Republic of Iraq

Ministry of Higher Education and Scientific Research

University of Basra

College of Pharmacy

Synthesis and Characterization of Metformin and Gabapentin Derivatives and Comparison the Anti-inflammatory Drugs with Their Derivatives in Vitro

Project

Submitted to the Council of the College of Pharmacy - University of Basra

In Partial Fulfillment of the Requirements for degree B.Scs. in pharmacy

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Dedication…

To my lord "The owner of my soul and the time" who gives me the power to go on. To my heroes who were in my back all the time, who spend effort and time to make me who I am today to (mom and dad).To my brothers and friends who encourage me shared with me all good and bad days throughout my life. To all the people whom I love, and they love, support and stand beside me all the time. To the nights and hard work, I spend to witness this moment.

With respect…

Acknowledgments…

Thanks to Allah, lord of whole creation who made all this work possible. I would like to express my deepest thanks and sincere gratitude to my supervisor, ***Dr. Huda Salih and Dr. Osama Hammed*** for her scientific guidance, patience, support and encouragement during the entire stages of this work, wishing to her continuous progress. In addition, there are no words adequate to express thanks to my parents who have given me care and assistance in my life.

(بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ)

(وَلَقَدْ آتَيْنَا دَاوُودَ وَسُلَيْمَانَ عِلْمًا وَقَالَا الْحَمْدُ لِلَّهِ الَّذِي فَضَّلَنَا عَلَىٰ كَثِيرٍ مِنْ عِبَادِهِ الْمُؤْمِنِينَ)

(سورة النمل: آية ١٥)

(صدق الله العظيم)

Abstract

The aim of the research was to evaluate the anti-inflammatory effect of gabapentin and metformin compared to celecoxib. The human red blood cell (HRBC) membrane standardization method was chosen to evaluate the anti-inflammatory effect and evaluation the protection ratio. The two drugs had different levels of protection from celecoxib 71.07% while the other drugs were gabapentin 80.32% and metformin 78.33%, so our study revealed the anti-inflammatory effect of four common drugs that are heavily used by patients these days.

The level of protection was increased by the use of 2-hydroxy-4-methoxybenzaldehyde to convert these drugs to their derivatives. The protection rate for the gabapentin derivative was increased to 99.30%, and for the metformin derivative, the protection rate was 98.85% compared to the original drug.

Introduction

injury. The primary signs of inflammation can be explained by increased blood flow and cell elevated inflammation is a section of the non-particular immune response that occurs in reaction to any kind of bodily on metabolism, vasodilation, the of soluble mediators, extravasation of fluids, and cellular influx. [1]

Under normal conditions, the inflammatory process is Self-healing; it becomes persistent, and chronic inflammatory diseases develop later. The prime signs of inflammation can be explained by increased blood flow, elevated cellular

metabolism, vasodilatation, the release of soluble mediators, extravasation of fluids, and cellular influx. In some disorders, the inflammatory process is under normal conditions [2]. Inflammation is considered as a primary physiologic defense mechanism, this helps the body to protect itself against infection, burn, toxic chemicals, allergens, or other noxious stimuli uncontrolled and persistent. inflammation may act as an etiologic factor for many chronic illnesses.[3-5].

While acute inflammation is a necessary response to help the body heal, chronic inflammation can lead to various health problems, including arthritis, heart disease, and even cancer. [6].

Anti-inflammatory agents are medications or natural substances that help to reduce inflammation in the body. They can be used to treat conditions that involve inflammation, such as arthritis, asthma, and inflammatory bowel disease. [7]. There are two main types of anti-inflammatory agents: non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. NSAIDs work by blocking the production of prostaglandins, which are substances in the body that cause inflammation. Examples of NSAIDs include aspirin, ibuprofen, and naproxen. Corticosteroids, on the other hand, work by suppressing the immune system and reducing inflammation. They are often used to treat conditions such as asthma, allergies, and autoimmune diseases.[8]

There are also natural anti-inflammatory agents that can be found in certain foods and supplements. These include omega-3 fatty acids, which are found in fatty fish such as salmon and mackerel, as well as in flaxseed and chia seeds. Turmeric, ginger, and green tea are also natural anti-inflammatory agents that have been shown to reduce inflammation in the body.[9]

It's important to note that while anti-inflammatory agents can be effective in reducing inflammation, they may also have side effects, especially when used over a long period of time. Common side effects of NSAIDs include stomach upset and gastrointestinal bleeding, while corticosteroids can cause weight gain, high blood pressure, and mood changes. Therefore, it's important to talk to your doctor before starting any anti-inflammatory treatment to determine the best course of action for your individual needs.

