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Some bromohexine's derivatives: Synthesis and characterization by I.R, autodock and antimicrobial test

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Abstract :

An amine and carboxylic acid react to form the amide group, an organic group with a variety of biological properties including antibacterial, antifungal, anti-inflammatory, and anticancer properties. the conventional and simple process of making amides, which involves first converting carboxylic acid to acyl chloride and then reacting that acyl chloride with amine to produce the amide.Using thionyl chloride and triethylamine as catalytic agents and stirring for an hour, the objective is to synthesize new amide derivatives from a single pot reaction involving two drugs: one is an amine (bromohexine, which acts as a mucolytic) and the other is a carboxylic acid, such as anti-inflammatory NSAIDs (diclofenac, naproxen, indomethacin, and mefenamic acid). Melting point, infrared spectroscopy, and physical inspection will be used to characterize the final products. Since amide derivatives have a wide range of biological activities, the antibacterial activity will be examined using two g+ve (Streptococcus and Staph. aureus) and two g-ve (E. coli and Klebsiella) bacterial species. In order to determine each derivative's inhibition score and compare it with both the reference inhibitor and the antibacterial test, a docking research will be conducted on the synthesized compounds on the suggested targets.

Key words: bromohexine, amides, molecular docking, NSAIDs, antibacterial.

تحضير وتشخيص بعض مشتقات البروموهيكسين بواسطة الأشعة تحت الحمراء وبرنامج الاوتودوك واختبار مضادات الميكروبات

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مستخلص:

يتفاعل الأمين مع حمض الكربوكسيل لتكوين مجموعة الأميد، وهي مجموعة عضوية ذات خصائص بيولوجية متنوعة بما في ذلك خصائص مضادة للبكتيريا، ومضادة للفطريات، ومضادة للالتهابات، ومضادة للسرطان. عملية تقليدية وبسيطة لصنع الأميدات، والتي تتضمن أولًا تحويل حمض الكربوكسيل إلى كلوريد الأسيل ثم تفاعل كلوريد الأسيل مع الأمين لإنتاج الأميد. باستخدام كلوريد الثيونيل وثلاثي إيثيل أمين كعوامل محفزة والتحريك لمدة ساعة، يكون الهدف هو التجانس مشتقات أميد جديدة من تفاعل وعاء واحد يتضمن عقارين: أحدهما أمين (بروموهيكسين، الذي يعمل كحال للبلغم) والآخر حمض كربوكسيلي، مثل مضادات الالتهاب غير الستيروئيدية (ديكلوفيناك، نابروكسين، إندوميتاسين، وحمض الميفيناميك). سيتم استخدام نقطة الانصهار، والتحليل الطيفي للأشعة تحت الحمراء، والفحص المادي لتوصيف المنتجات النهائية. نظرًا لأن مشتقات الأميد لها نطاق واسع من الأنشطة البيولوجية، فسيتم فحص النشاط المضاد للبكتيريا باستخدام نوعين من البكتيريا g+ve (المكورات العقدية والمكورات العنقودية الذهبية) ونوعين من البكتيريا g-ve (E. coli وKlebsiella). من أجل تحديد درجة تثبيط كل مشتق ومقارنتها مع كل من المثبط المرجعي واختبار مضاد البكتيريا، سيتم إجراء بحث على المركبات المصنعة على الأهداف المقترحة.

الكلمات المفتاحية: بروموهكسين ، امايدات ، موليكيولار دوكنك ، ادوية مضادات التهاب غير ستيرويدية، مضاد التهاب.

