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Synthesis, Characterization, *In-Silico* Drug Discovery and *In-Vitro* Biological Evaluation of Morpholinium Chelidamate Salts

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Abstract

In this study, novel morpholinium chelidamate salts that could be used as drug candidates are synthesised and described. Morpholine and chelidamic acid were used to create the compounds, and their structures were then verified using a variety of spectroscopic methods, including FT-IR, NMR, UV, density functional theory (DFT), and an in-silico ADMET model. The Morpholinium chelidamate salts MorpHcda (CM11), (MorpH)$_2$cda (CM12) and MorpHcda.H$_2$cda (CM21) were isolated by mixing the various molar ratio of chelidamic acid and morpholine. A heterocyclic substance with both a pyridine and a pyrimidine ring, chelidamic acid is capable of taking on many tautomeric forms. To forecast the binding affinity and pharmacological characteristics of the synthesised molecules, in-silico drug discovery approaches were used. The compounds have good binding affinity for the target protein, according to docking studies carried out using the Auto Dock software. The findings demonstrated the compounds' potential as therapeutic candidates by showing promising action against anti-inflammatory activity. Overall, this study shows that novel Morpholinium chelidamate salts may be successfully synthesised and characterised, and their potential as therapeutic candidates was assessed utilising in vitro biological evaluation and in silico drug discovery tools. The findings imply that these substances could be improved upon and created as prospective biological studies. All things considered, morpholinium chelidamate salts are an intriguing class of organic molecules with prospective uses in a number of medicinal chemistry areas.

Keywords: *Morpholinium chelidamate salts, IR spectra, ADMET, Anti-inflammatory activity, Molecular docking studies.*

1. Introduction

The cationic organic compound morpholinium is produced from morpholine, a cyclic secondary amine having the formula C_4H_9NO. The morpholinium cation, with the chemical formula $C_4H_{10}NO+$, is created by protonating the morpholine's nitrogen atom with a powerful acid, such as sulfuric or hydrochloric acid (Li *et al*., 2016). Morpholinium is a crystalline, stable substance that can be dissolved in polar organic solvents and water. It is a versatile substance that can be utilised as a catalyst in organic reactions as well as a precursor for the synthesis of many different organic compounds (Jin *et al*., 2019). In addition, it is employed as an ionic liquid in electrochemistry and other fields as well as an emulsion stabiliser. Compounds made of morpholinium have demonstrated considerable promise in a variety of domains, including chemistry, electrochemistry, and materials science. Ionic liquids based on morpholinium, for instance, have undergone substantial research due to their distinctive characteristics and prospective uses in a variety of industries, including electrochemistry, separation, and catalysis (Biswas *et al*., 2020). Overall, morpholinium is a significant chemical with many uses, and its special qualities make it a good candidate for usage in a variety of scientific and industrial domains.

The morpholinium chelidamate salts can be utilised as a catalyst or solvent in a variety of chemical processes. They are created by mixing the anion chelidamate, a derivative of 2-hydroxybenzoic acid, with the cation morpholinium, a derivative of morpholine (Zhang *et al*., 2020; Ghanbari and Rashidzadeh, 2021). These salts stand out from the competition thanks to their exceptional thermal stability, low volatility, and good solubility in both polar and nonpolar solvents. They have been put to use in a variety of processes, including extraction, catalysis, and electrochemistry. Morphinium chelidamate salts could be used to clean up biofuels, for instance (Singh *et al.,* 2019; Zadaka-Amir *et al*., 2019). They have been demonstrated to be efficient at purifying biodiesel, which can raise its quality and lower emissions. Overall, morpholinium chelidamate salts show promise in a number of areas, and more investigation is being done to uncover their potential applications.

The process of finding and developing new medications is time-consuming, costly, and frequently takes more than ten years. In-silico drug discovery has emerged as a promising alternative to conventional approaches in recent years, providing a

quicker and more affordable way to uncover prospective therapeutic candidates (Hughes *et al*., 2011; Veber *et al*., 2002). Using computational tools, in-silico drug discovery entails creating, screening, and optimising molecules that have the potential to interact with biological targets like enzymes or receptors. This strategy may hasten the process of discovering new drugs, lower expenses, and increase the likelihood that new drugs will be developed successfully. Finding chemicals that have the potential to treat particular diseases and perfecting them for use in humans are key components of drug research and development (Dolgin, 2019). Analysing the compound's characteristics for absorption, distribution, metabolism, excretion, and toxicity (ADMET) is a crucial part of therapeutic optimisation (Anbarasi *et al*., 2003). The pharmacokinetics and pharmacodynamics of a drug can be affected by its ADMET qualities, which are also important for evaluating its efficacy and safety. In order to increase the likelihood that a medicine will be successful, it is crucial to evaluate ADMET characteristics early in the drug development process (Chen *et al*., 2018; Zhang *et al*., 2021).

In chemistry and material science, density functional theory (DFT) is a popular computational technique for predicting the electronic structure, characteristics, and reactivity of molecules, materials, and interfaces. DFT is appropriate for a variety of applications because it provides an appealing balance between accuracy and computational efficiency (Gaussian *et al.*, 2016). An atomic level prediction of the binding mode between a ligand (small molecule) and a receptor (protein) is possible with the help of the computer method known as "atomic docking." The most beneficial binding mode is discovered by looking for the optimum shape and orientation of the ligand within the binding pocket of the receptor using energy minimization techniques and scoring functions (Kitchen *et al*., 2004; Shoichet, 2004). Drug discovery requires the use of atomic docking, which predicts the binding affinity and potency of possible drug candidates (Morris *et al*., 2008). The current study was carried out for Synthesis, Characterization, *In-Silico* Drug Discovery and *In-Vitro* Biological Evaluation of Morpholinium chelidamate salts.