Certainly! NSAIDs, which stand for nonsteroidal anti-inflammatory drugs, are a class of medications commonly used to relieve pain, reduce inflammation, and lower fever. They are widely available over the counter and also available in prescription form. NSAIDs are commonly used to alleviate symptoms of various conditions such as headaches, muscle aches, menstrual cramps, arthritis, and injuries.

Some key points about NSAIDs:

Mechanism of Action: NSAIDs work by inhibiting the production of certain enzymes called cyclooxygenases (COX), which are involved in the synthesis of prostaglandins. Prostaglandins are chemical substances in the body that contribute to inflammation, pain, and fever.[10,11]

Types of NSAIDs:

There are several types of NSAIDs available, including ibuprofen (Advil, Motrin), naproxen (Aleve), aspirin, diclofenac, indomethacin, meloxicam, and many others. Each NSAID has its own characteristics, including dosing frequency, duration of action, and potential side effects.

Pain Relief and Anti-inflammatory Properties: NSAIDs are effective in reducing pain by blocking the production of prostaglandins, which helps to alleviate inflammation and swelling. They can be particularly helpful for conditions such as arthritis, where inflammation plays a significant role.

Common Uses: NSAIDs are commonly used to manage a wide range of conditions, including headaches, toothaches, back pain, menstrual cramps, sports injuries, osteoarthritis, rheumatoid arthritis, gout, and other inflammatory conditions.

Potential Side Effects: While NSAIDs can be effective, they are not without risks. Common side effects include stomach irritation, heartburn, indigestion, and in some cases, stomach ulcers or bleeding. Long-term or high-dose use of NSAIDs may also increase the risk of cardiovascular problems or kidney damage. It is important to use NSAIDs as directed and consult with a healthcare professional if you have any concerns.

Precautions and Interactions:

Certain individuals, such as those with a history of stomach ulcers, gastrointestinal bleeding, or kidney disease, may need to avoid or use NSAIDs with caution. NSAIDs can also interact with other medications, so it is essential to inform your healthcare provider about all the medications you are taking.

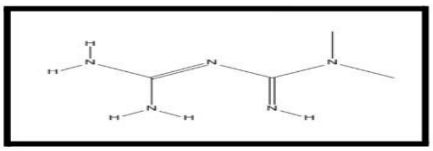
OTC (over-the-counter) drugs are medications available without a prescription. vs. Prescription: Some NSAIDs are available (OTC) in lower doses, while others require a prescription for higher doses or specific formulations. It is important to follow the recommended dosage and seek medical advice if necessary.

Cyclooxygenase (COX; prostaglandin synthase catalyzes the first two steps in

the biosynthesis of prostaglandins (PGs). COX-1 and COX-2 are widely considered targets use of non-steroidal anti-inflammatory drugs, suggesting a role for these enzymes in pain, fever, inflammation, and formation of tumors. Constitutive ubiquitination expression of COX-1 and inducible expression of COX-2 for them led to the popular belief that COX-1 produces homodimers of PG particles, whereas the PGs produced by COX-2 are primarily pathophysiology.

Metformin:

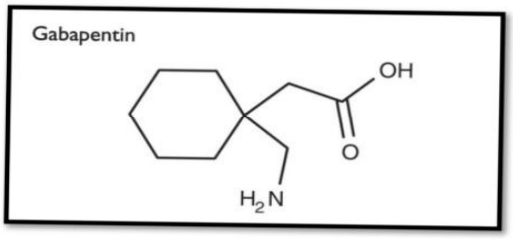
Metformin hydrochloride is an N, N-dimethyl biguanide glucose-lowering agent that was extracted from the plant Galega officinalis in the 1920s; further, metformin (Mf) has been widely used to control noninsulin-dependent diabetes mellitus (NIDDM) [2-4]. Diabetes mellitus (DM), generally called diabetes, is a group of metabolic disorders characterized by high blood sugar levels over a long time; this results in symptoms such as increased thirst and hunger as well as frequent urination. Metformin improves liver sensitivity to insulin, decreases glucose production in the liver, increases the absorption of insulin, and induces the consumption of glucose by peripheral tissues; therefore, it is effective in treating the loss of appetite, which leads to a decrease in weight. Besides its utilization as an antidiabetic, metformin has been shown to have anti-cancer and anti-aging effects and is a risk factor reducer for cardiovascular disease [5-8]. Although other biguanide drugs induce lactic acidosis, metformin does not; however, nearly 30% of patients on metformin therapy suffer from gastrointestinal side effects [9].



