Ministry of Higher Education

and Scientific Research

Journal of Kufa for Chemical Sciences

A refereed

Research Journal Chemical Sciences

Vol.2 No.8 Year 2022

ISSN 2077-2351

Synthesis and Characterisation of New Hexahydropyrimidine Derivatives, and Study of Their Effect as Anti-parasite.

Meaad K. Al-Shuraify, Fatima S. Sabah, Hanoy K. Al-Amood.

Department of chemistry/ College of Science/ University of Basrah/ Iraq.

E-mail: ***hanoy.alamood@uobasrah.edu.iq***

الخلاصة

تم تحضير مركبين من مشتقات البيريميدينات الجديدة (هكساهيدروبيريميدين) من خلال تفاعل الجالكونات مع الكوانيدين امين ، حيث كان الناتج بحصيلة عالية وتمت متابعة سير التفاعل من خلال كروماتوغرافيا الطبقة الرقيقة بإستخدام مزيج من الاسيتالديهايد والهكسان بنسبة (3:1). تم تشخيص المركبات من خلال طرق التشخيص الطيفية (الاشعة تحت الحمراء ، الرنين النووي المغناطيسي و مطيافية الكتلة) حيث اثبت من خلالها تحضير هذين المركبين. تم تطبيق المركبين كمضادات للطفيليات ، وكانت نسبة (78.9%) للبيريميدين غير المعوض و للبيريميدين المعوض بمجموعة الامين بالموقع ميتا بنسبة التثبيط (66.6%).

Abstract

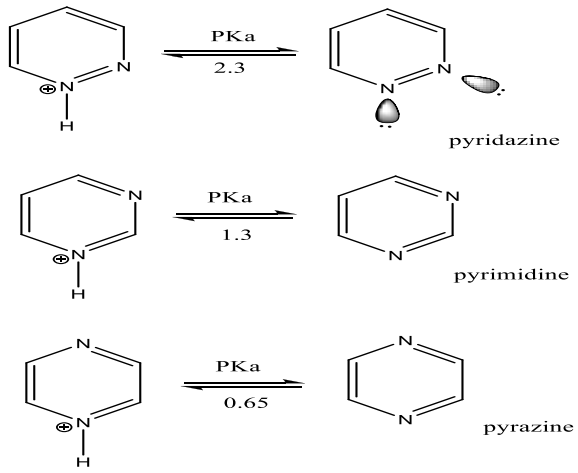
New pyrimidine derivatives (hexahydropyrimidine), 4-(2,4-dichlorophenyl)-6-phenyl-1,3-hexahydropyrimidine-2-amine (N_H), and 6-(3-aminophenyl)-4-(2,4-dichlorophenyl)-1,3-hexahydropyrimidine-2-amine ($N_{m\text{-}NH2}$) were synthesised and identified with FT-IR, Mass and H^1-NMR spectra. The new hexahydropyrimidine (N_H, $N_{m\text{-}NH2}$) applied to the parasite echinococcosis granulosus gave a different result. The prepared compounds' inhibition ratio was N_H (78.9%) and $N_{m\text{-}NH2}$ (66.6%).

Keywords:

Pyrimidine derivatives, hexahydropyrimidine, echinococcosis granulosus, antiparasite.

Introduction

Pyrimidine is the essential six-membered ring with two nitrogen atoms in position 1,3[1]. It has three isomer structures according to the nitrogen atom's position in the hexagonal ring. When comparing the three positions of the nitrogen atoms (1,2, 1,3, 1,4), all three compounds are very weak bases. Pyridazine is slightly more basic than the other two because the two adjacent lone pairs repel each other and make the molecule more nucleophilic (the α effect)[2]. (Scheme 1):



Scheme (1): pk_a values of pyridazine, Pyrimidine, and pyrazine.

Pyrimidine is more vital than other because of its involvement in DNA and RNA. They include several nucleic acid constituents (Cytosine, Thymine, and Uracil) and form the basic structure of the barbiturates. Pyrimidine has many biological applications such as antibacterial, antimalarial, anti-inflammatory, antifungal, anticancer, and antiviral [3]. One of the famous pyrimidine derivatives is hexahydropyrimidine, and their derivatives have pharmacological activities such as anticancer, antiviral, antimalarial, and antibacterial [4]. They were also used as a valuable reagent in many organic syntheses. FAD (Figure 1) is an important example of the hexahydropyrimidine group. It is a redox-active coenzyme associated with various proteins involved with several enzymatic reactions in metabolism [5].

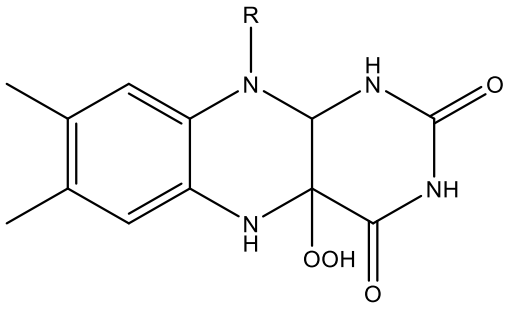


Figure (1): Flavin adenine dinucleotide (FAD-4ά-OOH).