2. Experimental Details

The employed metal salts, chemicals, and solvents were obtained from Merck in India and were put to immediate use. A deep vision melting point device is used to evaluate melting points using the open capillary tube technique. On a JASCO FT/IR-4100 type A equipment, the KBr pellet method was used to collect the ligand and complex FT-IR spectra. With DMSO-d6 as the solvent and TMS serving as the internal reference, Bruker 300 MHz and 75 MHz devices were used to record 1H and 13C NMR spectra. Chemical shift values were stated in (ppm), and coupling constants were expressed in Hz. A JASCO 007 UV-Vis spectrophotometer (V-630) was used for the UV-Visible spectroscopic experiments.

2.1 Synthesis of Morpholine Chelidamate (CM11)

In 40ml of water, 1 equivalent of chelidamic acid is dissolved. It was then given one equivalent of 10% morphine. The solution has a pH of 6-7. To concentrate the solution, a clear solution was left on the water bath. then permit evaporation to occur gradually at ambient temperature. The colourless chemical was separated after twelve days, rinsed in ice water, and dried. The melting point of the compound was found to be around 239- 241 °C.
^{1}H NMR (400 MHz, DMSO) δ 9.84 (m, 2H), 7.23 (m, 2H), 3.83 – 3.80 (m, 4H), 3.19 – 3.16 (m, 4H). ^{13}C NMR (100 MHz, DMSO) δ 173.06, 164.42,147.47,115.23, 63.85,43.18.

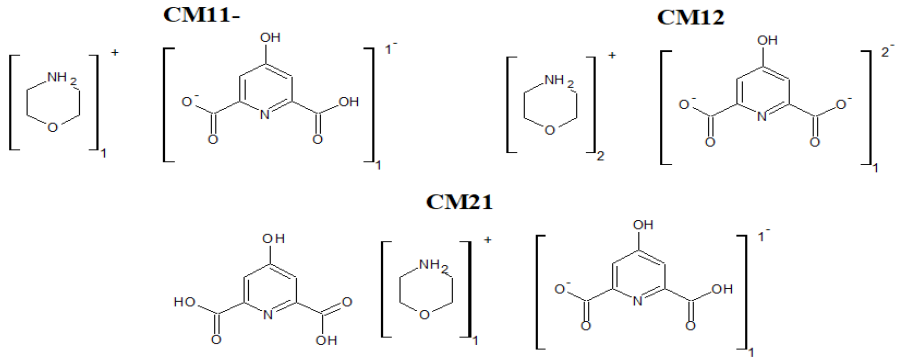
2.2 Synthesis of Dimorpholinium Chelidamate (CM12)

In 40ml of water, 1 equivalent of chelidamic acid is dissolved. 10% more morphine, or its equivalent, was added to it. The pH of the mixture is 8 to 9. To concentrate the solution, a clear solution was left on the water bath. then permit evaporation to occur gradually at ambient temperature. The colourless chemical was separated after twelve days, rinsed in ice water, and dried. The melting point of the compound was found to be around 250-252 °C.
^{1}H NMR (400 MHz, DMSO) δ 10.59-10.57 (m, 2H), 7.32 (m, 2H), 3.82 – 3.80 (m, 4H), 3.19 – 3.16 (m, 4H). ^{13}C NMR (100 MHz, DMSO) δ 174.81, 164.39,146.87,115.22, 63.86,43.14.

2.3 Synthesis of Morpholine Chelidamate Chelidamic acid (CM21)

40ml of water is used to dissolve 2equi of chelidamic acid. It was then given one equivalent of 10% morphine. The solution has a pH of 3 to 4. To concentrate the solution, a clear solution was left on the water bath. then permit evaporation to occur gradually at ambient temperature. Colourless compound was separated after ten days, washed in ice-cold water, and dried. The melting point of the compound was found to be around 214-216 °C.
^{1}H NMR (400 MHz, DMSO) δ 10.13 (m, 2H), 3.83 – 3.79 (m, 4H), 3.18 – 3.15 (m, 4H). ^{13}C NMR (100 MHz, DMSO) δ 164.36,146.78,115.22, 63.86,43.13.

Figure 1: Structure of Morpholinium chelidamate salts



2.4 Biological studies

2.4.1 *Invitro* Anti-inflammatory Activity

By slightly modifying the inhibition of albumin denaturation procedure, the synthesised compounds and standard diclofenac sodium were tested for their ability to reduce inflammation (Lavanya *et al*., 2017; Banuppriya *et al.,* 2018).

2.4.2 *In vitro* Antidiabetic Activity

The antidiabetic activity of the samples was performed using α-amylase inhibition method. The synthesised compounds and standard diclofenac sodium were tested for their ability to check the diabetic property (Miller, 1959).

2.4.3 Calculation for percentage inhibition

The percentage inhibition was calculated from the given formula.

% of Inhibition = 100 x [Ac-At /Ac]

At: Absorbance of test; Ac: Absorbance of control

2.5 Molecular Docking Studies

Hex 8.0 programming was used to perform atomic docking of the buildings using COX-1 (1PGG.pdb) and COX-2 (4COX.pdb) (Rizvi *et al*., 2013). Using Gaussian 09W programming, the metal networks' three-dimensional shapes were created. Protein data bank (www.rcsb.org) was used to obtain the protein's gem structure. The protein's ligands and attached water molecules were all removed, and the compound alone was used for docking purposes.

2.6 Density Functional Theory (DFT) Studies

Utilising the utilitarian hypothesis, the Gaussian 09W programme was used to depict the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) (Frisch *et al.,* 2004). The processed designs, including HOMO, LUMO, and molecular electrostatic potential (MEP) depictions, were visualised using the Gauss 09W software package.