Gabapentin :

(amino methyl) cyclohexane acetic acid, Neurontin (Gpn) structurally belongs to thegamma-aminobutyric acid (GABA), widely studied for its significant inhibitory action in thecentral nervous system [1]. Gpn has been applied in the treatment of

It is a new generationused as add-on therapy and in patients with [2]. A safe and effective is a concern for anincreasing number of adults suffering from epilepsy. Epilepsy also imposes a remarkableeconomic load on society [3]. The selection of an antiepileptic drug depends on how itfunctions with regard to the specific tolerability, and safety. Furthermore, gabapentin can beconsidered as an emergent solution for the “pain riddle”. Starting from this point, more randomized, double blind studies, which compare with gabapentin, may be relevant to identifying the first choice therapy for acute and chronic pain relief [4].Thus, the search for novel chemical entities for the cure of epilepsy is essential [5,6].



Experimental

Materials and Methods: All chemicals used in this study were of analytical

Grade. They were procured from Sigma Chemical Co and Merck India Ltd,

Mumbai, India. Metformin drug (1000 mg) Gabapentin drug (100mg) Accord Company

General procedure

Preparing a mixture of an equimolar amount of 2- hydroxyl -4-methoxy Benz aldehyde (0.005)mmol, 0.76g) and the drug(metformin 0.645g) or (gabapentin 0.85g) in 30 ml of ethanol, 5 drops of glacial acetic acid was added with Stirring and the reaction mixture was heated and refluxed for 3h.

The detection of Schiff base reaction progression was monitored by TLC.

Drug	mmol ,g	Precipitation color
Metformin	0.005 mmol, 0.64 g	Gray
Gabapentin	0.005 mmol, 0.85 g	Brown

Tablet 1. General procedure

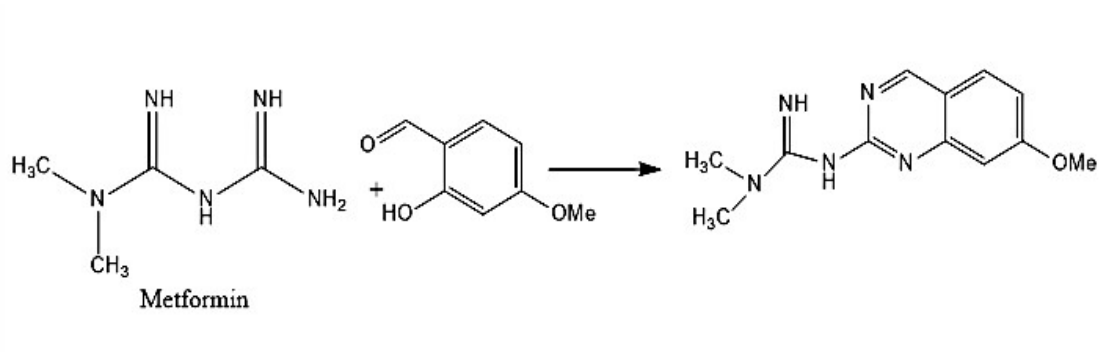


Figure 1: Schiff base of the Metformin with 2-hydroxy -4-methoxy benzaldehyde.

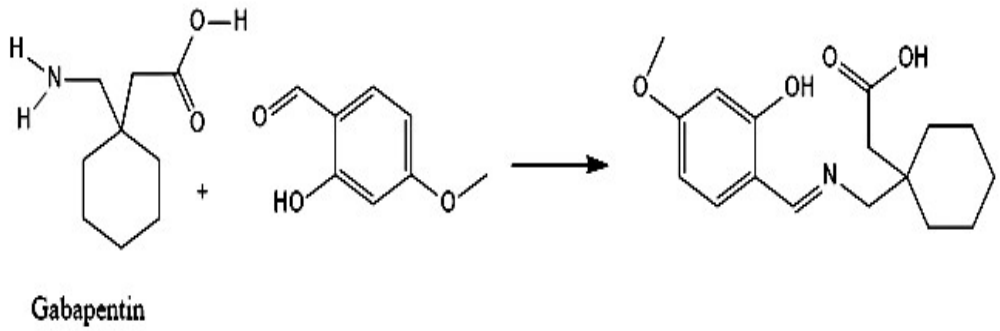


Figure 2: Schiff base of the Gabapentin 2-hydroxy -4-methoxy benzaldehyde.