Parasite:

The parasite is a sickness caused by polluted water and food. In several cases, parasites can spread through insects, such as mosquitoes [6]. There are many kinds of parasites, one of them a type named an echinococcosis granulosus that shown in Figure (2):

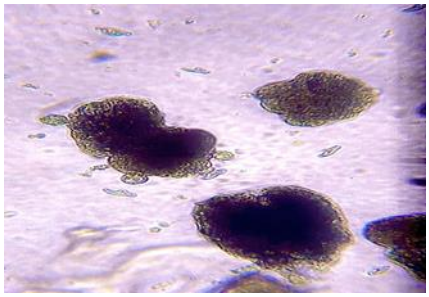


Figure (2): Echinococcosis granulosus parasite.

The life cycle of parasites:

The larval steps affect human echinococcosis or hydatid sickness [7]. In the small intestine of the dogs, The adult Echinococcosus granulosus (2-7mm long) was lived. The spawns approved in the faeces are proximately infectious. After eating an apposite intermediate, spawns hatch in the small intestine and release six-hooked that enter the intestinal wall and travel through the blood system into several organs, particularly the lungs and liver. Develops into a thick-walled hydatid cyst. It is creating spawn cysts that fill the cyst internal. after eating, the protoscolices evaginate, fasten to the intestinal mucosa.Grow into adult stage 1 in 32 to 80 days.

Humans are abnormal intermediate hosts and become infected by ingesting spawns that are free in the intestine, and hydatid cysts grow in a series of organs. The liberated protoscolices may create secondary cysts in other sites within the body (intermediate echinococcosis) If cysts rupture [8].

Materials and Methods:

The melting point was measured with the Electrothermal melting point apparatus. IR spectra were recorded using KBr disk on Shimadzu FT-IR-8300 spectrophotometer in Basrah University, Science college, Chemistry department. H^1-NMR spectra were measured in Tehran University (IRAN) on Avance DRX 500 MHz (from Bruker), using dimethylsulphoxide (DMSO) and ($CHCl_3$) as internal standards. The parasite experience was done on Biotech Technology in Basrah (IRAQ).

preparation of chalcones:

To a mixture of 2,4-dichlorobenzaldehyde (0.348 g, 2mmole) and acetophenone (2mmole) in Ethanol (96%, 15 ml), 10 ml of aqueous NaOH 10% was added. The reaction mixture was stirred at 0◦C for two hours. 100ml of cold water was added to the mixture and acidified with 5ml acetic acid. The precipitate formed was filtrated and recrystallised in absolute Ethanol [9]. Toluene: ethyl acetate: n-hexane (3:1:0.5) were used as eluent solvents. UV Lamp at 250 nm was used to visualise the product spot.

preparation of pyrimidine derivatives N_H and $N_{m\text{-}NH2}$ compounds:

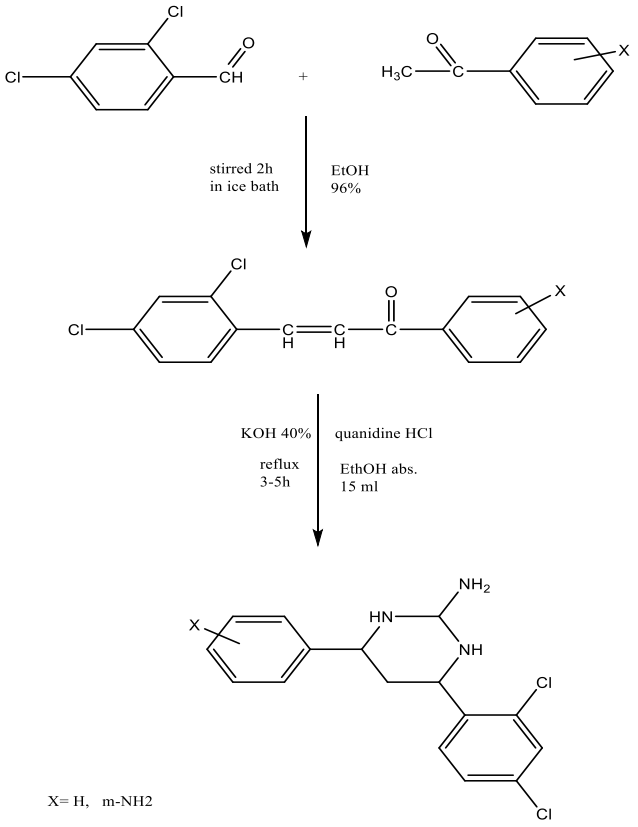
Un aqueous KOH 40% (1ml) was added to a mixture of chalcones' derivatives (2mmole), and guanidine hydrochloride (2mmole) in ethanol absolute (15 ml). The reaction mixture was heated with reflux for 3-5 hours, then powered into ice-cold water after being acidified with 3drops of (1:1) HCl: Water. The precipitate formed was filtered and recrystallised in an absolute Ethanol: water mixture. TLC was showed one spot, and the eluent was used (3:1: 0.5) ethyl acetate: normal hexane: H_2O

anti-parasite test [10]:

