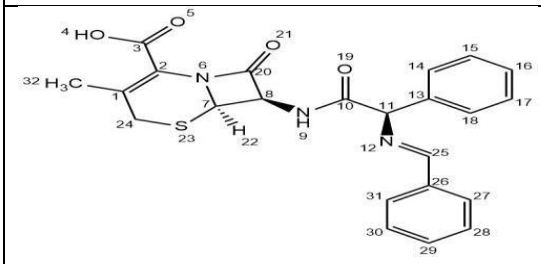


Figure 4: FT-IR Spectrum of A_3

Table 5: 13C, and ^{1}H-NMR spectra (DMSO-6d; ppm) of A_1, A_2, and A_3

Schiff Base structure	Item	^{13}C-NMR	^{1}H-NMR
	A_1	(C1, 131.08), (C2, 123.52), (C3, 166.70), (C7, 57.76), (C8, 60.15), (C10, 171.58), (C11, 7.64), (C13, 137.85), (C14, C18, 2C, 128.62), (C15, C17, 2C, 128.88), (C16, 127.85), (C20, 166.12), (C24, 29.08), (C25, 18.16), (C26, 151.04), (C27, 123.17), (C28, 146.11), (C29, 20.64), (C30, 36.12), (C31, 25.95), (C32, 124.12), (C33, 132.97), (C34, 24.06), (C35, 19.60).	δ12.11(s, 1H, H4, 1.01), 5.52(dd, 1H, H9, 1.01), 6.78(d, 1, H9, 1.01), 6.23(s, 1H, H11, 0.98), 7.52(m, 1H, H16, 1.10), 5.41(d, 1H,H22, 1.05), 3.58(d, 1H, H24, 0.68), 3.7(d, 1H, H24, 0.98), 2.18(s, 3H, H25, 2.99), 7.75(dp, 1H, H26, 0.95), 6.04(ddq, 1,H27,0.88), 1.96(m, 3H, H29, 2.76), 2.52(m, 2H, H30, 1.96), 2.32(dddt, 2H, H31,1.97), 5.29(dddt, 1H,H32,1.08), 1.84,1.80(ddt, 6H, H34,H35, 5.92), 7.70(ddd, 2H,H14,H18, 1.98), 7.60(m, 2H, H15, H 17, 1.88)
	A_2	(C1, 19.60), (C2, 131.95), (C3, 123.17), (C4, 165.99), (C8, 59.22), (C9, 61.22), (C11, 171.87), (C12, 72.17), (C14, 137.14), (C15, C1,127.95), (C16, C18, 128.60), (C17, 127.27), (C21, 163.19), (C25, 29.97), (C27, 159.02), (C28, 132.26), (C29, 116.26), (C30, 148.27), (C32, 150.32), (C33, 113.21), (C34, 122.87), (C35, 57.47).	δ2.18(s, 3H, H1, 3.01), 12.11(s, 1H, H5, 1.00), 5.79(s,1H,H9, 1.00), 6.41(s, 1H,H10,0.96), 6.59(s,1H,H12,0.99), 7.19(m, 1H,H17,1.16), 5.4(d, 1H, H23, 0.99), 3.58(d,1H, H25, 1.00), 3.66(d, 1H, H25, 1.03), 8.50(s, 1H, H27,0.99), 7.06(m, 1H, H29, 0.99), 7.6(s, 1H, H31. 0.99), 6.92(s, 1H, H33,1.00), 7.38(m, 1H, H34, 0.99), 4.04(s, 3H, H35, 2.96), 7.52(ddt, 2H, H15,H19, 2.10), 7.47(m, 2H, H16, H18, 1.92),
	A_3	(C1, 133.05), (C2, 123.60), (C3, 168.69), (C7, 59.05), (C8, 60.98), (C10, 173.79), (C11, 73.79), (C13, 138.01), (C14, C18, 130.05), (C15, C17, 131.05), (C16, 129.71), (C20, 163.15), (C24, 29.35), (C25, 161.15), (C26, 136.04), (C27, C31, 131.65), (C28, C30, 131.60), (C32, 18.79).	δ12.08(s, 1H, H4, 1.00), 5.79(dd, 1H, H8, 1.01), 6.92(d,1H,H9, 1.01), 6.59(d, 1H,H11,0.99), 7.52(m,1H,H16, 0.99), 5.40(d, 1H,H22,1.00), 3.57(d, 1H, H24, 1.00), 3.68(d,1H, H24, 1.00), 8.75(s, 1H, H25, 0.99), 7.65(m, 1H, H29,1.11), 2.18(s, 3H, H32, 2.99), 7.69(m, 2H, H14,H18, 2.11), 7.60(m, 2H, H15, H17, 2.11), 2.12(m, 2H, H27, H31, 2.12), 7.63(m, 2H, H28, H30, 2.12).