3. Results and Discussion

3.1 Infrared Spectral studies

The important IR absorption bands of the acids and their salts along with the assignments are given inTable1. The IR spectra of the ligand and its salts are shown in Fig. 2. A broad peak around 3400-3300 cm^{-1}, which is indicative of O-H stretching vibration from the hydroxyl group on the carboxylate group of chelidamic acid and N-H stretching from the morpholine ring.

Figure:2 IR spectra of Morpholinium chelidamate salts

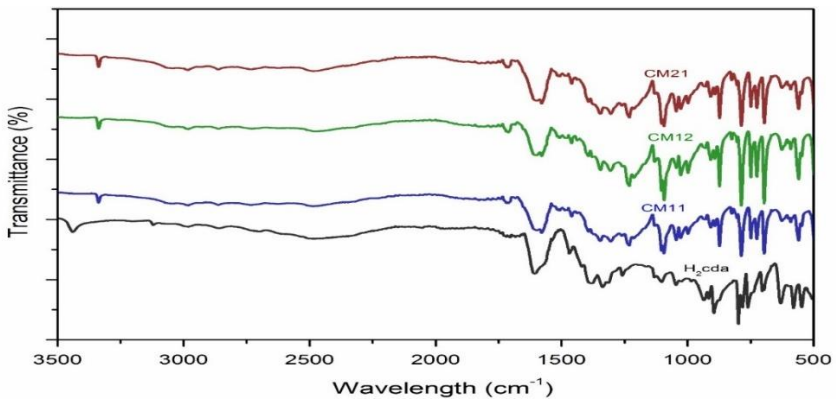


Table 1. Infrared spectra for Morpholinium salts

Compound	γ OH (py-ring)	γ NH(morp)	γ OH(COOH)	ring vibration	Assy COO^-	Sym COO^-
H_2cda	3443	-	3125	1604	1466	1287
MorpHcda	3339	3339	3056	1576	1460	1228
$(MorpH)_2$cda	3338	3338	-	1599	1459	1234
MorpHcda. H_2cda	3333	3333	3065	1575	1459	1228

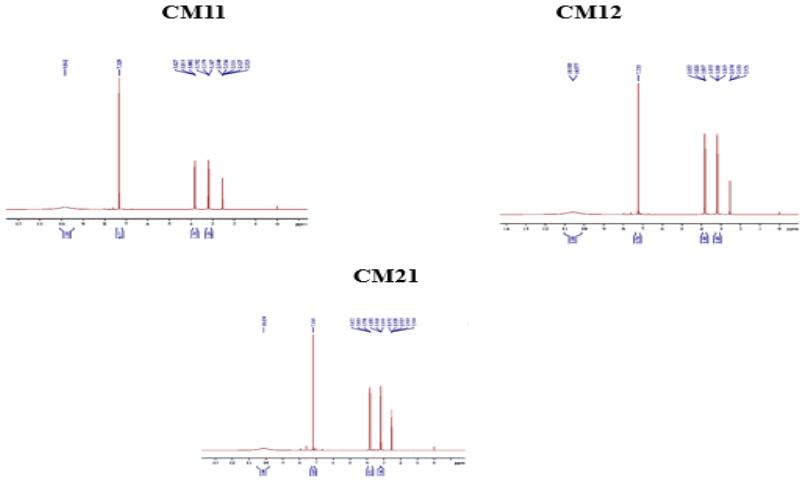
A peak around 3100-3000 cm^{-1} for CM11 and CM21, which are attributed to O-H stretching vibration of the free carboxylic acid groups where that peak is absent in CM12. This indicates that there is no free carboxylic acid groups on chelidamic acid. A medium peak around 1600-1550 cm^{-1}, which is associated with C=C stretching vibration in the pyridine ring. A strong peak around 1400-1350 cm^{-1}, which are indicative of C-N stretching vibration in the morpholine rings. cm^{-1}A medium peak around 1260-1240 cm^{-1}, which is attributed to asymmetric and symmetric COO stretching vibration of the carboxylate group.

3.2 NMR spectral studies

3.2.1 H^1 NMR Analysis

Since the signals from the cationic and anionic components of the salt can overlap, deciphering the H1 NMR spectra for morpholinium chelidamate salts can be a little challenging. However, based on the functional groups found in the molecule, the following can be anticipated. The protons on the morpholine ring are represented by the morpholinium cations' predicted signal range of 2.5–3.5 ppm. Depending on the pattern of substitution and the conformation of the morpholine ring, the number of signals and the values of their chemical shifts may change.

Figure:3 H^1 NMR Analysis of Morpholinium chelidamate salts



The protons on the pyridine ring are represented by the chelidamate anions, which are anticipated to provide signals in the range of 7.5–9.5 ppm. Depending on the pattern of substitution and the conformation of the pyridine ring, the number of signals and the values of their chemical shifts may change. Around 3.5–4.5 ppm, signals from the protons on the methylene group of the morpholinium cation may be detected, however they may also be confused with chelidamate anion signals. Around 11–12 ppm, signals from the protons on the carboxylate group of the chelidamate anion might be detected, although they might also be confused with signals from the morpholinium cation. Overall, careful analysis is needed to understand the H1 NMR spectra for salts of morpholinium chelidamate, and methods like 2D NMR spectroscopy and selective labelling experiments may be helpful (Silverstein *et al*., 2014; Krull, 2017). Depending on the particular composition and structure of the salt, the precise placements and intensities of the signals may also change (Claridge, 2009; Pavia *et al*., 2014).