Echinococcus granulosus protoscoleces were isolated from liver hydatid cysts obtained from infected sheep with phosphate-buffered saline (PBS). Protoscoleces were washed five periods containing 100 U/ml penicillin and 100 µg/ml streptomycin. The viability of protoscoleces was determined by staining with 0.4% (w/v) trypan blue, dead protoscoleces staining blue. Protoscoleces with > 95% viability were subsequently cultured in RMPI 1640 average complemented by 10% (v/v) FBS (fetal bovine serum), 100 U/ml penicillin and 100 µg/ ml streptomycin in a 25 cm flask at 37˚C in a CO_2 incubator for 24 hours. To evaluate the cytotoxicity of prepared compounds against the protoscoleces. The protoscoleces maintained as described above were seeded in 96-well plates (200 protoscoleces/250 µl/ well) in a complete RPMI 1640 culture medium at 37˚C in a CO_2 incubator three days. (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) assay (MTT) was used to determine viable protoscoleces. Viable protoscoleces with active metabolism reduce the MTT tetrazolium into a purple-coloured formazan product. Therefore, 25 µl of 5m/ml MTT prepared in BPS was added to each well and protoscoleces were incubated at 37˚C in a CO_2 incubator in the dark for two hours. The medium was removed, and formazan crystals formed by the protoscoleces were dissolved using 250 µl of 100% (v/v) dimethyl sulfoxide (DMSO). The absorbance was read at 570 nm using an ELISA reader. The half-maximal inhibitory concentration (IC_{50}) value was calculated using GraphPad Prism version 8.

Results and discussion:

pyrimidine derivatives were prepared from the reaction of chalcone derivatives with guanidine amine hydrochloride to give the products (scheme 2).



Scheme (2): preparation of chalcone and Pyrimidine derivatives.

The most suitable method is the Claisen-Schmidt [11] condensation of equimolar quantities of aryl ketone (acetophenone, and 3-aminoacetophenone) with aryl aldehyde (2,4-dichlorobenzaldehyde) in the presence of alcoholic alkali.

Hexahydropyrimidine was prepared by the cyclisation between the chalcone and guanidine hydrochloride in the presence of an alkali catalyst (KOH). The yield was excellent in the product that did not contain the substituted group (N_H), but it decreased when the substituted groups were an amino group in the meta position. The products were a powder and melted in different ranges. The physical properties are shown in Table1. The products were characterised by infra-red (Table2), H-NMR (Table3), and Mass spectra (Table 4).

Table (1) physical properties of synthesised compounds.

No. of comp.	IUBAC name	M.wt	Yield	Colour	M.P
N_H	4-(2,4-dichlorophenyl)-6-phenyl-1,3-hexahydropyrimidine-2-amine	322	80%	Brown	120
$N_{m\text{-}NH2}$	6-(3-aminophenyl)-4-(2,4-dichlorophenyl)-1,3-hexahydropyrimidine-2-amine	337	75%	Brown	88

FT-IR spectral data:

Infrared spectroscopy is the preferred method for detecting and determining the functional group positions in a given sample. This technique was performed to get information about the vibrational origin of all the prepared compounds.

The spectra of the prepared compounds show that two compounds have typical peaks, such as the stretching (N-H) group at (3414, 3336) cm^{-1}, which belongs to the primary NH_2 group in the heterocyclic ring. These facts enhance the correct expected chemical structure of these compounds. Also, the appearance of peaks at (3223) cm^{-1} due to stretching vibration of the heterocyclic ring's secondary (N-H) group. The strong band showed in the region (3093, 3090) $cm^{-1,}$ which were attributed to the (C-H) aromatic group, and two very strong bands at (1589 and 1469 cm^{-1}) to the (C=C) stretching vibration aromatic ring. The absorption bands in the (1103-1099) cm^{-1} belong to the (C-Cl) stretching. The absorption bands data of these compounds are shown in Table (2) refer to the infrared spectra of the prepared compounds[12], and the infra-red spectrum of the compounds were shown in the Figures 3, and 4):

Table (2): FT-IR Spectra data of synthesised compounds.

Compounds	IR data(cm^{-1})
N_H	N-$H_{pry.}$(3414),N-$H_{sec.}$(3223), C-$H_{aliphatic.}$(2928), C=$C_{Aromatic}$ (1589 1469), Ar-H(3093),C-Cl (1103), C-N (1049).
$N_{m\text{-}NH2}$	N-$H_{pry.}$ (3336), N-H_{sec} (3223), C-$H_{aliphatic}$ (2925), C=$C_{Aromatic}$ (1589 1469), Ar-H(3090), C-Cl (1103) , C-N (1049).