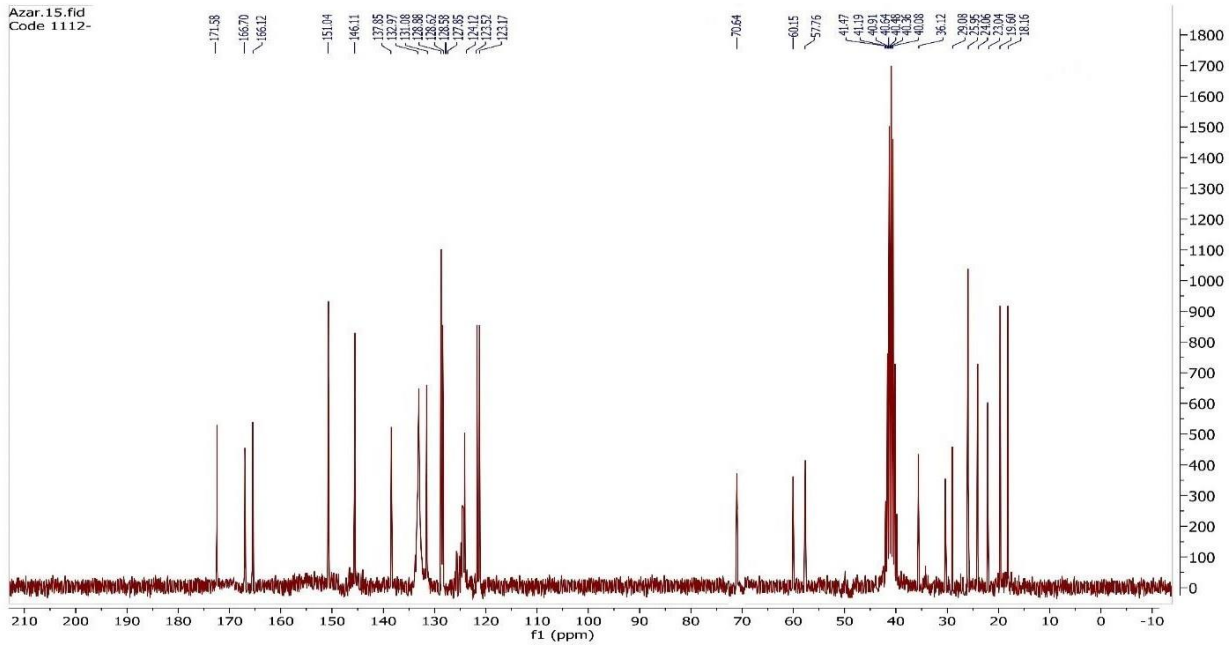


Figure 5: ^{13}C-NMR Spectrum of Product A_1

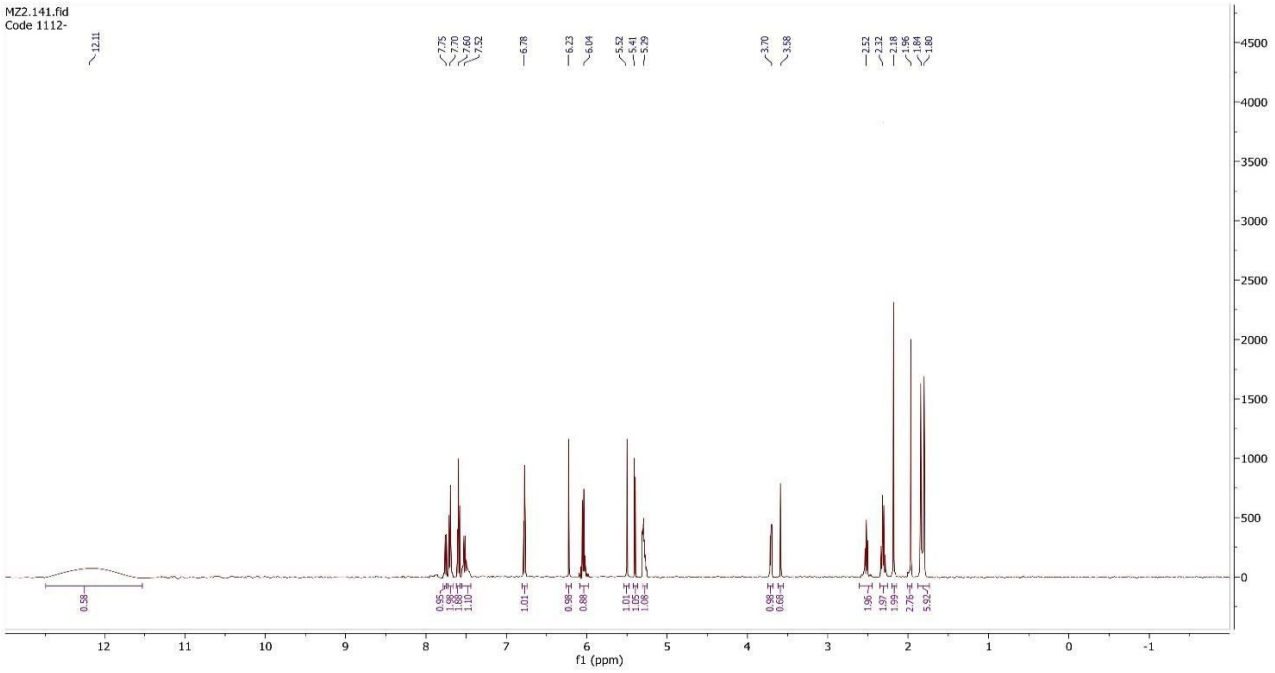


Figure 6: ^{1}H-NMR Spectrum of product A_1

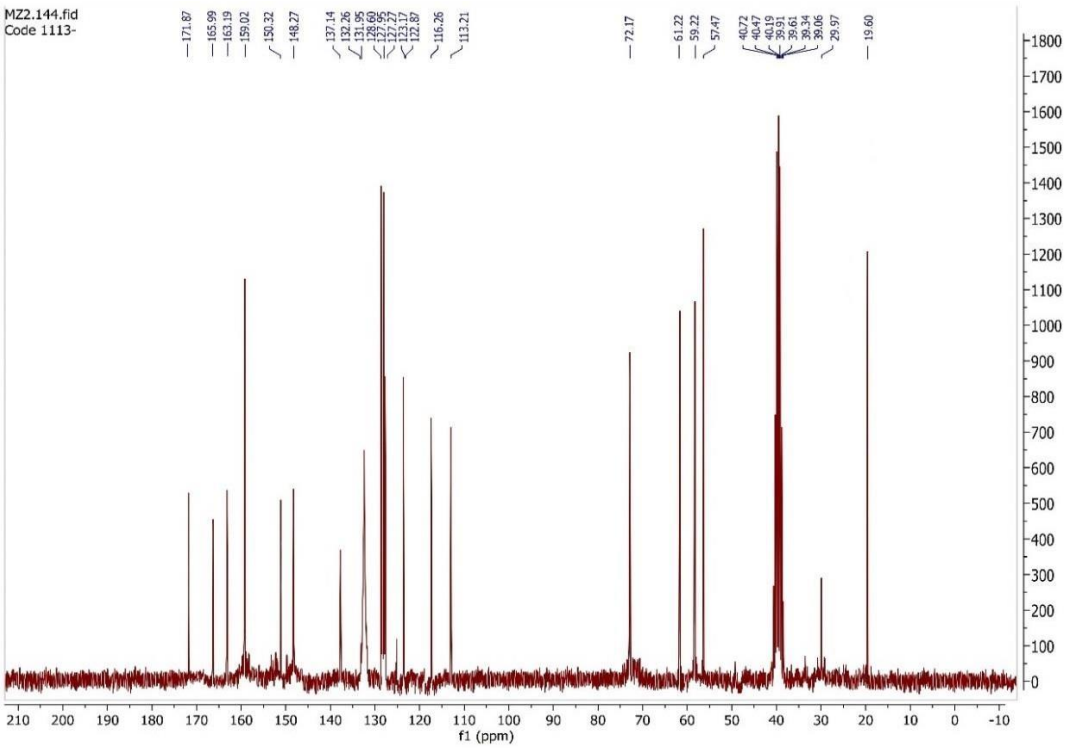


Figure 7: ^{13}C-NMR Spectrum of product A_2