1. Introduction:

Amides are a significant chemical class that is widely distributed in nature and is present in many biologically active substances. They constitute the fundamental link in natural proteins and peptides, as well as in polymers, agrochemicals, and medicinal drugs [1,2]. Derivatives of amides have a wide range of pharmacological effects. Convulsions, pain, TB, inflammation, tumors, insects, fungal, and bacterial infections are all treated with them [3]. It is crucial for medicinal chemists to understand that amide groups play a role in the binding contact between a medication and its target because they operate as hydrogen bond donors and acceptors in hydrogen bonding interactions. [4] Typically, to prepare amide products, an amine compound react with carboxylic acid after converting the last one to acyl chloride compound . The resulting product is then reacted with the amine compound to produce the corresponding amide, which causes the water molecule to be expelled [5,6]. The objective of this research is to create novel amide compounds through a direct reaction between an amine drug (bromohexine) and carboxyl medicines, such as several non-steroidal anti-inflammatory drugs (NSAIDs), such as naproxen, indomethacin, diclofenac, and mefenamic acid. These medications have analgesic, antipyretic, and anti-inflammatory properties [7,8]. NSAIDs work by inhibiting the cyclooxygenase (COX) enzyme, which has two isoforms (COX 1 and 2) and is involved in the creation of prostaglandins, which are a class of lipids that function similarly to hormones [9,10]. NSAIDs are prescribed to treat headaches and conditions like osteoarthritis, gouty arthritis, and rheumatoid arthritis [11,12]. A synthetic benzyl amine derivative of vasicine, bromohexine is a weak base. [13] By decreasing the septum's viscosity, bromohexine has a mucolytic effect that improves expectoration and lung ventilation [14] . Currently utilized as a co-treatment for COVID-19, the virus mostly enters cells by trans membrane protease serine 2 (TMPRSS2). In addition to its mucolytic and expectorant effects, bromohexine has been shown [15,16]. To interfere with this enzyme (TMPRSS2). This means that it disrupts the pathway by which the virus enters the cell and

causes cell damage [17-19].

2. Experimental:

2.1. Materials and Methods:

All of the solvents and solids that were employed were of the pure variety, and their sources were Thomas Baker and Alphchem in India. The SDI company supplied naproxen, bromohexine, mefenamic acid, diclofenac, indomethacin, and mefenamic acid to the drug industry in Samarra, Iraq. Melting points were measured using the capillary method using an electric melting point device (England) called the Bamstead / Electro-thermal 9100. Compounds were identified using FTIR spectra, which were recorded as KBr disks using an FTIR-spectrophotometer (FTIR-6100 Type A).

2.2. General procedure for the synthesis of amide derivatives (1a-d);

Add 3 mmol of triethylamine to a mixture of 1 mmol of amine and 5–10 ml of dichloromethane. Next, add 1 mmol of corrosive and 1 mmol of thionyl chloride ($SOCl_2$) at room temperature. Tightly pack the mixture near the holder and let it sit at room temperature for an hour.Vanish the dissolveable in order to achieve the desired outcome. The resulting fabric was broken up in dichloromethane and first treated with 1 N HCl, then with 1 N NaOH. Using (Na_2SO_4), the natural stage was dried and disappeared to reveal the corresponding carboxylic amide [20].

2.3. Antimicrobial activity assessment:

The in vitro antibacterial activity of the recently synthesized amide compounds (1a-d) was evaluated against a variety of isolates of Gram negative (Klebsiella pneumoniae) and Gram positive (Staphylococcus aureus, streptococcus aeruginosa) bacteria. The evaluated compounds were prepared at doses of 100 mg/ml using dimethyl sulfoxide (DMSO). The disk diffusion method was used to determine the bacteria' starting activity. Whatman No. 1 filter papers were used to create the 6.25 mm-diameter disks. Each screw-covered bottle received a batch disc, which was then sterilised for one hour at 140°C using dry heat. Agar media was perforated, fresh seeds were added, and 100µl of each concentration was placed within each well. DMSO was the control utilized in