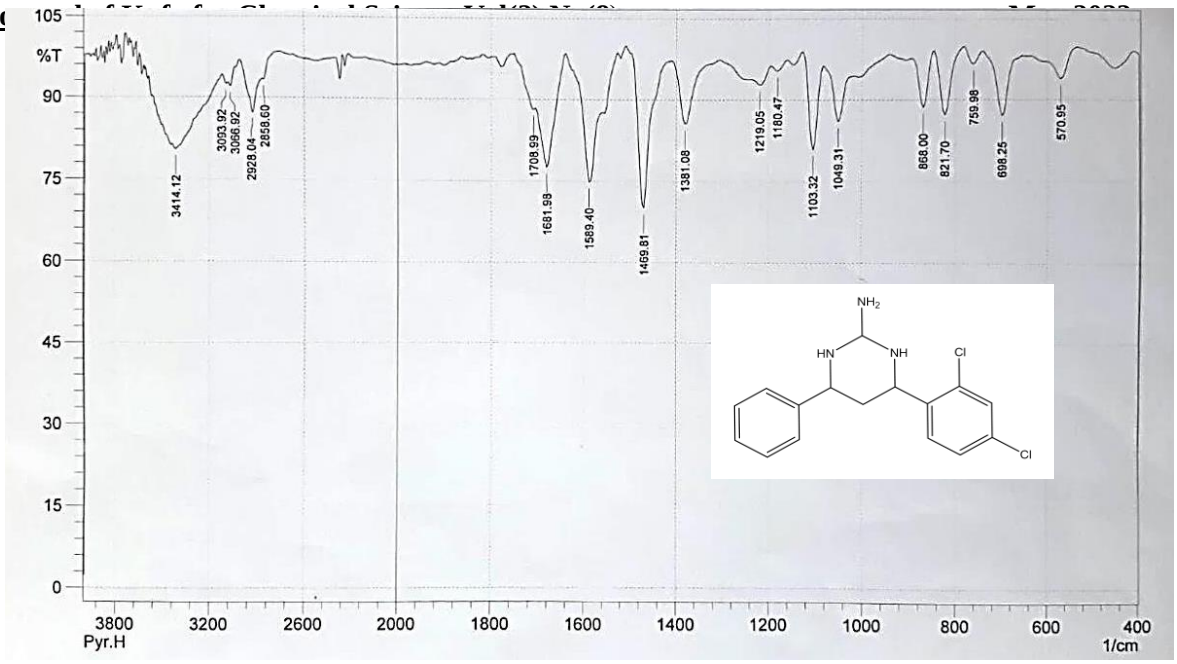


Figure (3): The infra-red Spectrum of the compound $N_{(H)}$.

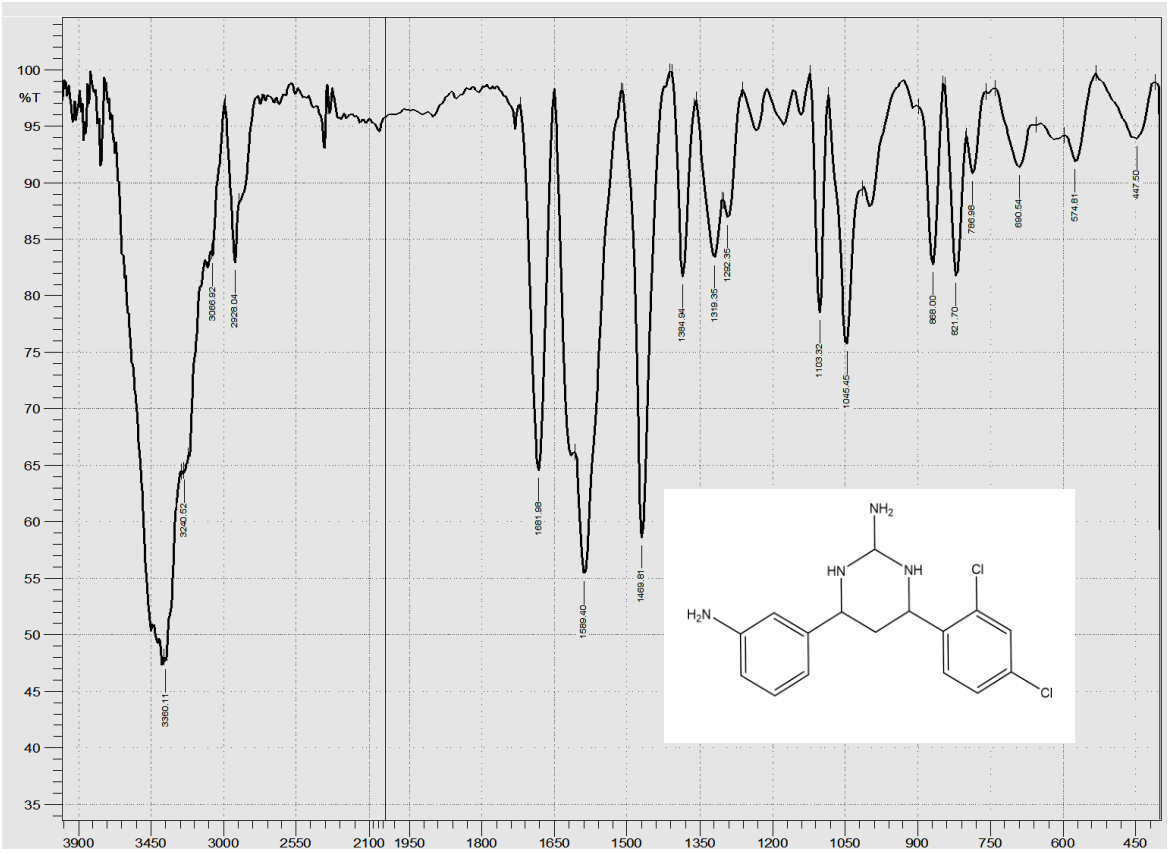
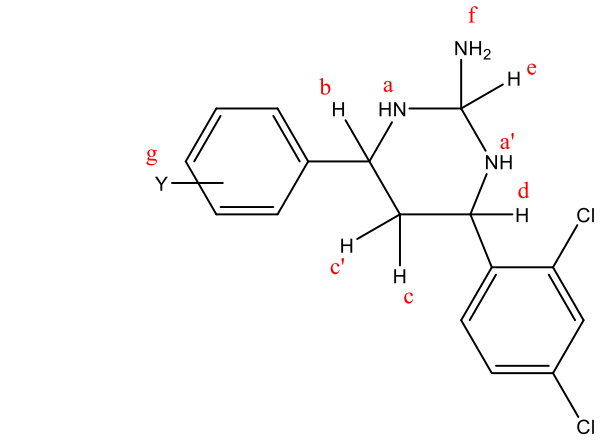