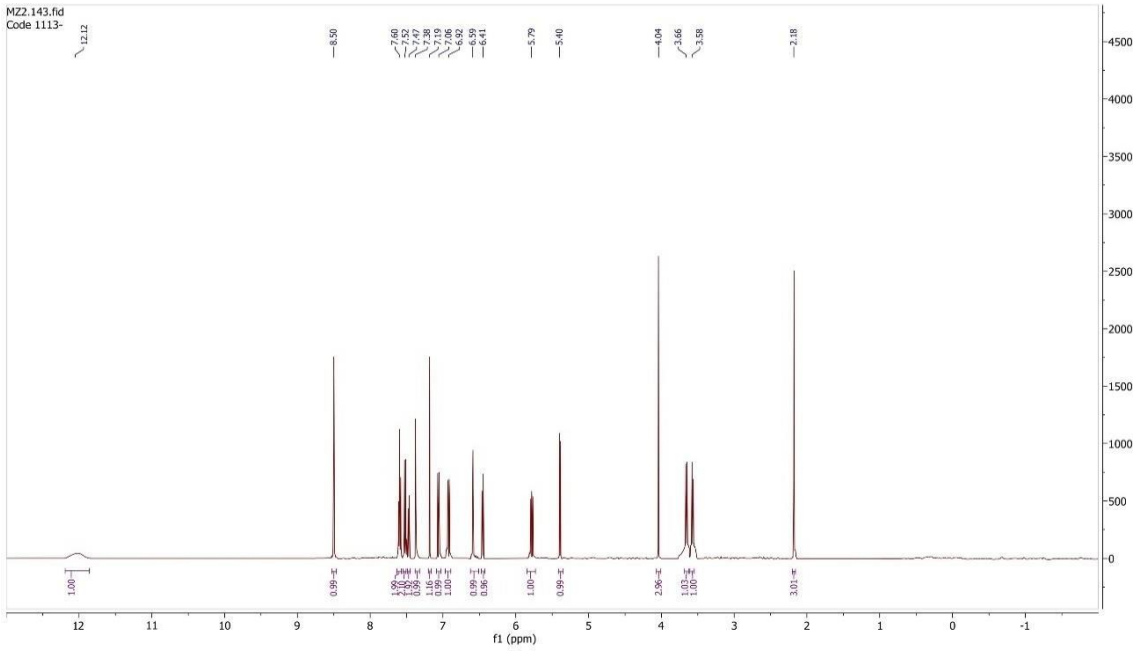


Figure 8: ^{1}H-NMR Spectrum of product A_2

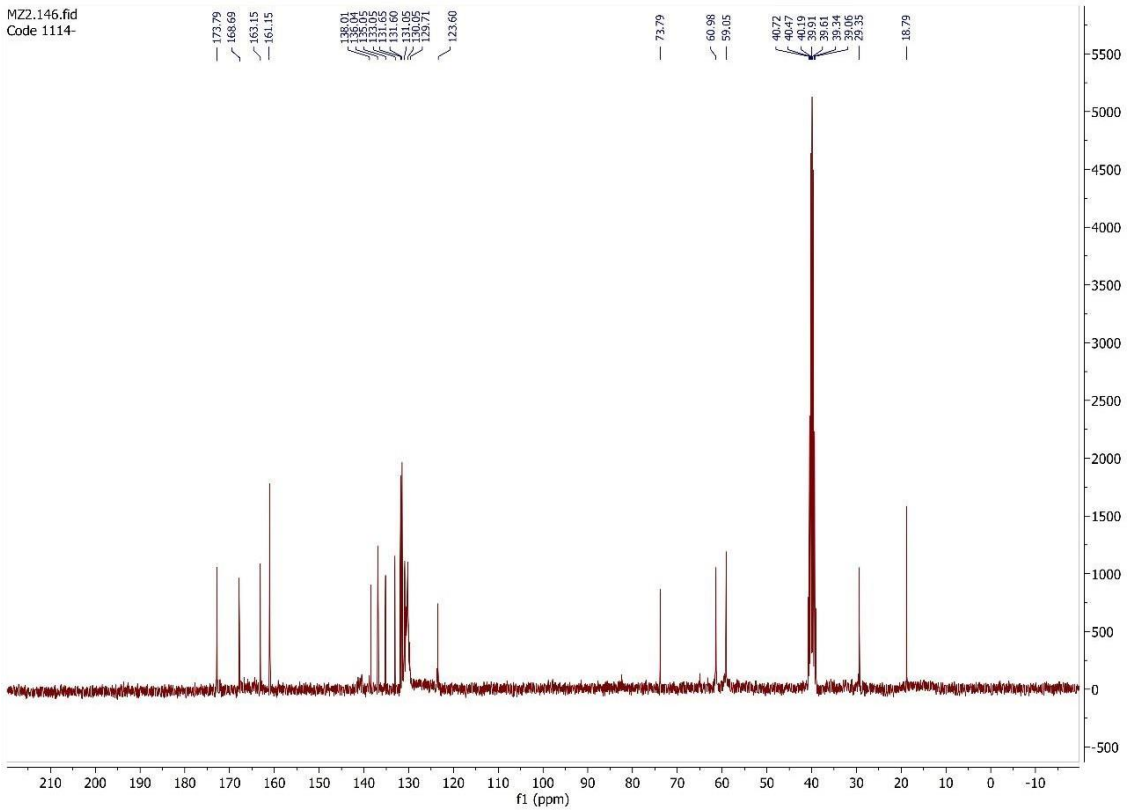


Figure 9: ^{13}C-NMR Spectrum of Product A_3

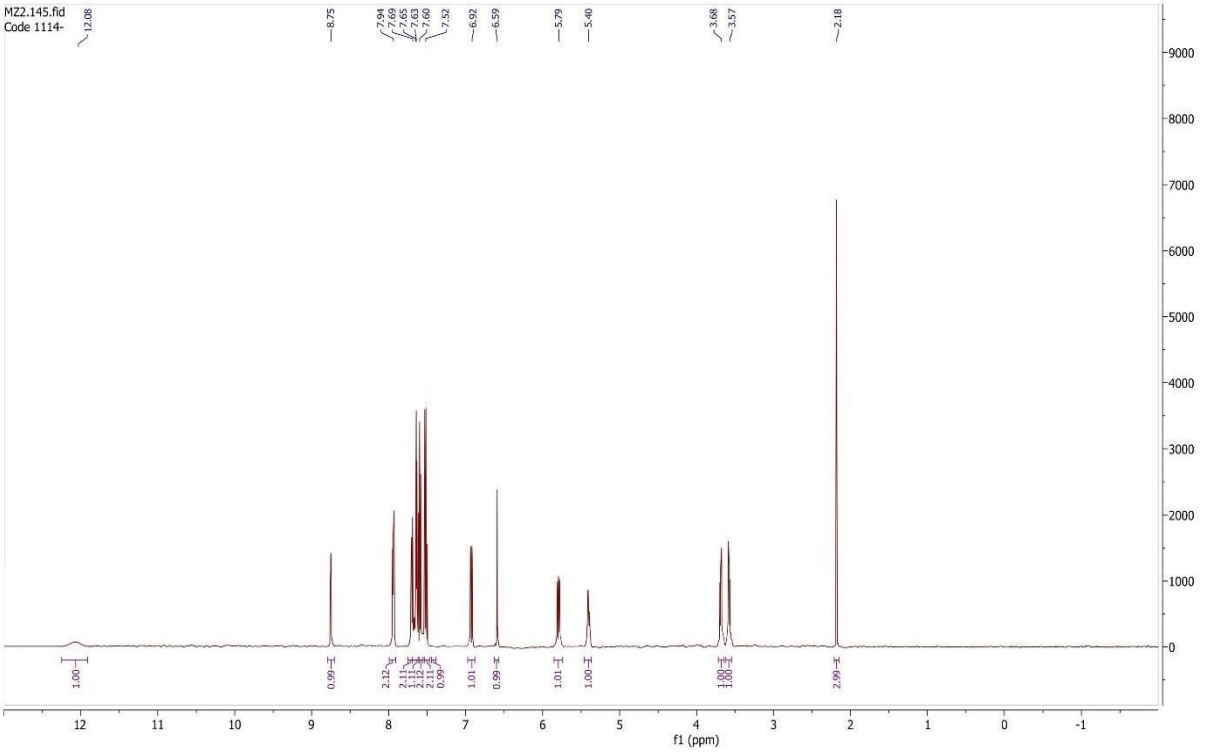
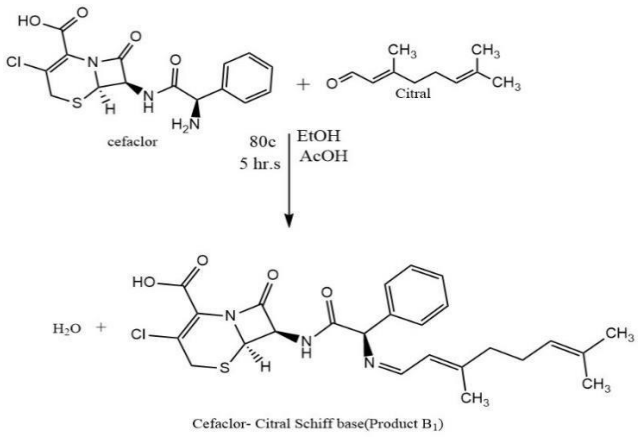