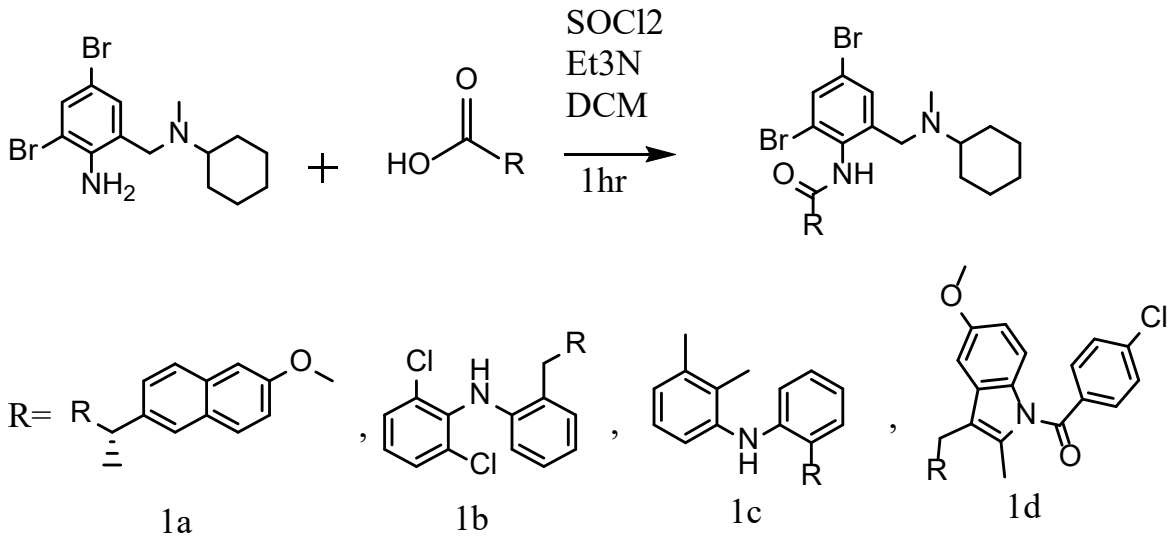
this experiment. For one day, the incubation was carried out at (37°C). In the current test, conventional medications included trimethprim, gentamycin, erythromycin, and sulfamethaxazole. The investigated compounds' antibacterial activity was evaluated using the diameter of the observed inhibitory area [21,22].

2.4. Molecular docking:

The autodock tool was used to create the molecular docking in order to verify that the derivatives bound to the intended target and to compare the outcome with both the in vitro and standard antibacterial tests. Autodock was used to measure the binding energy (Kcal/mol) of the tested chemical; the compound with the lowest binding energy is the preferred compound [23,24].

3. Results and discussion:

The synthetic pathway for the newly synthesized amide derivatives (1a-d) described in scheme 1.



Scheme 1: synthesis of amide derivatives. SOCl2(thionyl chloride), Et3N(tri ethyl amine), DCM(di chloromethane) .

N-(o,p-dibromo-6-((cyclohexyl(methyl)amino)methyl)phenyl)-2-(6-methoxynaphthalen-2-yl)propanamide (1a) : m.wt (588), m.p (83-86), IR (KBr) υ (cm−1): 470 (C-Br), 1454 (C-N), 1554 (aromatic C=C), 1672 (amide C=O), and 3430 (amide N-H). [25] Brown powder (63% yield).

N-(o,p-dibromo-6-((cyclohexyl(methyl)amino)methyl)phenyl)-2-(2-((2,6-dichlorophenyl)amino)phenyl)acetamide (1b) : m.wt (654) , m.p (112-115) , IR (KBr) υ (cm−1): 45۷ (C-Br), 557 (C-Cl), 1450 (C-N), 1506 (aromatic C=C),1580 (amide C=O), 3255 (amine N-H), 3380 (amide N-H).reddish brown powder (88% yield).

N-(o,p-dibromo-6-((cyclohexyl(methyl)amino)methyl)phenyl)-2-((2,3-dimethylphenyl)amino)benzeneamide (1c) : m.wt (599) , IR (KBr) υ (cm−1): 500 (C-Br), 1452 (C-N), 1506 (aromatic C=C),1647 (amide C=O), 3314 (amine N-H), 3353 (amide N-H). sticky yellow material (43% yield).

2-(1-(p-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)-N-(2,4-dibromo-6-((cyclohexyl(methyl)amino)methyl)phenyl)acetamide (1d) : m.wt (715.9) , m.p (90-93) , IR (KBr) υ (cm−1): 513 (C-Br), 554 (C-Cl), 1450 (C-N), 1484 (aromatic C=C),1555 (indomethacin amide C=O), 1579 (amide C=O), 3435 (amide N-H). dark brown powder (82% yield).

Table 1: antimicrobial test results.