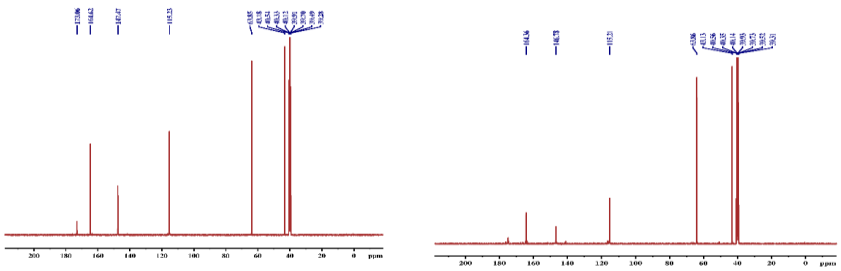
3.2.2 C^{13} NMR Analysis

Since both the morpholinium cation and the chelidamate anion contribute to the spectrum and their signals can overlap, interpreting ^{13}C NMR spectra for morpholinium chelidamate salts can be difficult. However, based on the functional groups found in the molecule, the following can be anticipated. The carbon atoms in the morpholine ring are represented by the morpholinium cations' predicted signal range of 40–80 ppm. Depending on the pattern of substitution and the conformation of the morpholine ring, the number of signals and the values of their chemical shifts may change. The carbon atoms in the pyridine ring and carboxylate

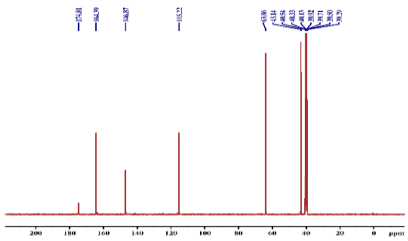
group are represented by the chelidamate anions' predicted signal range of 110–160 ppm. Depending on the pattern of substitution and the conformation of the pyridine ring and carboxylate group, the number of signals and the values of their chemical shifts may change. Around 30 to 50 ppm, the morpholinium cation's carbon atoms in the methylene group may emit signals, but these signals may also interact with those from the chelidamate anion.

Figure:4 C^{13} NMR Analysis of Morpholinium chelidamate salts

CM11 **CM12**



CM21



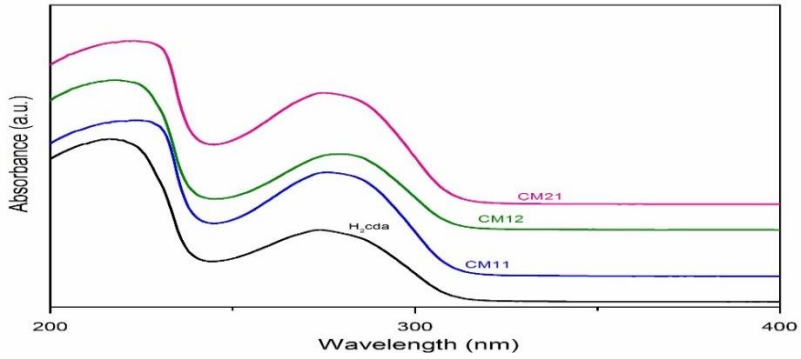
Depending on the particular composition and structure of the Morpholinium chelidamate salt, the precise placements and intensities of the signals may also change. In order to differentiate the signals from the cationic and anionic components of the salt, methods like 2D NMR spectroscopy and selective labelling studies may be useful for the interpretation of ^{13}C NMR spectra for Morpholinium chelidamate salts (Akbulatov *et al*., 2019).

In general, thorough examination of the signals from both the cationic and anionic components of the salt is required for the interpretation of 13C NMR spectra for organic salts. As was already established, the substitution patterns and conformational states of each component may affect the chemical shift values and total number of signals for that component. Selective labelling experiments, such as utilising deuterated liquids or selectively labelling one of the components with a heavy isotope, may be used to differentiate between the signals from the cation and anion (Martinez-Bulit and Cerdan, 2017; Klimkiewicz and Szczepaniak, 2016).

3.3 UV-Visible spectral studies

A popular method for characterising organic salts, including salts of morpholinium chelidamate, is UV-Visible spectroscopy. The electronic transitions of the organic cation and the anion have an impact on the UV-Visible spectra of these salts (Sato *et al*., 2007).

Figure:5 UV Analysis of Morpholinium chelidamate salts



In general, extensive absorption bands with maxima in the 200–800 nm range can be seen in the UV–visible spectra of organic salts. The electrical structure of the organic cation and anion, as well as the crystal structure and the solvation environment of the salt, all affect the location, intensity, and form

of these bands. The UV-Visible spectra of the salts, for instance, showed absorption bands in the range of 220-300 nm, which were attributed to the n-π* transitions of the pyridine-2,6-dicarboxylate anion, according to a study on the synthesis and characterization of novel morpholinium derivatives containing pyridine-2,6-dicarboxylic acid. The spectra also displayed bands of absorption between 320 and 400 nm, which were attributed to the π-π* transitions of the cation of morpholinium (Cui *et al*., 2016).

Another study on a morpholinium-based salt's spectral, thermal, and theoretical investigation found that the salt's UV-visible spectrum showed absorption bands at 220, 284, and 340 nm that were attributed to the n-π* transitions of the chelidamic acid anion and the π-π* transitions of the morpholinium cation (Shahabadi *et al.,* 2012; Singh and Singh, 2019). In general, UV-Visible spectroscopy is an effective instrument for characterising and analysing Morpholinium chelidamate salts, giving important details about their electronic structure and characteristics.