Figure 10: ^{1}H-NMR Spectrum of Product A_3

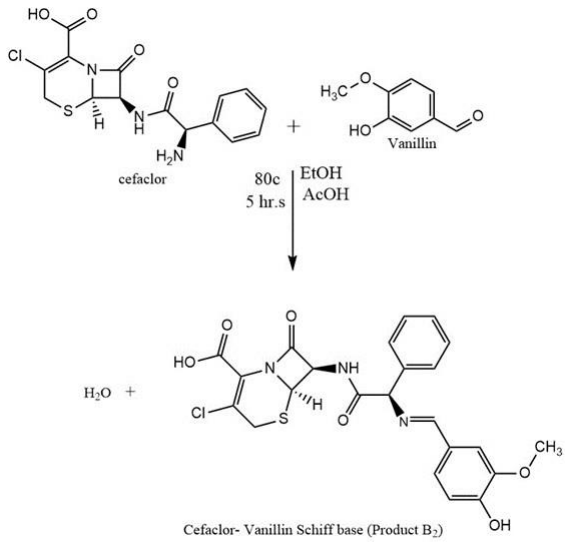
Cefaclor – Aldehyde Schiff bases (B Products):

Schiff Bases of Cefaclor produced implementing the general method mentioned previously. (Schemes 5, 6, and 7). Physical properties of the products written down in (Table 6) with consideration that melting points of cefaclor and B products could not be precisely determined due to combustion before reaching melting temperatures. Equimolecular weight (1 mmole, 0.347 gm) of Cefaclor added to the same amount of corresponding aldehydes (0.171 ml citral for B_1, 0.1521 Vanillin for B_2, and 0.1020 ml of Benzaldehyde for B_3), each separately in a separate procedure mixed in ethanol using round bottom flask and acetic acid as catalyst.

TLCs were well developed using a mobile phase of (ethyl acetate 1: 9 cyclohexane). The FT-IR spectrums asset to formation of Imine functional group (1672 cm^{-1} in B_1, 1668 cm^{-1} in B_2, and 1654 cm^{-1} in B_3), also absence of cephalexin primary amine double peaks (3334 cm^{-1} and 3205 cm-1 (Table 7, and Figures 11, 12, 13, and 14). ^{13}C-NMRs recorded in DMSO-d6, revealed the Characteristic peaks for imine carbon (151.11 ppm for C10 in B_1, 160.55 ppm for C33 in B_2, and 162.02 ppm for C32 in B3) (Table 8), while ^{1}H-NMRs show characteristic imine Carbons (7.85 ppm in B_1, 8.50 ppm in B_2, and 8.71 ppm in B_3)(Table 8).



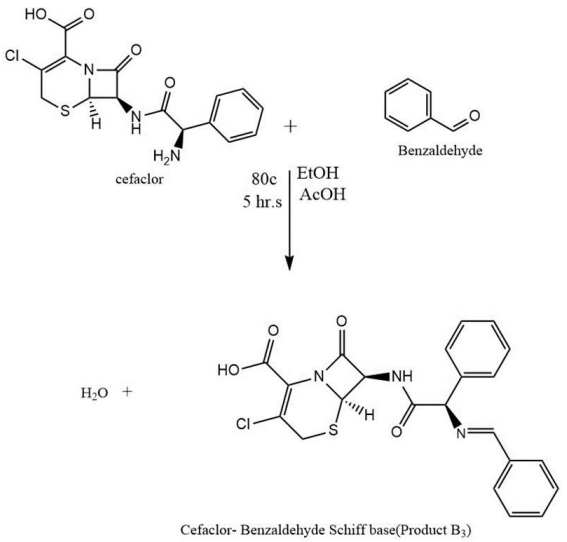
Scheme 5: Cefaclor and Citral reaction (Product B_1)



Scheme 6: Cefaclor and Vanillin reaction (Product B_2)

Table 6: physical properties of Cefaclor and its Schiff bases

Item	Reactants	Colour	Yeild %
Cefaclor	none	White	none
B_1	Cefaclor + Citral	light yellow	88
B_2	Cefaclor + Vanillin	dark yellow	80
B_3	Cefaclor + Benzaldehyde	Dark yelloe	86



Scheme 7: Cefaclor and Benzaldehyde reaction (Product B_3)

Table 7: FT-IR Spectral characterization of Cefaclor, B_1, B_2, and B_3

Item	ν Carboxylic O-H (cm^{-1})	ν Carboxylic C=O (cm^{-1})	ν Imine C=N (cm^{-1})	ν Primary amine NH_2 (cm^{-1})	ν Carbonyl C=O (cm^{-1})	ν Citral Aliphatic C=C (cm^{-1})	ν C-S-C (cm^{-1})	ν C-O Phenyl alkyl (cm^{-1})	ν Aryl-Chloride (cm^{-1})	N Citral CH_3 & CH_2 bending (cm^{-1})
Cefaclor	3053	1786	NA	3334, 3205	1696	NA	1164	NA	1112	NA
B1	3042	1746	1672	NA	1708	1647	1179	NA	1108	1452, 1381
B2	3037	1732	1668	NA	1691	NA	1165	1264, 1053	1112	NA
B3	3008	1726	1654	NA	1688	NA	1160	NA	1115	NA