Pathogenic Bacteria	Inhibition zone diameter (mm)																				
	1d				1b				1c				1a				CON +				
	1	2	3	4	1	2	3	4	1		3	4	1	2	3	4	1 SMT	2 TRM	3 ERT	4 AMP	5 GNT
Staph.	2	-	-	-	15	15	13	11	12	11	12	12	13	19	13	17	15	15	0	11	11
Strep.	-	-	-	-	11	10	10	8	9	9	8	9	9	9	9	9	-	-	17	11	-
E.coli	-	-	-	-	-	-	-	-	14	13	13	11	-	-	-	-	15	17	-	-	11
Klebs.	-	-	-	-	-	10	10	10	9	9	9	9	9	9	9	9	17	15	13	-	17

Note; SMT (sulfamethaxazole), TRM (trimethoprime), ERT (erythromycin), AMP (ampicillin), GNT (gentamicin). (-) mean no inhibition zone. (1,2,3,4) refer to concentrations of 1000,500,250,125 Mcg/ml respectively.

Table 2: autodock results on dihydrofolate reductase target (PBD ID: 2w9g)

Compound	Binding score
trimethoprime	-7.4
1a	-9.0
1b	-9.0
1c	-9.4
1d	-7.7

Table 3: autodock results on dihydropteroate synthase target (PBD ID: 1ad4)

Compound	Binding score
sulfamethaxazole	-6.4
1a	-7
1b	-6.4
1c	-6.7
1d	-5.9

Chemically, one-pot amide synthesis is made possible by the employment of catalytic agents SOCl2 and Et3N. SOCl2 first reacts with carboxylic acid to form acid chloride, and then it reacts with amine to get the corresponding amide derivative.

These derivatives exhibit some antibacterial action, according to the antimicrobial test (table 1), with 1a and 1b having greater activity against staph. than every medicine that was examined. (1a, 1b, and 1c) do better on strep. compared to gentamicin, trimethoprim, and sulfamethaxazole. (1c) are somewhat more effective against E. Coli than erythromycin and ampicillin. (1a, 1b, and 1c) are more effective against Klebsila than ampicillin.

The structure of the products suggests that the antibacterial effect occurs by inhibition of either the dihy-

dropteroate synthase enzyme, which is inhibited by sulfamethaxazole, or the dihydrofolate reductase enzyme, which is inhibited by trimethoprim. The docking results (tables 2 and 3) demonstrate that all derivatives have higher inhibition scores than the corresponding medicines, with the exception of 1d on the 1ad4 enzyme, and that 1d has the lowest overall activity among the derivatives. Because the suggested process may not be the same mechanism by which the derivatives function as antibacterial, the results do not entirely fit the antibacterial test.

4. conclution:

1. The intended chemicals have been successfully synthesized.
2. Physical property determination (melting point and description) was used to confirm the synthesized compounds' identity and characterization.
3. The end products' antibacterial evaluation shows that the amide group added antibacterial activity to the reacted drug—one is NSAID, the other is mucolytic.
4. The docking analysis demonstrates that all of the derivatives, with the exception of 1d on the 1ad4 enzyme, have higher inhibition scores than the respective medicines, and that 1d has the lowest overall activity among the derivatives (based on the postulated mechanism of action).

5. Further study:

Since such measurements are not available in Iraq, verify the HNMR and CNMR spectra on the produced substances for additional research. Examine additional actions such as anti-inflammatory, anti-tumor, anti-fungal, and other properties for amide compounds.

6.Acknowledgments:

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7. references :

1. Alandini N, Buzzetti L, Favi G, Schulte T, Candish L, Collins KD, Melchiorre P. Amide Synthesis by Nickel/Photoredox-Catalyzed Direct Carbamoylation of (Hetero) Aryl Bromides. Angewandte Che-

mie. 2020 Mar 23;132(13):5286-91.