Figure (4): The infra-red Spectrum of the compound $N_{(m\text{-}NH2)}$.

Proton Nuclear Magnetic Resonance (^{1}HNMR)

The ^{1}H-NMR spectrum of the Hexahydropyrimidines shows a signal at (1.25-1.26, 4.76) ppm, which is ascribed to each of the protons of amine groups (NH_2 f), and (NH_2 g), respectively. The NH protons of the hexahydropyrimidine ring (a) at (0.85, and 0.87)[13], as such we can have observed signal at which ascribed to each the proton of hexahydropyrimidine ring (as appears in the Figure(5)), ($1H_b$,$2H_{c\&c'}$,$1H_d$, and $1H_e$) (there were shown in Table 3). In addition to the appearance of signals for the aromatic ring, also it was observed that bundles of dimethyl sulfoxide DMSO-d^6 solvent in their specific locations upon displacement (2.50-2.53) ppm, and for the chloroform $CHCl_3$ at 7.26 ppm. The chemical shifts of H^1-NMR spectra of the prepared compounds are shown in Table 3, and the H^1-NMR spectea of the compounds were shown in the Figures 6, and 7):



Y= H, m-NH2

Figure (5): The chemical structure of the prepared compounds.

Table(3): Chemical Shifts H^1.NMR Spectra of the prepared compounds.

Compounds	H^1-NMR(ppm)
N_H	0.85 (dd, 1H). (a)), 1.08 (dd, 1H) (a')), 3.51(t.,1H(b)), 2.65 (dd, 1H, c), 2.38 (dd,1H(c')), 4.34 (d, 1H, (d)), 6.75 (d, 1H (e)), 1.25 (s, 2H) (f)), (7.97 – 7.53 (m, 8H) Ar-H)), DMSO-d_6 (2.50-2.53).
$N_{m\text{-}NH2}$	0.87 (d, 1H), (a)), 1.11 (d, 1H), (a')), 3.76 (t, 1H, (b)), 2.33(dd,1H), (c) , 2.01 (dd, 1H), (c')), 5.29 (d, 1H), (d)), 6.80 (d, 1H), (e)), 1.26 (s, 2H), (f)), 4.76(s, 2H), (g)), (7.59 – 7.32 (m, 4H), 7.19 – 7.07 (m, 3H), Ar-H)), (Chloroform-*d*), (7.26).