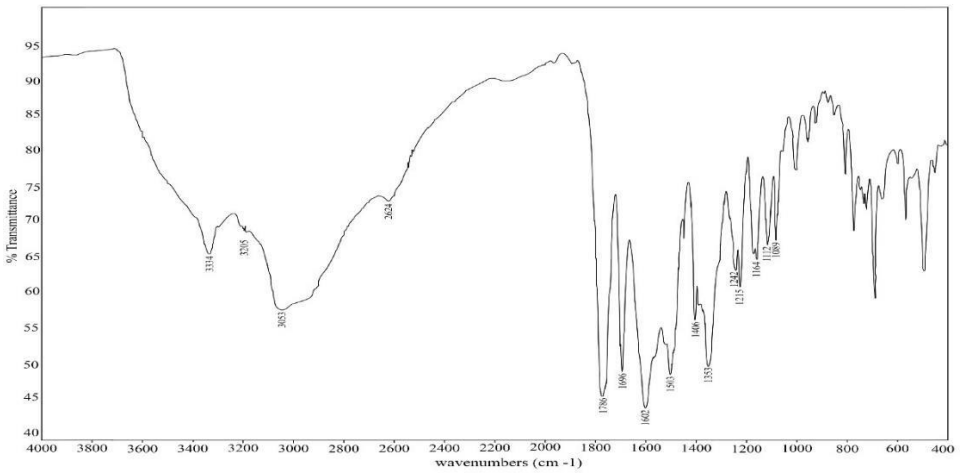


Figure 11: FT-IR spectrum of Cefaclor

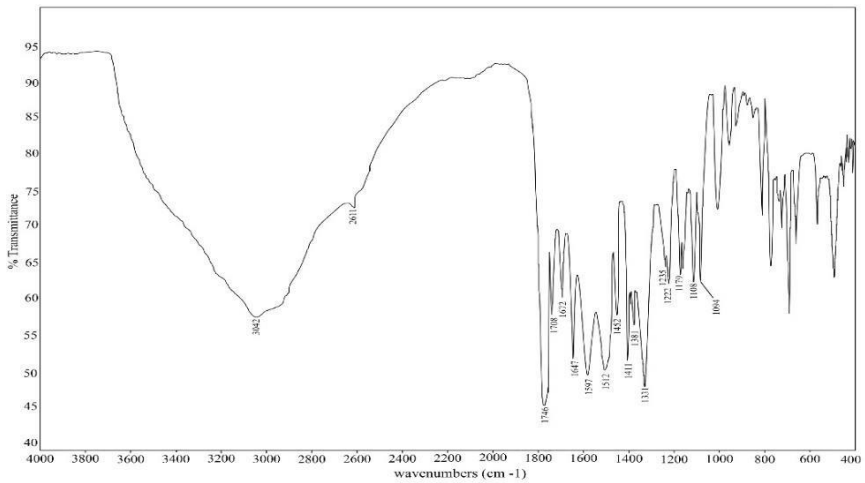


Figure 12: FT-IR spectrum of B_1

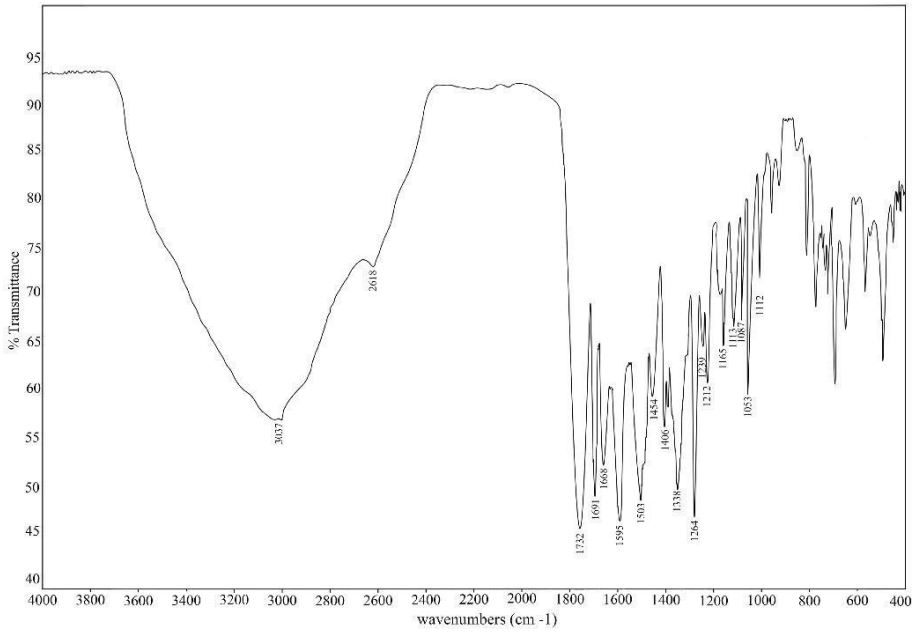


Figure 13: FT-IR spectrum of B_2

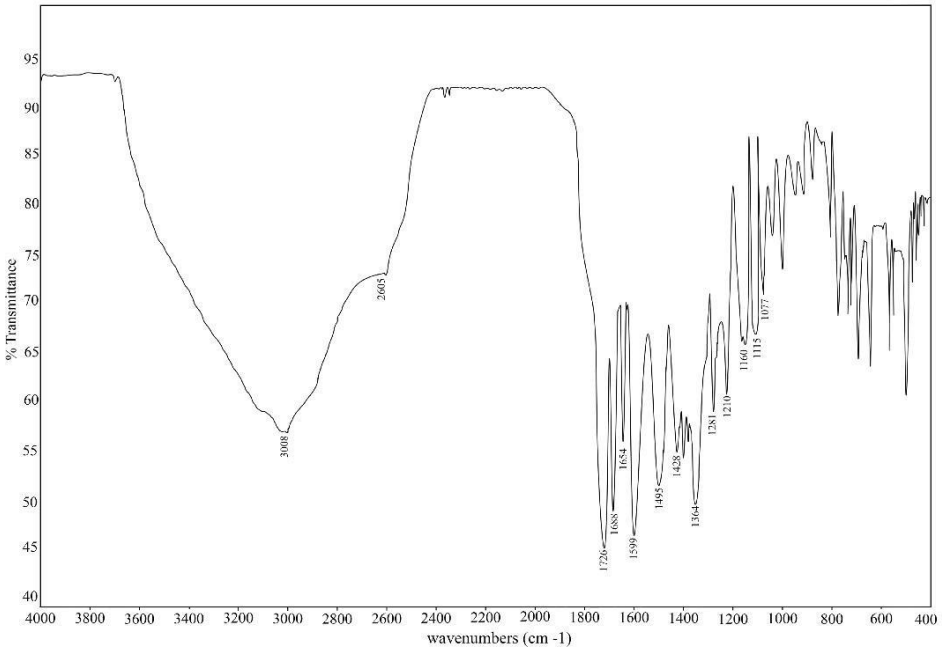
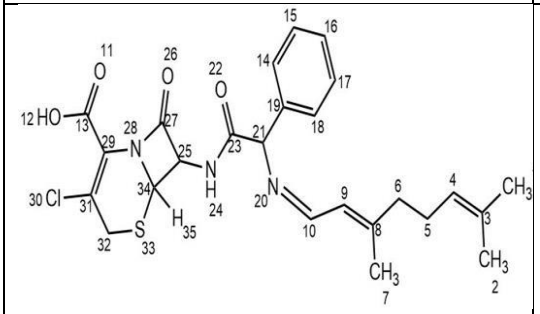
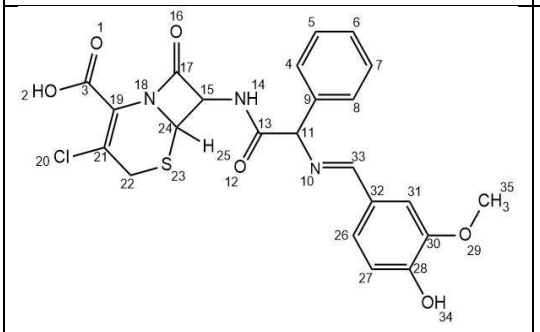
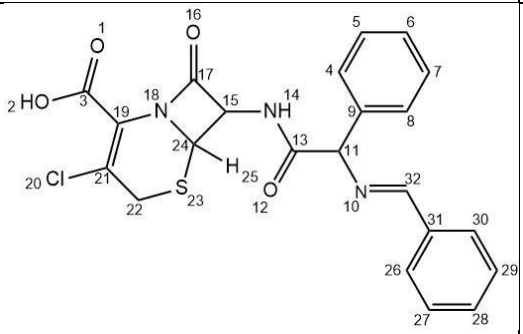