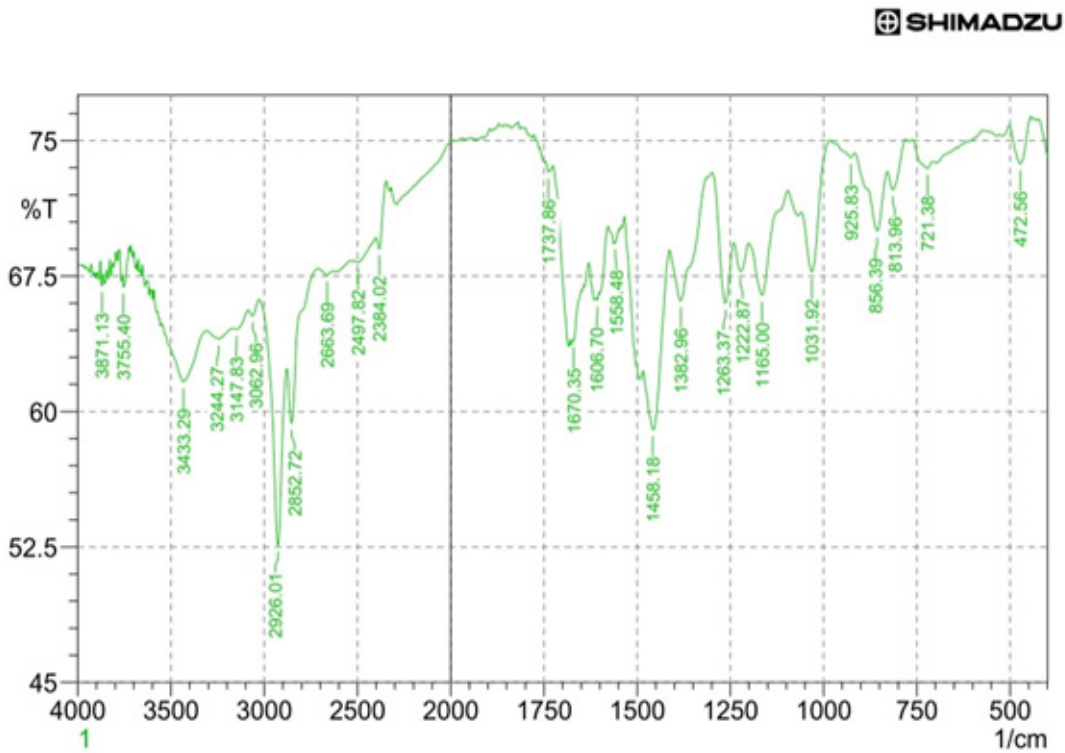
2. Sun S, Jia Q, Zhang Z. Applications of amide isosteres in medicinal chemistry. Bioorganic & medicinal chemistry letters. 2019 Sep 15;29(18):2535-50.
3. Kushwaha N, Saini RK, Kushwaha SK. Synthesis of some amide derivatives and their biological activity. International J. of Chem Tech Research. 2011 Jan;3(1):203-9.
4. Kumari S, Carmona AV, Tiwari AK, Trippier PC. Amide bond bioisosteres: Strategies, synthesis, and successes. Journal of medicinal chemistry. 2020 Jul 20;63(21):12290-358.
5. Massolo E, Pirola M, Benaglia M. Amide bond formation strategies: Latest advances on a dateless transformation. European Journal of Organic Chemistry. 2020 Aug 16;2020(30):4641-51.
6. Seavill PW, Wilden JD. The preparation and applications of amides using electrosynthesis. Green Chemistry. 2020;22(22):7737-59.
7. Karim HA, Raauf AM, Ali KF. Synthesis and preliminary pharmacological evaluation of some new triazole derivatives bearing nabumetone moiety targeting cyclooxygenase enzyme. Systematic Reviews in Pharmacy. 2021 Jan 1;12(1):190-5.
8. Schjerning AM, McGettigan P, Gislason G. Cardiovascular effects and safety of (non-aspirin) NSAIDs. Nature Reviews Cardiology. 2020 Sep;17(9):574-84.
9. Karim HA, Raauf AM, Ali KF. Synthesis and preliminary pharmacological evaluation of some new triazole derivatives bearing nabumetone moiety targeting cyclooxygenase enzyme. Systematic Reviews in Pharmacy. 2021 Jan 1;12(1):190-5.
10. Kushner P, McCarberg BH, Grange L, Kolosov A, Haveric AL, Zucal V, Petruschke R, Bissonnette S. The use of non-steroidal anti-inflammatory drugs (NSAIDs) in COVID-19. npj Primary Care Respiratory Medicine. 2022 Sep 21;32(1):35.
11. Musa LA, Karim HA, Ahmed AM. Selection of the best solvent and pH indicator for the acid-base titration of indomethacin in laboratories for pharmacy college students.

Eurasian Medical Research Periodical. 2022 Aug 2;11:1-5.