Table 3: The physical properties of synthesis compounds.

Sample	Mp.^{o}C	Eluent	R_f
Schiff base M	232-233	MeOH: Chloroform 2:8	0.55
Schiff base G	162-163	EtAC: Hexane 4:6	0.85

DISCUSSION ;

The FTIR spectra [14] of these compounds, Figure (1) showed

In vitro anti-inflammatory activity [12-14]

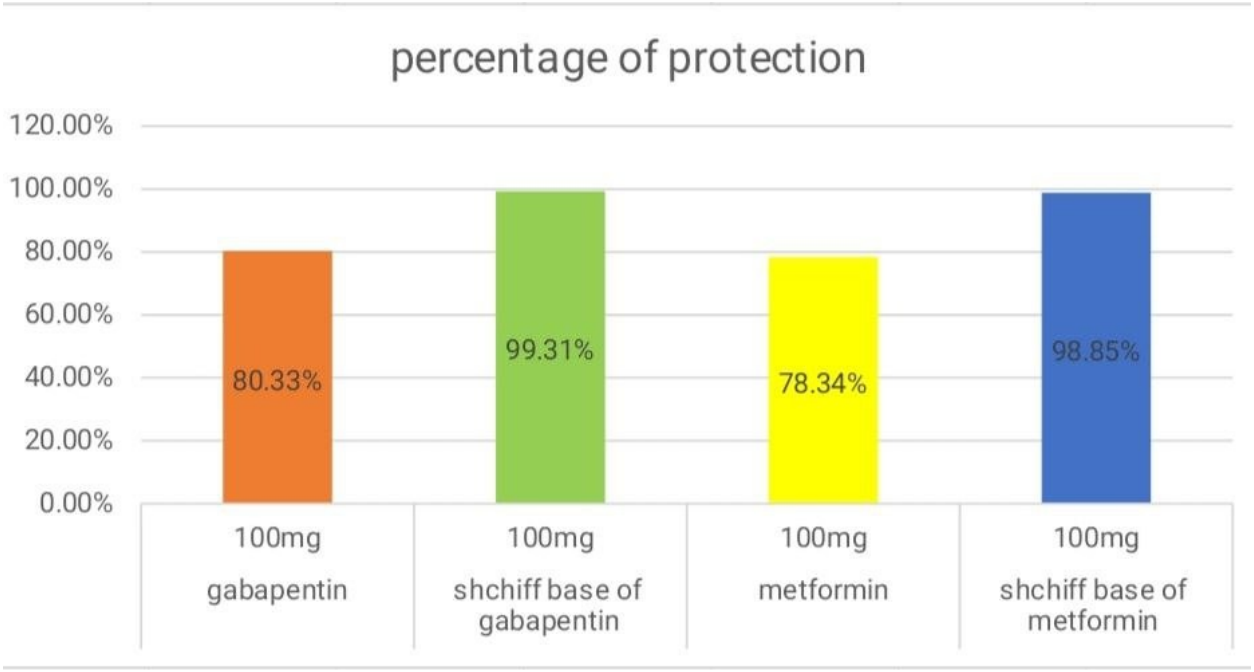
Anti-inflammatory activity was estimated in vitro by the HRBC method. Blood samples were collected from healthy volunteers and were mixed with an equal volume of sterilized Alsever's solution. Alsever's solution was prepared of 2.05% glucose, 0.41% NaCl, 0.81% trisodium citrate, and 0.056% citric acid, all dissolved in water. Other solutions were used in this method Hypo-saline (0.7% NaCl), Isosaline (0.9% NaCl), phosphate buffer (pH 7), and ethanol. Blood sample 5ml was washed with isosaline and 10% v/v suspension was complete with isosaline. A standard solution was prepared for Schiff bases of gabapentin and metformin (100mg in 1 ml of ethanol) with phosphate buffer (1 ml), hypo-saline (2 ml), and HRBC (0.5 ml). Both standards and blank were incubated at 37ºC for 30 minutes and centrifuged at 3000 rpm for 10 min. The supernatant liquid was

decanted and hemoglobin content was estimated by UV-visible spectrophotometer at 560 nm. The percentage of hemolysis was estimated by assuming the hemolysis produced in the control as 100%, according to the following equation.