Figure 14: FT-IR spectrum of B_3

Table 8: ^{13}C, and ^{1}H-NMR spectra (DMSO-6d; ppm) of B_1, B_2 and B_3

Schiff Base structure	Product	^{13}C-NMR	^{1}H-NMR
	B_1	(C1, 25.13), (C2, 18.13), (C3, 133.20), (C4, 123.11), (C5, 26.22), (C6, 39.13), (C7, 17.61), (C8, 145.62), (C9, 119.99), (C10, 151.11), (C13, 166.11), (C14, C18, 128.12), (C15, C17, 129.48), (C16, 127.11), (C19, 137.20), (C21, 70.22), (C23, 172.16), (C25, 60.48), (C27, 164.58), (C29, 123.62), (C31, 130.22), (C32, 32.18), (C34, 56.44).	δ1.84, 1.80(ddt, 6H, H1, H2, 6.22), 5.45(ddtd, 1H, H4, 0.99), 2.31(dtdd, 2H, H5, 2.24), 2.87, 3.06(m, 2H, H6, 2.21), 1.97(t, 3H, H7, 3.14), 6.23(dp, 1H,H9, 0.92), 7.85(dddd, 1H, H10, 1.12), 12.10(s, 1H, H12, 1.11), 7.74(m, 2H, H14, H18, 2.26), 7.71(m, 2H, H15, H17, 2.29), 7.60(m, 1H,H16, 1.22), 6.35(d, 1H, H21, 1.24), 6.62(d, 1H, H24, 0.96), 6.04(dd, 1H, H25, 1.11), 4.06, 4.17(dd, 2H,H32, 1.95), 5.8(d, 1H, H35, 1.12).
	B_2	(C3, 166.25), (C4, C8, 127.43), (C5, C7, 128.12), (C6, 127.28), (C9, 136.44), (C11, 70.22), (C13, 172.25), (C15, 59.84), (C17, 164.82), (C19, 123.54), (C21, 128.33), (C22, 31.58), (C24, 58.54), (C26, 123.33), (C27, 114.65), (C28, 148.61), (C30, 146.99), (C31, 112.54), (C32, 127.62), (C33, 160.55), (C35, 55.80s).	δ11.83(s, 1H, H2, 1.00), 7.73(m, 2H, H4, H8, 1.81), 7.64(m, 2H, H5, H7, 1.82), 7.52(m, 1H, H6, 0.91), 6.92(s, 1H, H11, 0.99), 6.29(d, 1H, H14, 0.91), 5.80(dd, 1H, H15, 0.92), 3.96(d, 1H, H22, 0.92), 4.01(d, 1H, H22, 0.92), 5.45(d, 1H, H25, 0.91), 7.58(dd, 1H, H26, 0.90), 7.10(d, 1H, H27, 0.97), 7.51(d, 1H, H31, 0.90), 8.50(s, 1H, H33, 0.94), 8.73(s, 1H, H34, 0.92), 3.99(s, 3H, H35, 2.74).
	B_3	(C3, 166.64), (C4, C8, 128.51), (C5, C7, 129.14), (C6, 128.21), (C9, 138.18), (C11, 71.45), (C13, 172.70), (C15, 60.14), (C17, 164.06), (C19, 125.20), (C21, 129.30), (C22, 32.10), (C24, 58.68), (C26, C30, 130.07), (C27, C29, 129.17), (C28, 131.48), (C31, 134.51, (C32, 162.02).	δ10.94(s, 1H, H2, 1.01), 7.75(m, 2H, H4, H8, 2.23), 7.60(m, 2H, H5, H7, 2.23), 7.52(m, 1H, H6, 1.14), 6.92(d, 1H, H11, 1.12), 6.29(d, 1H, H14, 1.14), 5.80(dd, 1H, H15, 1.11), 3.95(d, 1H, H22, 1.12), 4.01(d, 1H, H22, 1.13), 5.45(d, 1H, H25, 1.12), 7.94(m, 2H, H26, H30, 2.24), 7.66(m, 2H, H27, H29, 2.22), 7.72(m, 1H, H28, 1.12), 8.71(s, 1H, H32, 1.10).