12. Bindu S, Mazumder S, Bandyopadhyay U. Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: A current perspective. Biochemical pharmacology. 2020 Oct 1;180:114147.
13. Lad M, Jain V, Raj H. A review of analytical methods for determination bromhexine hydrochloride in pharmaceutical and biological samples. PharmaTutor. 2014 Nov 1;2(11):35-41.
14. Wang Y, Zhang Y, Chen X, Xue K, Zhang T, Ren X. Evaluating the efficacy and safety of bromhexine hydrochloride tablets in treating pediatric COVID-19: A protocol for meta-analysis and systematic review. Medicine. 2020 Sep 9;99(37).
15. Day T, Gandon S, Lion S, Otto SP. On the evolutionary epidemiology of SARS-CoV-2. Current Biology. 2020 Aug 3;30(15):R849-57.
16. Weatherbee BA, Glover DM, Zernicka-Goetz M. Expression of SARS-CoV-2 receptor ACE2 and the protease TMPRSS2 suggests susceptibility of the human embryo in the first trimester. Open Biology. 2020 Aug 5;10(8):200162.
17. Martins R, Ferreira I, Jorge DM, Almeida L, Souza JD, Pontelli MC, Castro IA, Melquiades T, Mendes Viana RM, Zamboni DS, Marcato P. In Vitro Inhibition of SARS-CoV-2 Infection by Bromhexine hydrochloride. bioRxiv. 2022:2022-12.
18. Depfenhart M, de Villiers D, Lemperle G, Meyer M, Di Somma S. Potential new treatment strategies for COVID-19: is there a role for bromhexine as add-on therapy?. Internal and Emergency Medicine. 2020 Aug;15:801-12.
19. Gholami A, Minai-Tehrani D, Eftekhar F. Bromhexine and its inhibitory effect on lipase–kinetics and structural study. Archives of Physiology and Biochemistry. 2022 Nov 2;128(6):1687-92.
20. Leggio A, Belsito EL, De Luca G, Di Gioia ML, Leotta V, Romio E, Siciliano C, Liguori A. One-pot synthesis of amides from carboxylic acids activated using thionyl chloride. Rsc Advances. 2016;6(41):34468-75.
21. Khan AK, Hamdee MD. New Hy-

drochlorothiazide Derivatives: Synthesis, Characterization and Anti-Microbial Assessments. Research Journal of Pharmacy and Technology. 2019;12(12):5848-54.

22. Lima LM, da Silva BN, Barbosa G, Barreiro EJ. β-lactam antibiotics: An overview from a medicinal chemistry perspective. European journal of medicinal chemistry. 2020 Dec 15;208:112829.
23. Achutha AS, Pushpa VL, Manoj KB. Comparative molecular docking studies of phytochemicals as Jak2 inhibitors using Autodock and ArgusLab. Materials Today: Proceedings. 2021 Jan 1;41:711-6.
24. Fan J, Fu A, Zhang L. Progress in molecular docking. Quantitative Biology. 2019 Jun;7:83-9.
25. Ji Y, Yang X, Ji Z, Zhu L, Ma N, Chen D, Jia X, Tang J, Cao Y. DFT-calculated IR spectrum amide I, II, and III band contributions of N-methylacetamide fine components. ACS omega. 2020 Apr 8;5(15):8572-8.