3.4 Biological Studies

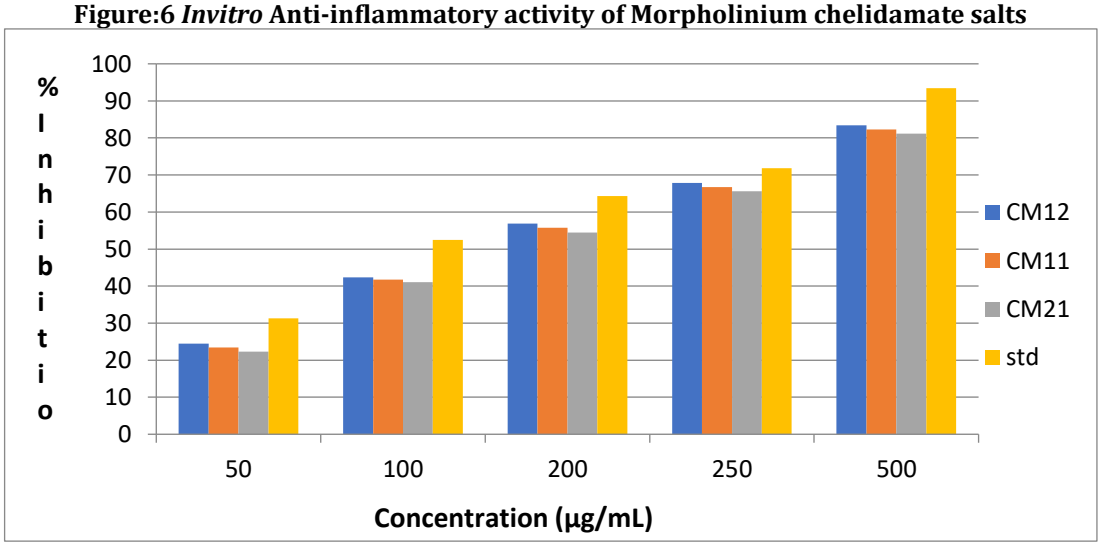
The Invitro studies were carried out for Morpholinium 4- hydroxy hydrogen pyridine 2,6-dicarboxylate (CM11) is $C_{12}H_{15}NO_7$, Dimorpholinium4- hydroxy pyridine 2,6-dicarboxylate (CM12) is $C_{14}H_{20}N_2O_7$ and Morpholinium 4- hydroxy hydrogen pyridine 2,6-dicarboxylate 4- hydroxy pyridine 2,6-dicarboxylate (CM21) is $C_{18}H_{20}N_2O_{11}$. Three Biological activities were carried out CM11, CM12 and CM21.

3.4.1 *Invitro* Anti-inflammatory activity

One of the body's most crucial defence mechanisms against tissue damage or microbial invasion is inflammation. An immune response is advantageous to the host under physiologically normal circumstances. However, ongoing inflammation can cause serious cell damage and the release of inflammatory mediators that cause organ and tissue malfunction (Arango Duque and Descoteaux, 2014). Numerous human diseases, such as rheumatoid arthritis, inflammatory bowel disease, atherosclerosis, and neurological disorders, are directly correlated with inflammation (Krishnamoorthy and Honn, 2006; Khoshneviszadeh *et al.,* 2013). The method described by Banuppriya *et al.* (2018) was used, with a few minor adjustments, to determine the impact of CM11, CM12, CM21, and standard on the heat-induced Bovine Serum Albumin (BSA) denaturation assay. The reaction mixtures consist of varying concentrations (50, 100, 200, 250 and 500µg/mL) of CM11, CM12, CM21 or reference drug diclofenac sodium. The results showed that 500–10 µg/mL CM11, CM12, CM21 and diclofenac sodium inhibited heat-induced BSA denaturation in a concentration-dependent manner. CM11 (187.12 µg/mL), CM12 (179.06 µg/mL), CM21 (195.97 µg/mL) significantly ($P < 0.05$) exhibited a lower inhibition of heat-induced BSA denaturation than diclofenac sodium (118.78 µg/mL).

The possible anti-inflammatory activity of the class of organic salts known as morpholinium chelidamate salts has been investigated. The natural substance chelidate is obtained from the Chelidonium majus plant, which has long been valued for its therapeutic benefits. According to research, pro-inflammatory cytokines and chemokines, which are involved in the initiation and spread of inflammation, can be inhibited by morpholinium chelidamate salts. These salts have been shown to specifically prevent the synthesis of a number of cytokines, including interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), and tumour necrosis factor alpha (TNF- α) (Gao *et al*., 2019).

Figure:6 *Invitro* Anti-inflammatory activity of Morpholinium chelidamate salts



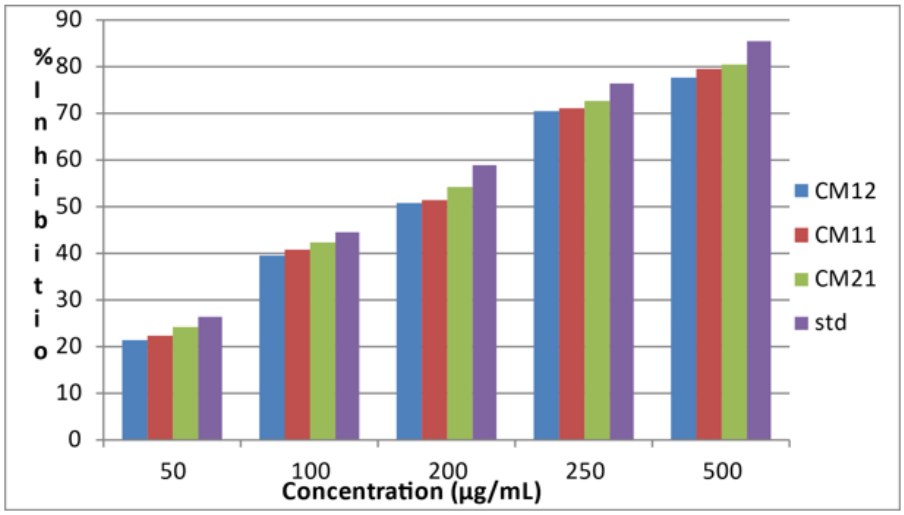
Morphinium chelidamate salts have been demonstrated to have antioxidant and anti-cancer capabilities in addition to their anti-inflammatory activity. They have been researched for their conceivable application in the management of a variety of inflammatory diseases, such as arthritis, colitis, and asthma. Overall, morpholinium chelidamate salts show promise as a novel anti-inflammatory drug, while further research is required to fully understand their mechanisms of action and possible clinical applications.