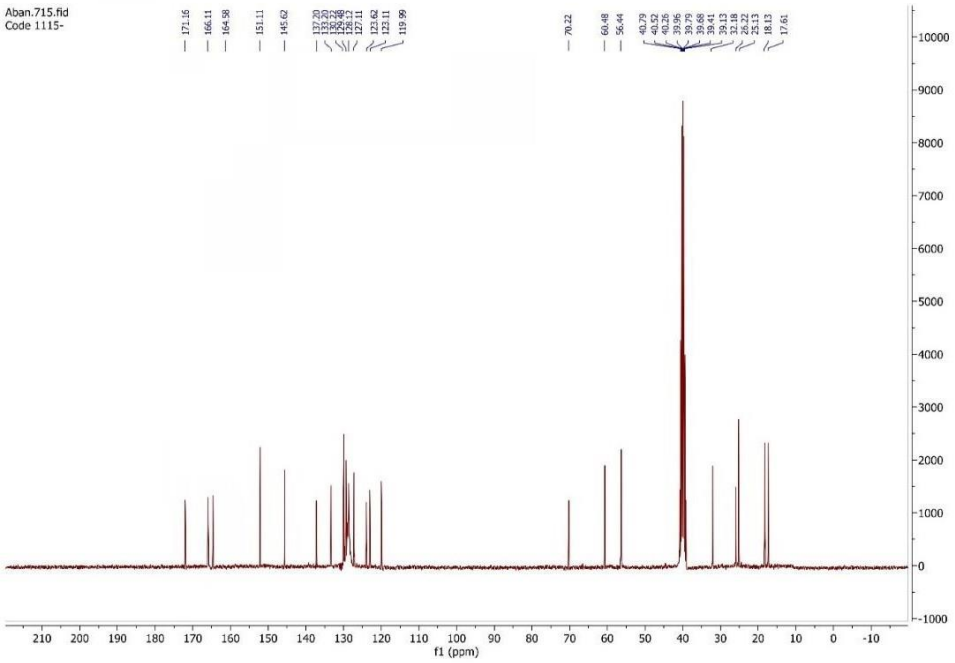


Figure 15: ^{13}C-NMR spectrum of product B_1

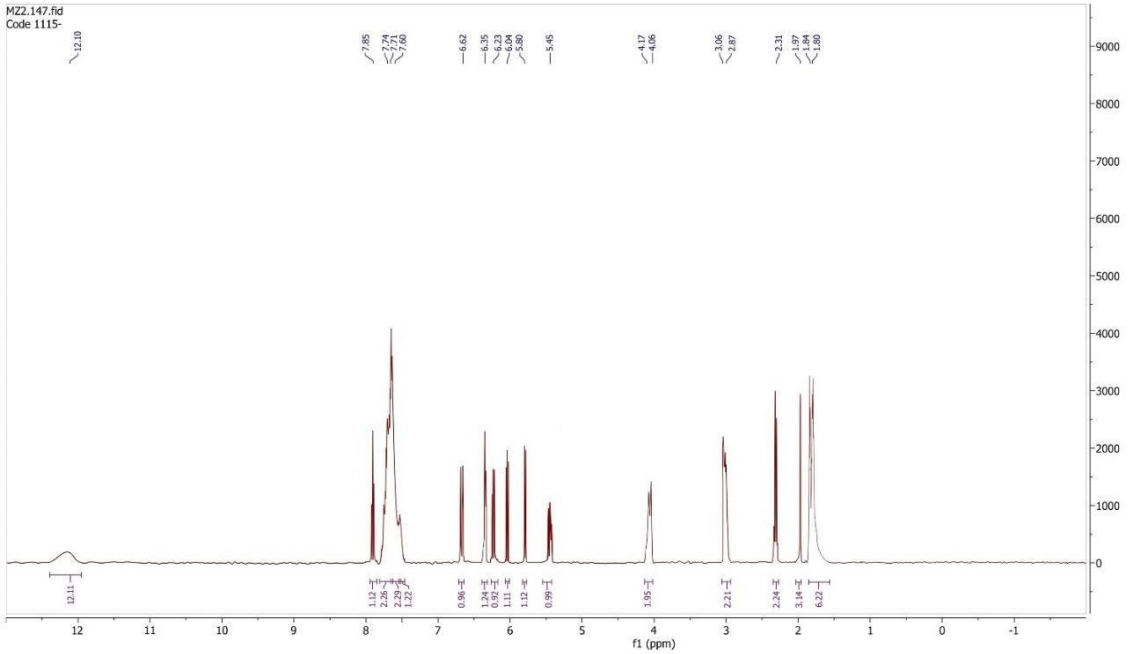


Figure 16: ^{1}H-NMR Spectrum of Product B_1

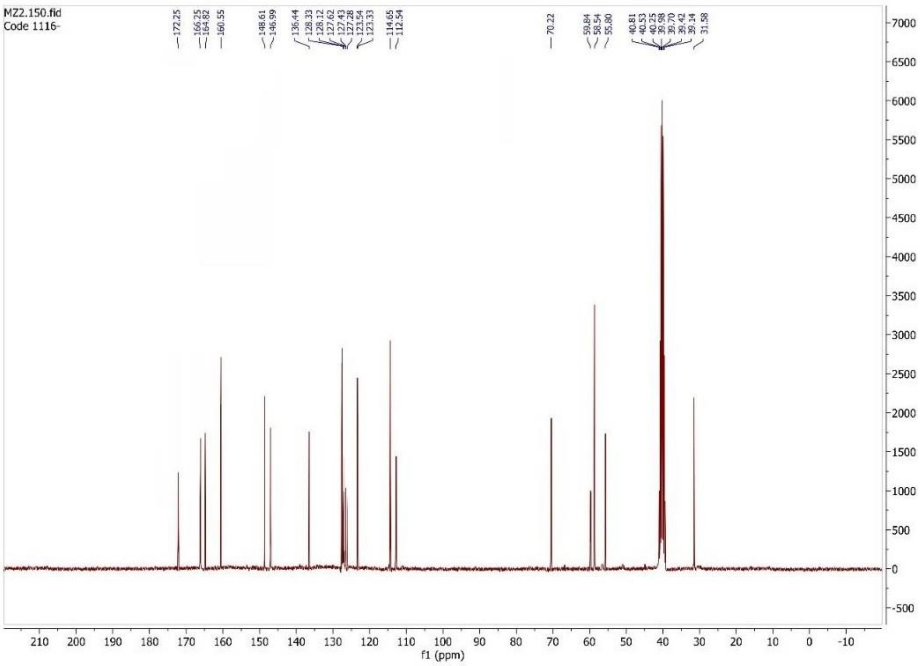


Figure 17: ^{13}C-NMR Spectrum of Product B_2

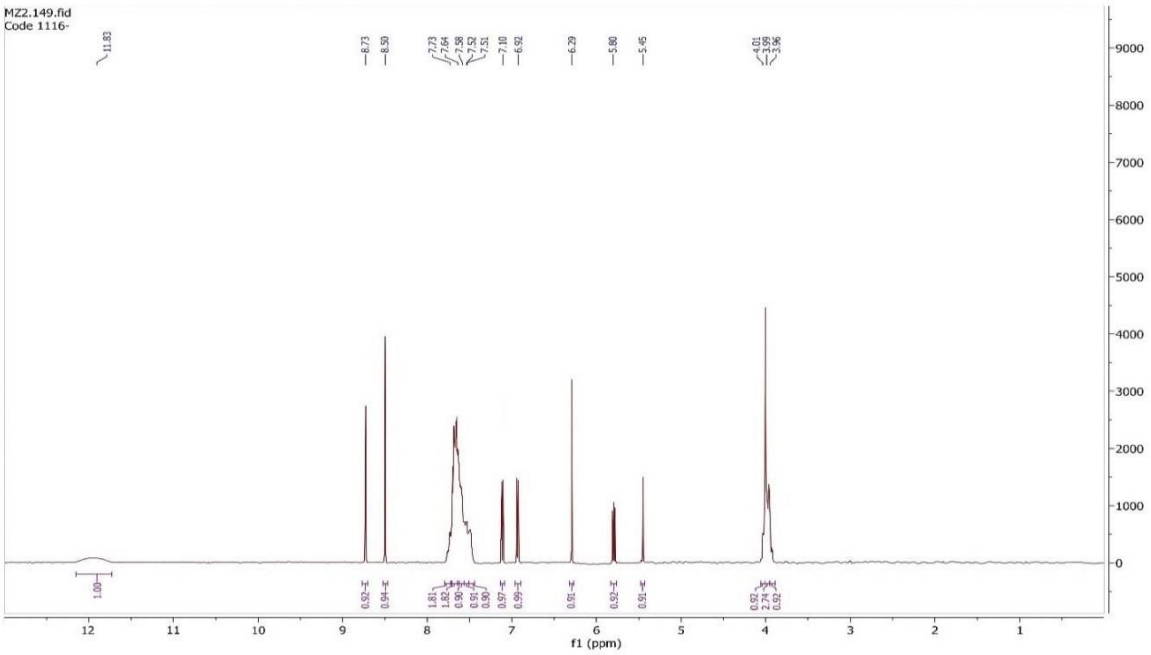


Figure 18: ^{1}H-NMR Spectrum of Product B_2

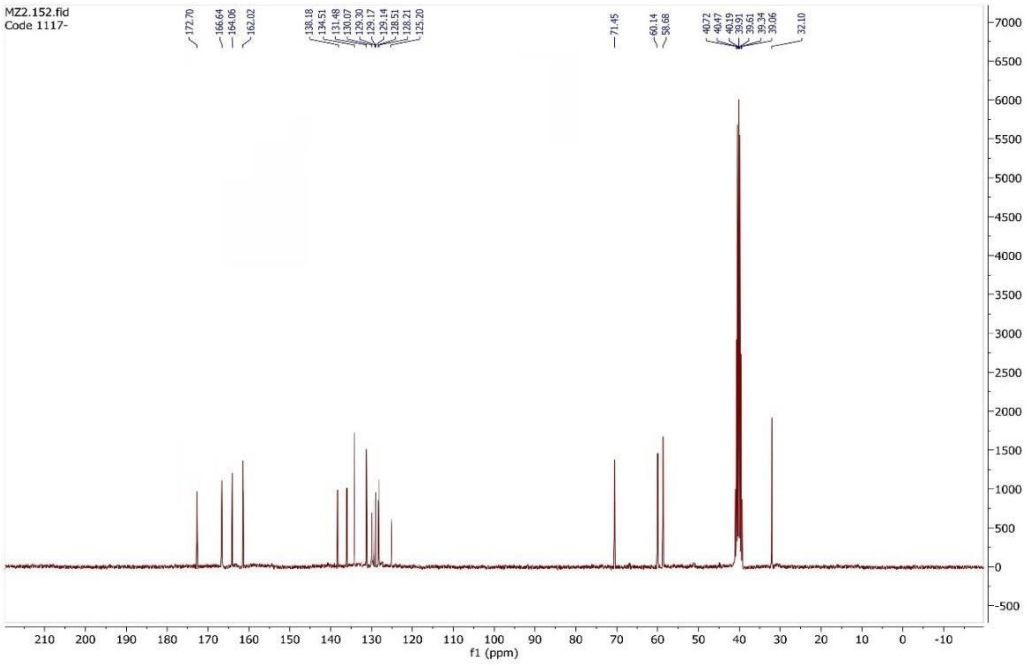


Figure 19: 13C-NMR Spectrum of Product B3

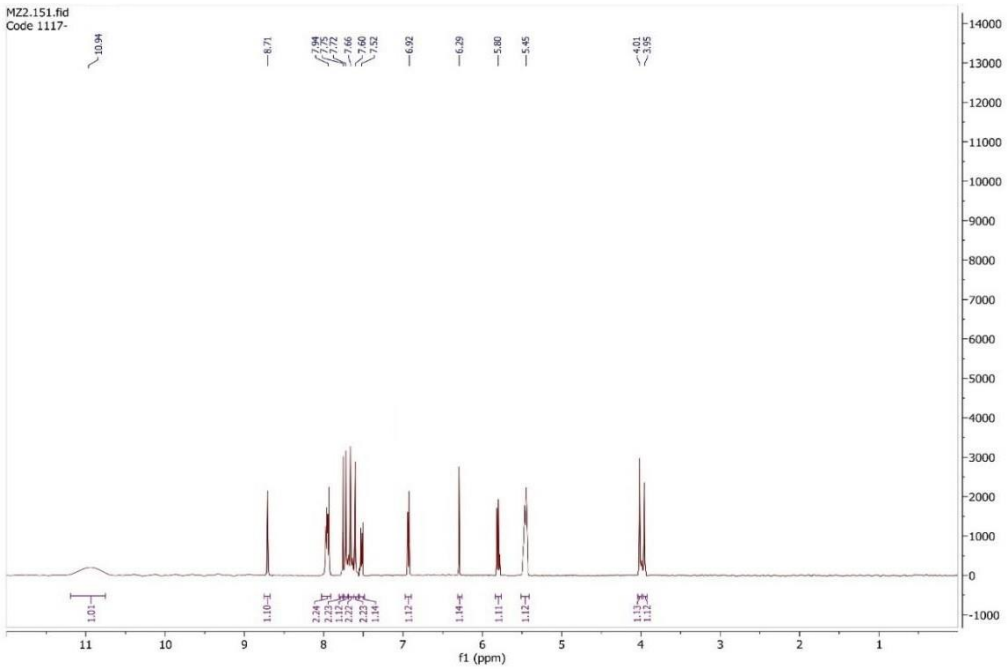


Figure 20:1H-NMR Spectrum of Product B3

DISCUSSION

FT-IR, ^{13}C-NMR, and ^{1}H-NMR of each of the products of the experiment reactions, as mentioned previously, characterize Imine formation in place of Primary amine groups of Cephalosporins (Cephalexin and Cefaclor), affirming that the goal of the reactions achieved. Table 9 illustrates the data collected from tastants after tasting each of the two starting cephalosporins, in addition to the Schiff base products. the participants involved in gustatory taste panel assessments perceived the bitterness of the products to possess a diverse spectrum of unpleasant bitter flavours.

As indicated in Table 9, the participants involved in gustatory taste panel assessments perceived the bitterness of the products to possess a diverse spectrum of unpleasant bitter flavours

Table 9: change in bitterness through blocking primary amine

Taste alteration means (on 10-unit scale)

Item (code)	rank (form 1)	rank (form 2)	rank (form 3)	rank (form 4)	rank (form 5)	rank (form 6)	rank (form 7)	rank (form 8)	rank (form 9)	rank (form 10)	Mean	Taste alteration
A	8	7	8	8	8	7	8	7	7	7	7.5	0
A_1	3	3	3	3	3	3	3	3	3	3	3	4.5
A_2	3	3	3	3	3	3	3	3	3	3	3	4.5
A_3	3	3	3	3	3	3	3	4	4	3	3.2	4.3
B	9	8	9	9	9	9	9	9	8	8	8.7	0
B_1	7	7	7	6	7	6	6	6	6	6	6.4	2.3
B_2	6	6	6	7	7	7	7	7	6	6	6.5	2.2
B_3	7	7	7	7	7	7	9	6	6	6	6.9	1.8