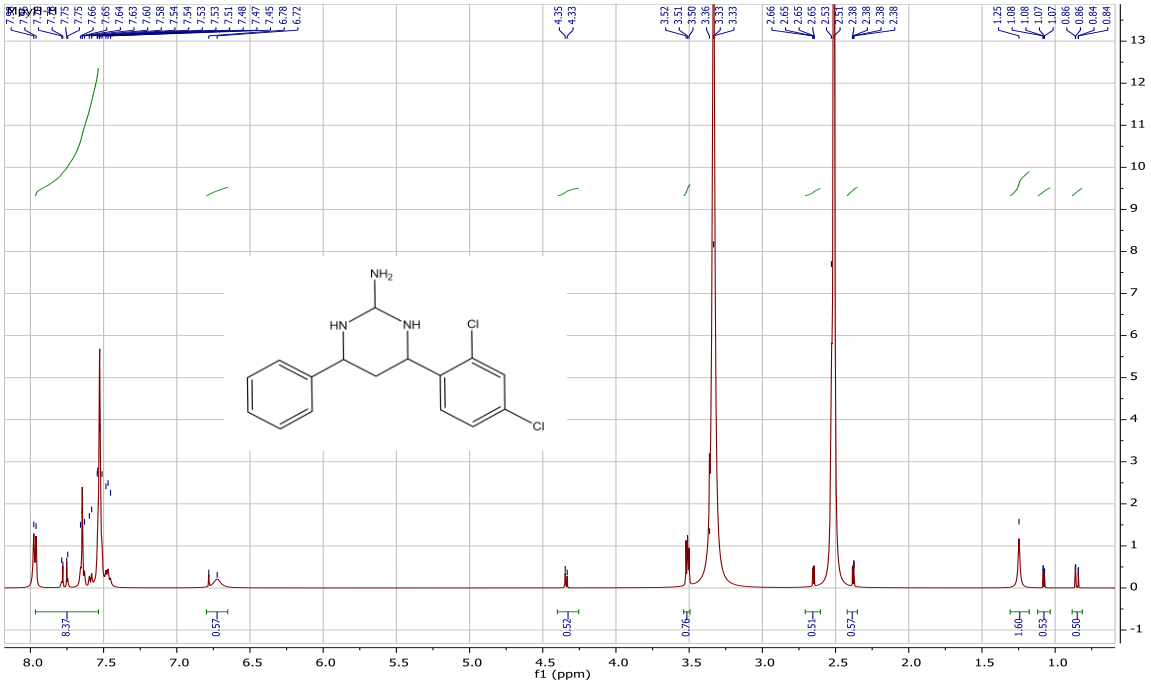


Figure (6): H^1-NMR spectea of $N_{(H)}$ compound.

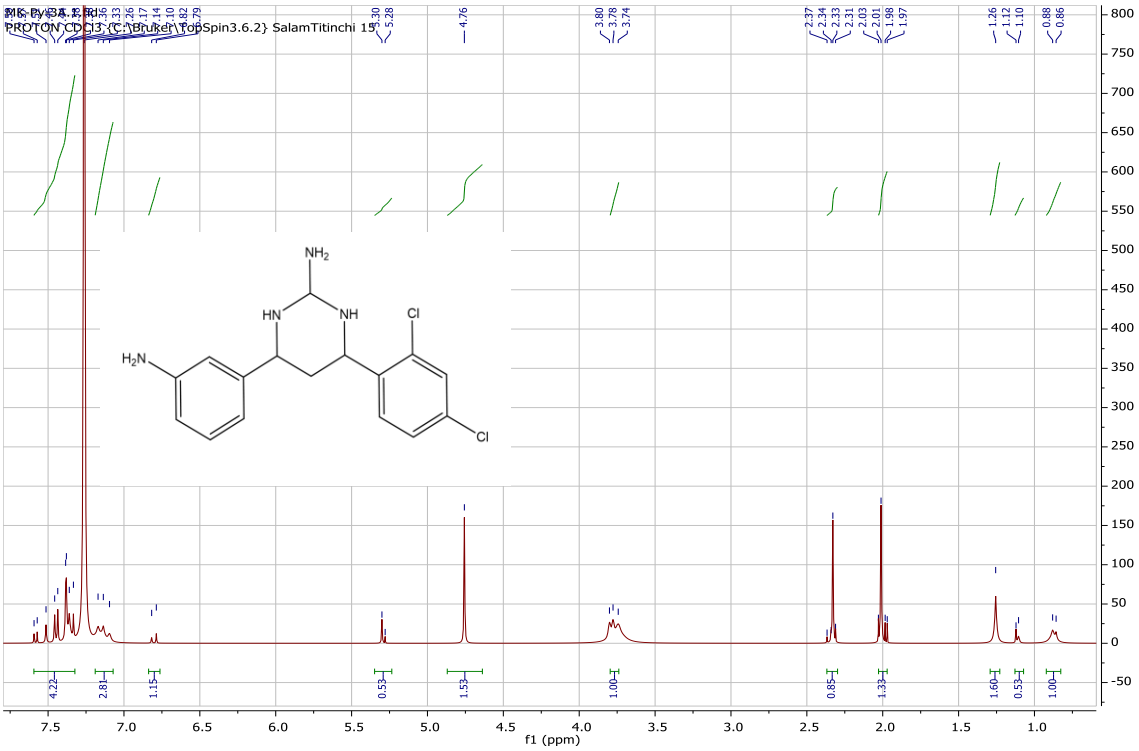
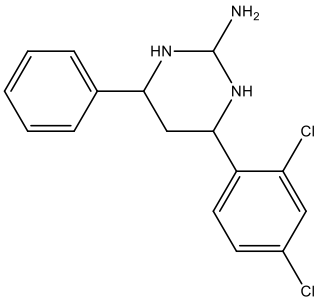
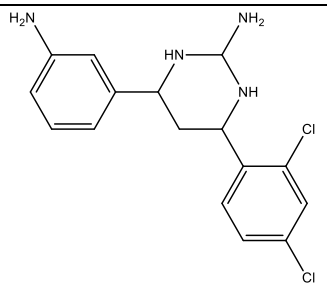


Figure (7): H^1-NMR spectea of $N_{(m\text{-}NH2)}$ compound.