Percentage of protection= 100 - (Absorbance of sample/Absorbance of control) x100%.

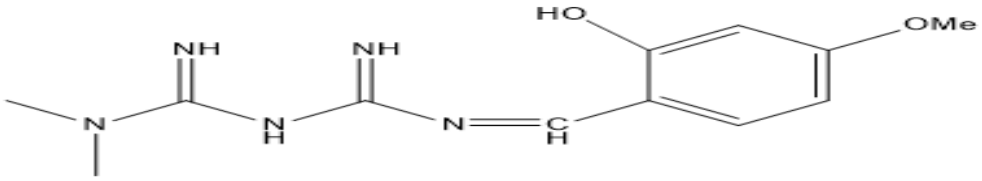
Table 4: The Result Absorbance and percentage of protection.

Sample	Concentration	Absorbance	Percentage of protection
Gabapentin	100 mg	0.168	80.33%
Schiff base of Gabapentin	100 mg	0.433	99.30%
Metformin	100 mg	0.185	78.34%
Schiff base of Metformin	100 mg	0.721	98.85%

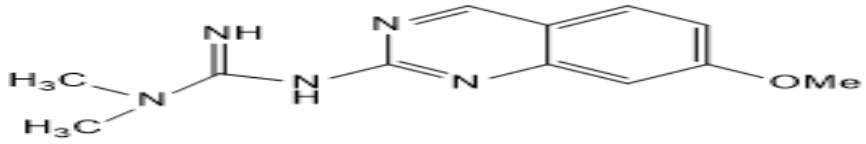


DISCUSSION

FTIR spectra [15] of these compounds, Fig. (3) and Fig. (4) showed the ranges 1581–1590 cm^{-1}, due to the presence of C = C belonging to the synthesis compounds, as well as the appearance of peaks at 1627-1650 to C=N bond and peaks at range 2816-2947 refers to the aliphatic C-H. The FTIR spectrum of Schiff of gabapentin G compound showed two peaks 3450 and 3640 belonging to OH groups in the G compound but in the FTIR spectrum of the Schiff compound of metformin M, we noted disappeared of -OH and the appearance of the -NH band at 3325 this indicates getting a new compound different from what we believe (N compound) because of the condensation of gabapentin with aldehyde, and this is confirmed by the 1HNMR spectrum. As shown in Figure 3 and Figure 4 and listed in Table 5.



(N compound not formation)



(M compound)

Table 5: the main peaks of FT-IR spectra (cm^{-1}) of compounds

Symbol	*C=C*	*Ar-H*		*C=N*	*Aliphatic C-H*	*C=O*	*-OH*	*-NH*
M	1581	3093	3170	1627	2816 2947	--------	------	3325
G	1590	3016	3101	1650	2854 2931	1680	3450 broad 3640 sharp	-------