Regarding Cephalexin, the findings reveal that the formation of Schiff bases with Citral and Vanillin (A_1 and A_2 in the tabular data) elicited a notable elevation in bitterness by a substantial 4.5 degrees on the intensity scale. Conversely, the introduction of Benzaldehyde into Cephalexin through Schiff base linkage yielded a more restrained alteration of 4.3 degrees. Similarly, with regards to Cefaclor, the observed trend diverged, demonstrating a reduction in bitterness through the creation of Schiff base complexes. Notably, the complexes with Citral and Vanillin managed to curtail the bitterness by 2.3 and 2.2 degrees, respectively. In a parallel vein, the Schiff base comprising Benzaldehyde exhibited a comparatively modest effect, yielding a bitterness decrement of 1.8 degrees.

CONCLUSION

The bitterness scale of Cephalexin and Cefaclor influenced by shifting their primary amine functional group into corresponding Imines through Schiff's reaction with aldehydes (particularly Citral, Vanillin, and Benzaldehyde).

These empirical results seamlessly align with established insights from various disciplines, accentuating the pivotal role played by the primary amine group in governing the bitterness of both edibles and medicaments. However, when examining the broader panorama, Cephalexin's

overall bitterness underwent a more substantial alteration, marking a change of 4.35 degrees, whereas Cefaclor displayed a milder shift of 2.05 degrees. This discrepancy signifies that while the primary amine group exerts a more pronounced influence on the bitterness of Cephalexin, other underlying factors are concurrently at play.

Additionally, it's noteworthy that even subsequent to imine formation facilitated by Schiff base reactions, a noticeable residue of bitterness persists. This observation underscores the multi-dimensional nature of bitterness in Cephalosporins, indicating the involvement of diverse functional groups in influencing overall taste perception. A comprehensive understanding of cephalosporin bitterness mandates an exhaustive evaluation of these contributing functional groups.

In summation, this study's revelations underscored the significant role of blocking the primary amine through Schiff bases in shaping taste modifications within Cephalexin and Cefaclor, thereby exposing the intricate interplay between chemical adjustments and sensory experience.

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