Mass spectrum

The mass spectrum found that the peaks at (m/z = 322, 337) represented the molecular ion [M·+] for hexahydropyrimidines compounds ($N_{(H)}$ and $N_{m\text{-}NH2}$), respectively. These peaks support our study while the structures of the synthesised heterocyclic compounds are correct. The significant fragmentation of hexahydropyrimidines compounds was shown in Table 4, and the mass spectra of the compounds were shown in the Figures 8, and 9):

Table(4): m/z of the main fragments of mass Spectra of the prepared compounds.

Name of sample	Structure	m/z of the main fragments
N_H		322, 307, 177, 147, 101, 86.
$N_{m\text{-}NH2}$		337, 322, 192, 147, 101, 86.

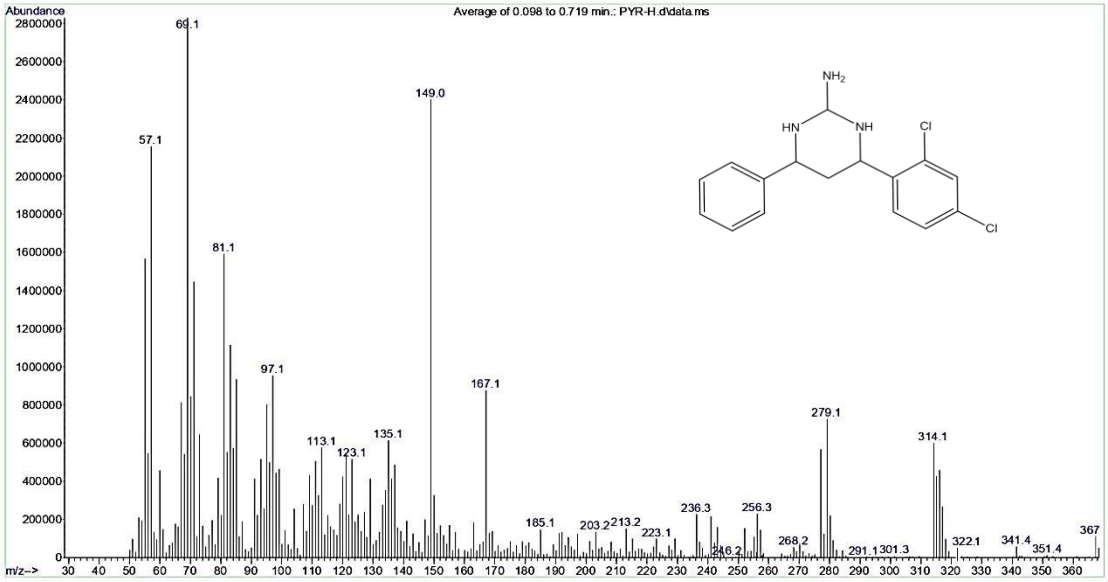


Figure (8): Mass spectra of $N_{(H)}$ compound.

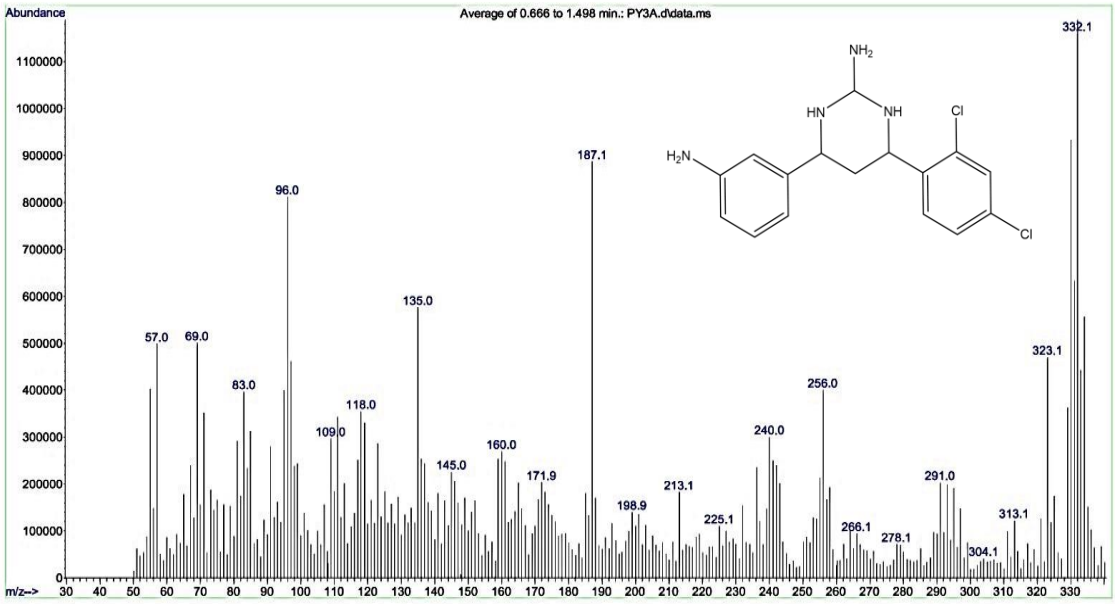


Figure (9): Mass spectra of $N_{(m\text{-}NH2)}$ compound.