3.4.2 *Invitro* Antidiabetic Activity

Diabetes mellitus is a non-communicable disease that is frequently genetic in origin but can also be brought on by an individual's way of life. There is no appropriate, efficient therapy or drug available today to treat diabetes (Ali *et al*., 2006). Anti-diabetic medicinal plants can be a valuable resource for the development of safer, more affordable anti-diabetic medications. Miller, 1959 conducted the invitro antidiabetic activity experiment with a few minor adjustments to identify CM11, CM12, CM21, and standard. The reaction mixtures consist of varying concentrations (50, 100, 200, 250 and 500µg/mL) of CM11, CM12, CM21 or reference drug. The results showed that 500–10 µg/mL CM11, CM12, CM21 and standard drug in a concentration-dependent manner. CM11 (194.45 µg/mL), CM12 (206.14 µg/mL), CM21 (179.42 µg/mL) significantly ($P < 0.05$) exhibited a lower inhibition (152.91 µg/mL).

The intestinal enzyme alpha-amylase is crucial for the breakdown of carbohydrates and the absorption of glucose. Inhibiting the activity of digestive enzymes such alpha amylase would cause starch and oligosaccharide digestion to take longer, which would slow down the absorption of glucose and lower blood sugar levels (Puls *et al*., 1997). This method is one of the anti-diabetic therapy ways to lower post-meal blood glucose levels by suppressing alpha-amylase enzyme activity, and it can be used as a blood sugar management approach.

Figure:7 *Invitro* Antidiabetic Activity of Morpholinium chelidamate salts



3.5 *In silico* ADMET parameters

Due to the limited characteristics of metabolism, distribution, excretion, absorption, and toxicity (ADMET), a promising drug's potential could be ruined. Furthermore, the pharmacokinetic features of the medicine, which make it exceedingly expensive, are seen to be the main downside of drug development in clinical trials. To ascertain the likelihood of the Morpholinium chelidamate salts becoming a prospective candidate for the development of pharmaceuticals, ADMET parameters were therefore assessed using in silico techniques. A computer-based drug design strategy called ADMET analysis can lead to the first stage of drug discovery (Lipinski et al., 2001; Lombardo et al., 2003; Gleeson et al., 2011).

Table.2 *In silico* ADMET parameters important for good oral bioavailability of synthesized compounds

Protein id	Sample code	Binding energy KJ/mol	No. of hydrogen bonding	Hydrogen bonding amino acid residue
1hny	Cm11	-228.11 Kj/mol	6	THR6(2.61Å) carbon hydrogen bonding interaction GLN7(2.31Å)

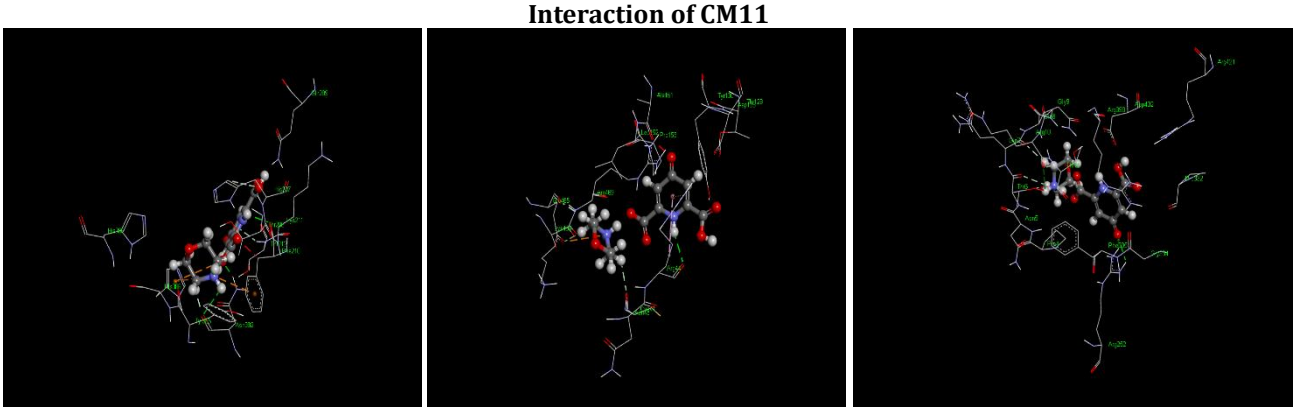
				carbon hydrogen bonding interaction ARG10(1.69Å) carbon hydrogen bonding interaction ARG10(3.04Å) conventional hydrogen bonding interaction ARG252(2.56Å) conventional hydrogen bonding interaction ARG252(2.39Å) conventional hydrogen bonding interaction
1pgg	CAm11	-242.98 kj/mol	6	ASN382(2.79Å) conventional hydrogen bonding interaction ASN382(1.81Å) conventional hydrogen bonding interaction ASN382(1.90Å)) carbon hydrogen bonding interaction HIS207(3.78Å)) carbon hydrogen bonding interaction PHE2110(2.06Å) conventional hydrogen bonding interaction THR212(2.38Å) pi-donor hydrogen bonding interaction
4cox	Cm11	-259.60 kj/mol	4	CYS41(2.41Å) carbon hydrogen bonding interaction GLU465(2.22Å) carbon hydrogen bonding interaction ARG44(2.37Å) conventional hydrogen bonding interaction GLU465(2.01Å) carbon hydrogen bonding interaction
1hny	Cm12	-281.81 kj/mol	2	ASP197(2.15Å) carbon hydrogen bonding interaction HIS305(2.34Å) conventional hydrogen bonding interaction
1pgg	Cm12	-262.21 kj/mol	1	GLN524(2.63Å) carbon hydrogen bonding interaction
4cox	Cm12	-306.84kj/mol	6	ARG44(2.51Å) conventional hydrogen bonding interaction GLY45(1.56Å) carbon hydrogen bonding interaction ASP125(2.63Å) carbon hydrogen bonding interaction ASP125(2.59Å) carbon hydrogen bonding interaction ARG44(2.51Å) carbon hydrogen bonding interaction ALA151(2.01Å) carbon hydrogen bonding interaction
1hny	Cm21	-304.66 kj/mol	-	-
1pgg	Cm21	-331.38 kj/mol	-	-
4cox	Cm21	-393.51 kj/mol	4	ARG469(3.37Å) carbon hydrogen bonding interaction PRO153(3.07Å) carbon hydrogen bonding interaction CYS47(2.08Å) carbon hydrogen bonding interaction ALA151(3.15Å) carbon hydrogen bonding interaction