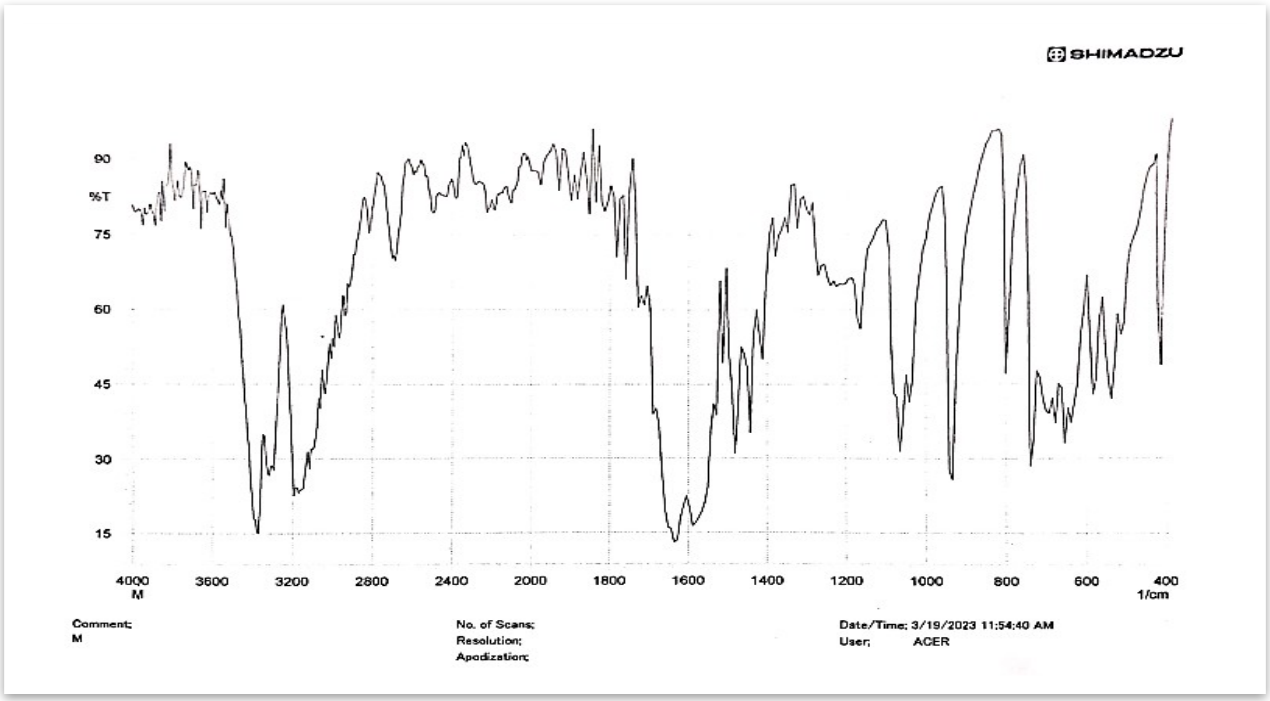


Figure (3): FTIR Schiff base of Metformin.