Results of antiparasite activity of pyrimidine derivatives:

The parasite was a disease caused by the drinking of polluted water and foods [14]. There are many kinds of parasites, one of them is an echinococcosis granulosus. The results that noticed were very good to inhibit the ratio of parasite, as it is shown in Table(5):

Table (5): the inhibition ratio of some prepared compounds applied to the echinococcosis granulosus parasite.

No. of sample	Name of sample	Inhibition Ratio
1	$N_{(H)}$	78.9
2	$N_{m\text{-}NH2}$	66.6

From table 5, the Pyrimidine with non-substituted ($N_{(H)}$) gave a higher inhibition ratio than the pyrimidine compound which substituted with meta amino group ($N_{m\text{-}NH2}$). The difference between the two compounds is only in the increasing of the nitrogen atoms in the last compound, indicating that the more nitrogen atoms in the pyrimidines, reduce the inhibition ratio of the parasite.

There are several reasons for interpreting these results, including:

1) The type of the group, whereas the amino group increased the electron density along the molecule, leading to molecular capacity to capture the parasite's cell [15].

2) Their ability to penetrate the membranes of the parasite [16].

Conclusion:

1- The compounds in meta substituted ($N_{m\text{-}NH2}$) had lower inhibition of parasite's cells than the compounds (N_H), which gives a much higher inhibition ratio.

2- Increasing the number of nitrogen atoms led to an increase in the electronegativity of the compound, thus reducing its ability to penetrate the parasite membrane.

References:

1- Naglaa M. Ahmed, Mahmoud M. Youns, Moustafa K. Soltan, and Ahmed M. Said, Design. [2021]: Synthesis, Molecular Modeling and Antitumor Evaluation of Novel Indolyl-Pyrimidine Derivatives with EGFR Inhibitory Activity. Molecules, 26, 1838.

2- Jonathan Clayden, Nick Greeve, Stuart Warren, and Peter Wothers. [2001]: organic chemistry. 2nd Edition. Oxford University.

3- Guanya Li, Dunhui Li, Tao Wang, and Shanping. [2021]: Pyrimidine Biosynthetic Enzyme CAD: Its Function, Regulation, and Diagnostic Potential. International Journal of Molecular Sciences. 22(19):10253.

4- Ivan V. Zhukov, Alexey S. Kiryutin, Mikhail S. Panov, Natalya N. Fishman, Olga B. Morozova, Nikita N. Lukzen, Konstantin L. Ivanov, Hans-Martin Vieth, Renad Z. Sagdeev, and Alexandra V. Yurkovskaya1. [2021]: Exchange interaction in short-lived flavine adenine dinucleotide biradical in aqueous solution revisited by CIDNP and nuclear magnetic relaxation dispersion. 2(05).03, p. 44.

6- Yohannes Alemu Belete, Tilahun Yemane Kassa, Minale Fekadie BayeID. [2021]: Prevalence of intestinal parasite infections and associated risk factors among patients of health centre requested stool examination Jimma, Ethiopia. PLOS ONE. 16(2): 0247063.

7- Armando Gonzalez, and Luis Gomez-Puerta . Echinococcus. [2018]: Book: Foodborne Parasites. p.245-267.

8- Khadeeja Abees Hmood Al-Khalidi, Hiba Riyadh Al-Abodi, Hayder Kamil Jabbar and Baheeja A. Hmood, intechopen. [2020]: Echinococcus granulosus. 90708.

9- Hanan F. Al-Shamsi, Hanoy K. Al-Amood, Hayat H. Abbas wijdan H. Al-Tamimi. [2019]: Synthesis and biological activity study of some new of the β -aminocarbonyl compounds. Bas.J.Vet.Res.Vol.18, No.2.

10- M.Nagoor Meeran, M.Shreelekha and R.P.Sotonye Campbell, Kristina Soman-Faulkner. [2021]: Antiparasitic Drugs. StatPearl.

11- Suresh Jeyakumar. [2015]: Synthesis, Characterisation and Antimicrobial Activity of Substituted Pyrimidines. Chemical Science Transaction, 4(4), 1139-1144.

12- Brennan, NF. [2006]: Infra-Red and Raman spectroscopy. Chapter 3. The University of Pretoria.

13- Philip Crews, Jaime Rodriguez, and Marcel Jaspars. [1998]: Organic structure analysis. University of California, New York Oxford. P 96.

14- Peter Irwin and Rebecca Traub. [2011]: Parasitic diseases of cats and dogs in the tropics. ResearchGate. V(1), No. 010.

15- Yen-Wen Huang, Yan-Cheng Lin., Chu-Chen Chueh. [2021]: Investigating the Backbone Conformation and Configuration Effects for Donor−Acceptor Conjugated Polymers with Ladder−Type Structures Synthesised Through Aldol Polycondensation . Journal of Materials Chemistry C. V(3). 9473-9483.

16- Kartikeya Tiwari and Vikash Kumar Dubey. [2018]: Fresh insights into the pyrimidine metabolism in the trypanosomatids. Parasites & Vectors.