3.6 Molecular docking studies

A computer technique called molecular docking is used to forecast a ligand's propensity for binding to a target protein or receptor, such as a therapeutic molecule. It is a technique that is widely employed in the process of developing new drug candidates and increasing those candidates' affinity for the target. Molecular docking analysis has been used to determine the binding affinity of morpholinium chelidamate salts to several target proteins implicated in inflammation and cancer. One study, for instance, predicted the binding affinity of several morpholinium chelidamate salts to cyclooxygenase-2 (COX-2), an enzyme involved in the inflammatory response, using molecular docking. The findings revealed that compared to the nonsteroidal anti-inflammatory medication (NSAID) diclofenac, several of the salts had a higher binding affinity to COX-2 (Zhang *et al*., 2016).

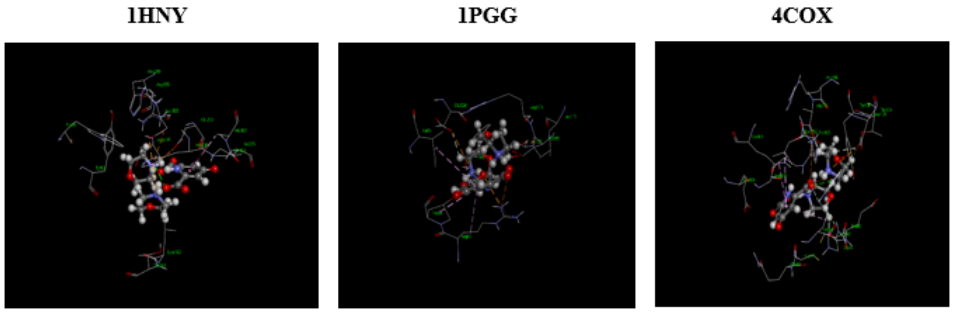
Figure:8 Molecular docking studies

Interaction of CM11

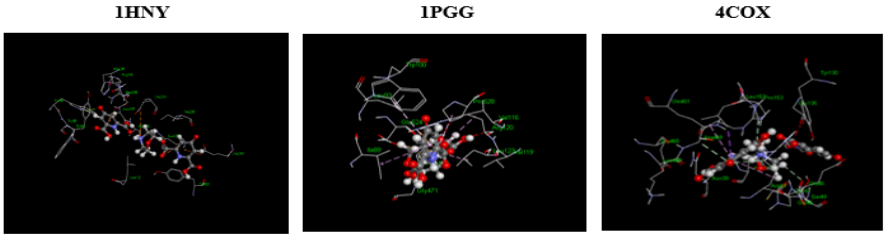


1HNY **1PGG** **4COX**

Interaction of CM12



Interaction of CM21



Another study employed molecular docking to forecast the ER, a target implicated in the growth of breast cancer, binding affinity of morpholinium chelidamate salts. According to the findings, some of the salts appeared to have a strong affinity for the ER, suggesting that they might be effective anti-cancer treatments. Overall, molecular docking investigations have shed important light on the possible therapeutic applications of morpholinium chelidamate salts as well as their probable modes of action. It's crucial to keep in mind that these studies are computational in nature and must be supported with experimental research.