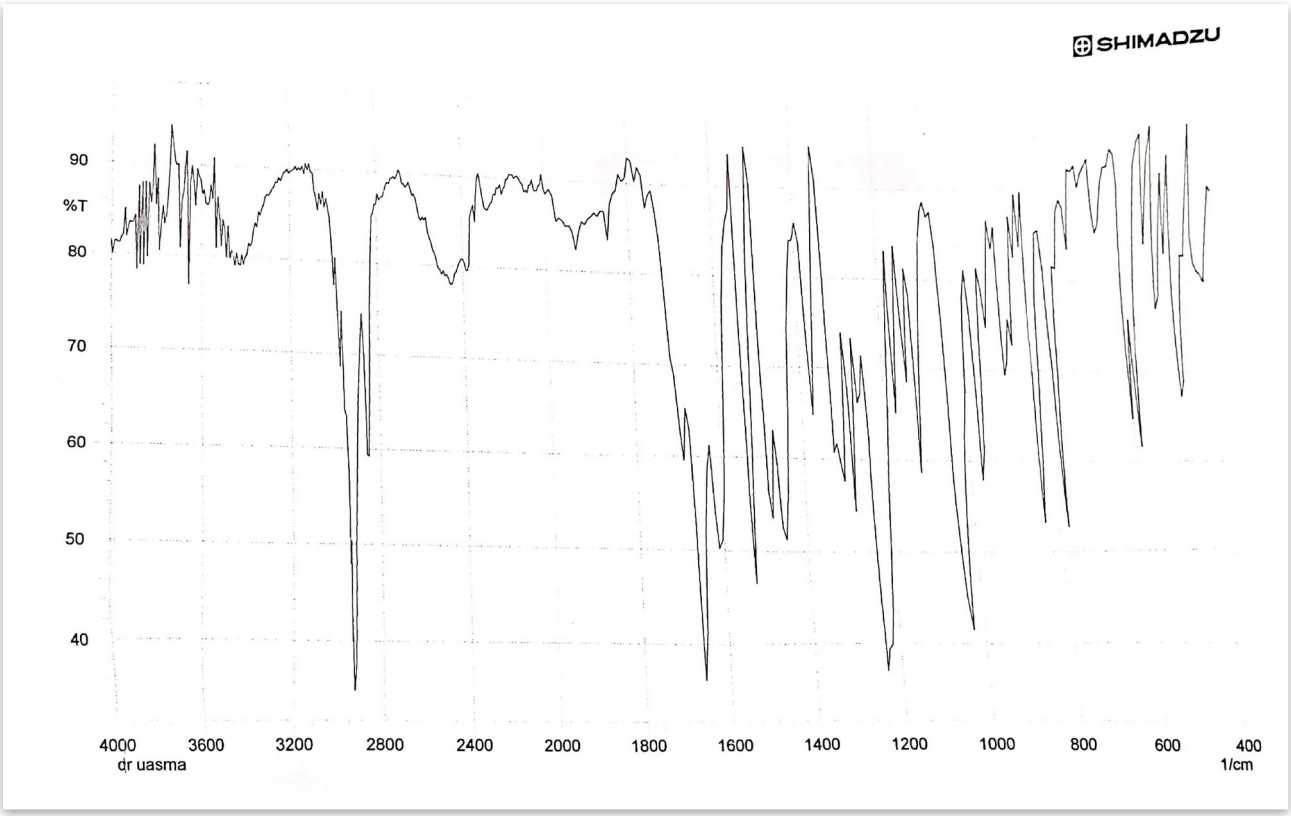


Figure (4): FTIR Schiff base of Schiff base of Gabapentin.