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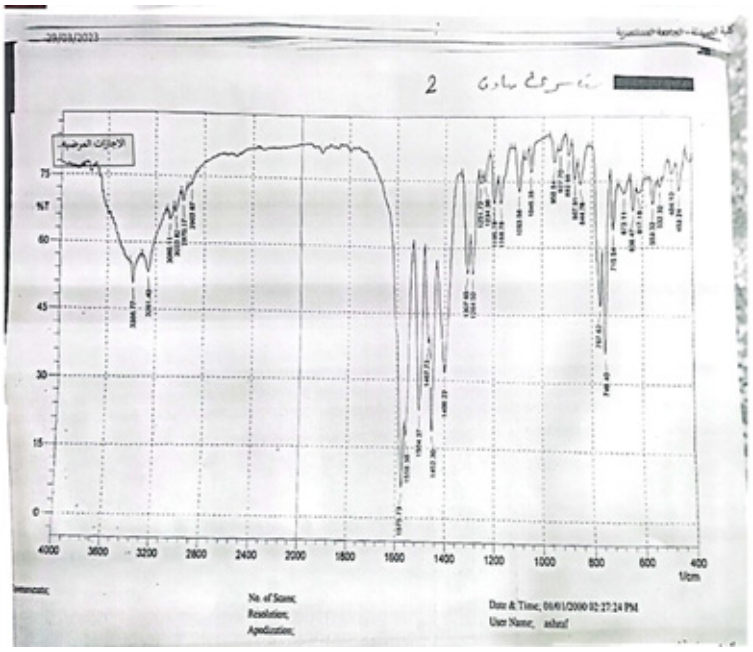
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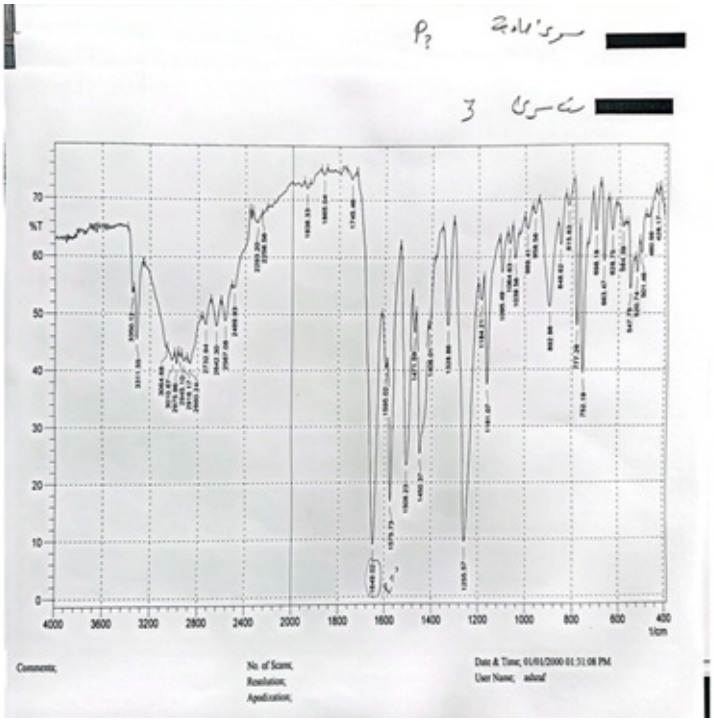
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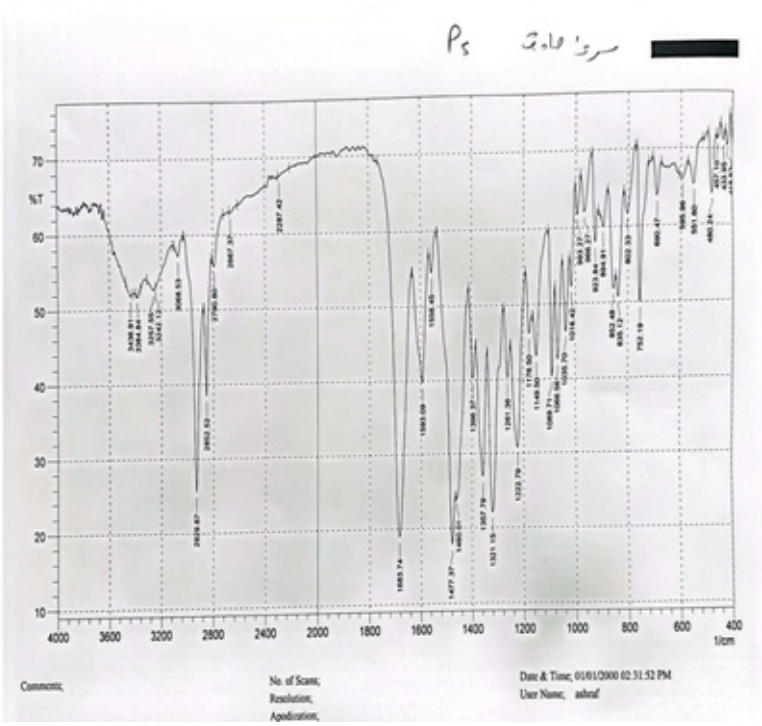
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