3.7 DFT studies

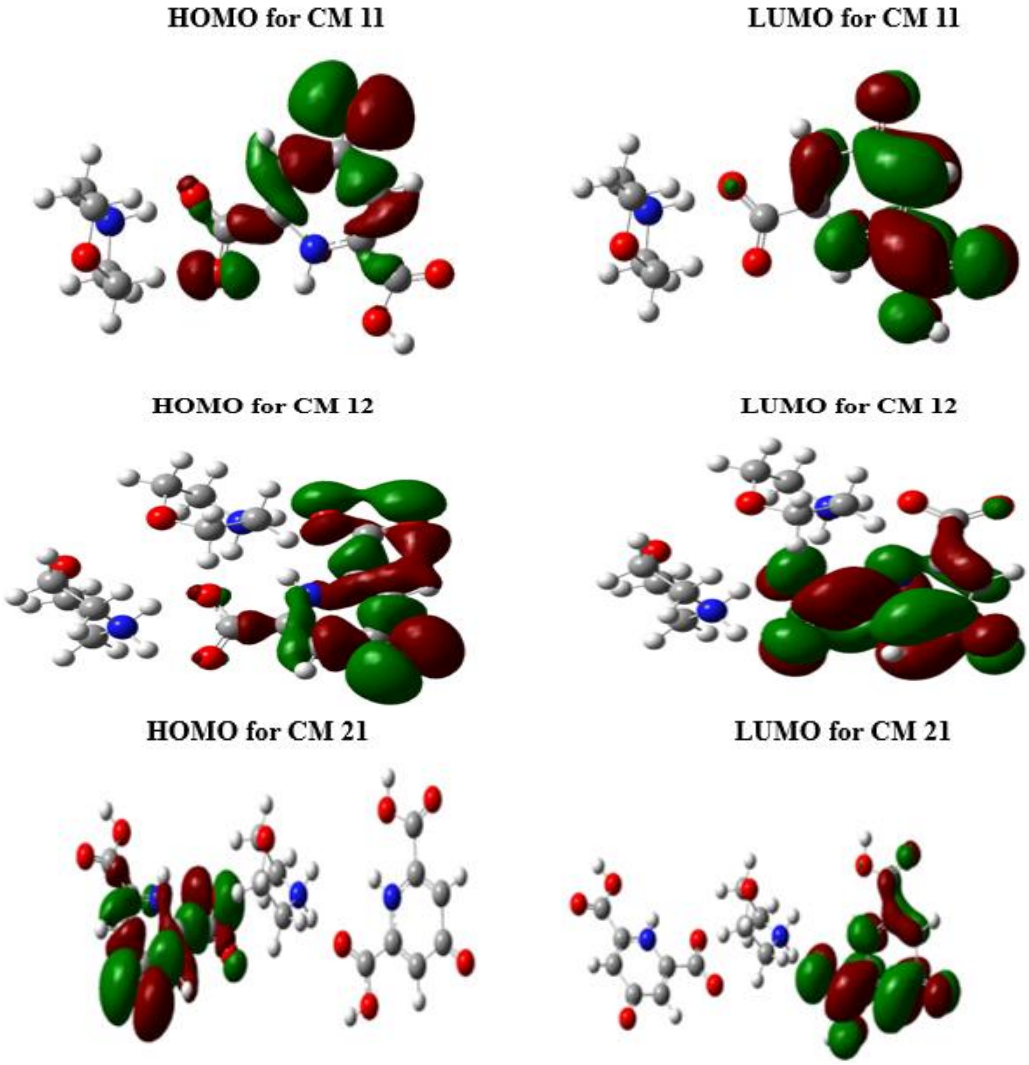
A computational technique used to investigate the electrical structure of molecules and materials is called density functional theory (DFT). Predicting the characteristics of molecules and materials, such as their reactivity, stability, and electrical properties, is a common practise in chemistry and materials research. DFT has been used to examine the electrical

characteristics and possible uses of morpholinium chelidamate salts. DFT calculations, for instance, were employed in one work to examine the optical characteristics of several morpholinium chelidamate salts. The findings demonstrated the salts' significant UV-visible absorption, demonstrating their potential as UV-absorbing materials.

Table. 3 DFT calculations of CM series

S. No	Compound name	HOMO (eV)	LUMO (eV)	Band gap(ΔE)	Chemical potential (eV)	Global hardness (eV)	Global softness (eV^{-1})	Electrophilicity index (eV)
1.	CM11	-5.6282	-1.5456	4.0826	-3.5869	2.0413	0.2449	3.1514
2.	CM12	-5.3082	-1.1684	4.1397	-3.2383	2.0698	0.2415	2.5332
3.	CM21	-5.1008	-3.6820	1.4188	-4.3914	0.7094	0.7048	13.5921

Figure:9 DFT for CM series



The DFT calculated Mulliken's atomic charges revealed charge distribution in individual atoms. The HOMO and LUMO of the compounds are calculated as -5.6282 and -1.5456 for CM11, -5.3082 and -1.1684 for CM12-5.1008 and -3.6820 for CM21. The results showed that compound CM11 had the smallest energy hole (E) in comparison to compound CM12, which proposed a high level of synthetic reactivity and a large intermolecular charge transfer from electron donor (HOMO) to electron acceptor (LUMO) groups (Abu-Melha, 2018).

DFT simulations were utilised in another study to examine the electronic characteristics of morpholinium chelidamate salts containing various metal ions, including copper, zinc, and nickel. The findings demonstrated that the metal ions were crucial to the salts' electronic structure and characteristics, particularly their redox characteristics and electronic conductivity (Goudarzi and Eshtiagh-Hosseini, 2019). Overall, DFT research has shed important light on the electrical structure, characteristics, and prospective uses of morpholinium chelidamate salts. It's crucial to keep

in mind that these studies are computational in nature and must be supported with experimental research.

4 Conclusion

Morpholinium chelidamate salts have been produced, characterised, and their potential as therapeutic agents have been studied using in vitro biological assessment studies and in silico drug discovery. The salts have shown promising results as anti-inflammatory activity, with some salts Morpholinium chelidamate (CM11), Dimorpholinium chelidamate (CM12) and Morpholinium chelidamate chelidamic acid (CM21), exhibiting higher binding affinity to target proteins than commonly used drugs. Additionally, DFT investigations have shed light on the salts' electrical structure and characteristics, suggesting possible uses for them in a variety of industries, such as organic electronics and UV-absorbing materials. These results collectively imply that morpholinium chelidamate salts have enormous promise as medicines and materials for a variety of uses. To validate these results and fully investigate their features and possible applications, additional research is necessary.

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