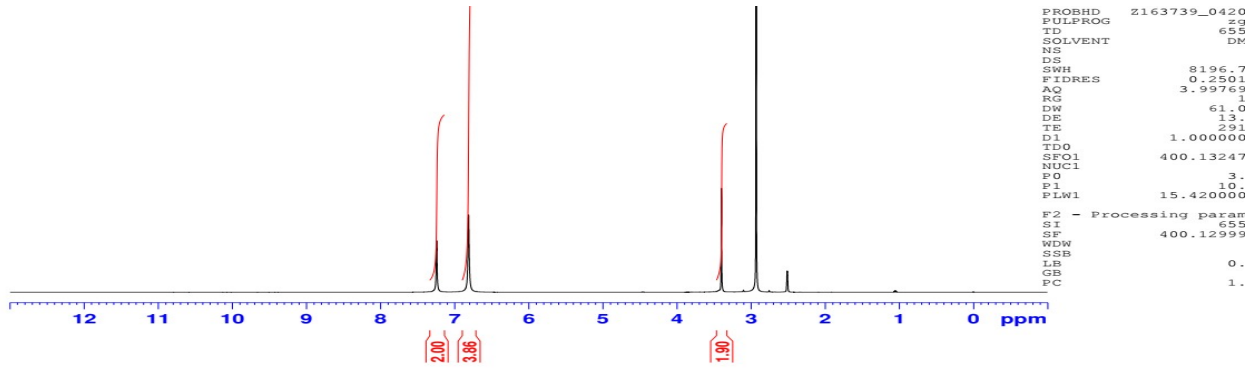


Figure 5: 1HNMR spectrum of Schiff base of Gabapentin

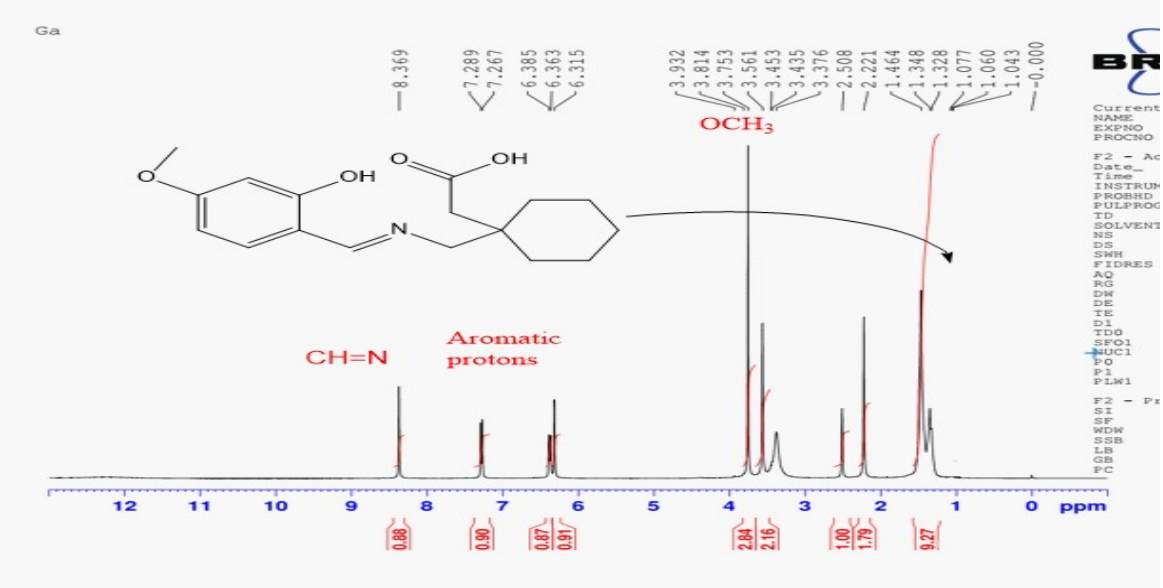


Figure 6: 1HNMR spectrum of Schiff base of Gabapentin

References

1. Ferrero-Miliani L, Nielsen O. H., Andersen P. S., and Girardin S. E. British Society for Immunology, Clinical and Experimental Immunology, 147: 227–235, 2006.
2. Xavire A. and Srividhya N, Synthesis and study of Schiff base ligands. J. Appl. Chem. 7(11), 6-15,2014.
3. Abood H, Ramadhan U, and Hamza H, Synthesis and anti-inflammatory activity study of Schiff bases complexes, Biochem. Cell. Arch. Vol. 20, No. 2, pp. 5627-5631, 2020.
4. Al-Noor T H, AL-Jeboori A T and Aziz M R, Preparation, Characterization and Antimicrobial activities of {Fe (II), Co (II), Ni (II), Cu (II), and Zn (II)} Mixed Ligand Complexes Schiff base derived from Cephalexin drug and 4 (dimethylamino) benzaldehyde with Nicotinamide. Adv. Physics Theories and Applications 18, 1-8,2013.
5. KshashAH, Synthesis some Schiff bases by direct condensation for cefotaxime (claforan) and benzaldehyde or its substitutions and study their antibacterial activity. Al-Anbar J. Vet. Sci. 3(2), 125-132,2010.
6. Cicero A.F., Tartagni E., Ertek S. Metformin and its clinical use: New insights for an old drug in clinical practice. Arch. Med. Sci. 2012;8:907. doi: 10.5114/aoms.2012.31622.
7. Kita Y., Takamura T., Misu H., Ota T., Kurita S., Takeshita Y., Uno M., Matsuzawa-Nagata N., Kato K.-i., Ando H. Metformin prevents and reverses inflammation in a non-diabetic mouse model of nonalcoholic steatohepatitis.
8. Nath N., Khan M., Paintlia M.K., Hoda M.N., Giri S. Metformin attenuated the autoimmune disease of the central nervous system in animal models of multiple sclerosis. J. Immunol. 2009
9. Lal M., Palepu N., Kessler D. Metformin salts of lipophilic acids. 20050182029A1. U.S. Patent. 2003 Feb14.
10.. Linlin Chen, Huidan Deng, Hengmin Cui, Jing Fang, Zhicai Zuo, Junliang Deng, Yinglun Li, Xun Wang, and Ling Zhao. Inflammatory responses and inflammation-associated diseases in organs, *Oncotarge*,23; 9(6): 7204–7218,2018.
11. Richard O. Day, Garry G Graham. Aust Prescr, The vascular effects of COX-2 selective inhibitor 27,142-145, 2004.
12.. Hameed BJ and Ramadhan UH. Xanthine oxidase inhibitory, anti hyperuricemic, anti-inflammatory, antinociceptive activity of α-lipoic

acid in gouty arthritis model. Asian Journal of Pharmaceutical and Clinical Research. 2018;11(12):483-7.

13. Siddiqa A, Abbasi MA, Siddiqui SZ, Rasool S, Sattar A, Khan KM, Ahmad I and Afzal S. Synthesis and antibacterial screening of S-substituted derivatives of 5-(3, 4-methylenedioxyphenyl)-1, 3, 4-oxadiazole-2-thiol. Pakistan journal of pharmaceutical sciences. 2016 1;29(1).
14. Xie X, Cong W, Zhao F, Li H,Xin W, Hou G and Wang C.Synthesis, physicochemical property and antimicrobial activity of noval quaternary ammonium salt. Journal of enzyme inhibition and medicinal chemistry. 2018 1;33(1):98-105.
15.. Silverstein RM, Webster FX and Kiemle DJ. Spetrometric identification of organic compounds. Publisher : Wiley; 7th Eddition(14,2005)