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The synthesis of alkyl Uracil by using Microwave irradiation and evaluated biological activity

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Abstract:

The aim of the present study was firstly to synthesize alkyle Uracil 1,3-di(prop-2-ynyl)pyriimide-2,4(1H,3H)-dione Using micro wave irradiation and to evaluate it's biological activity on serum alkaline phosphatase in breast cancer patients.

The preparation of the compound took only (20min) in DMF as solvent the identification was carried by FT-IR. The compound showed effect on serum alkaline phosphatase.

The type of inhibition was found to be non comparative with $Km=0.36\times10^{-2}$ M and V_{max} is 10.0 U/L for the uninhibited enzyme and 6.3 U/L for the inhibited enzyme.

الخلاصة:

الهدف من الدراسة الحالية هو تحضير يوراسيل الكيل باستخدام أشعة المايكروويف وتقييم فعالية المركب على انزيم الفوسفاتيز القاعدي في مصل الدم لمرضى سرطان الثدي. ان تحضير المركب استغرق وقت قصير لا يتجاوز (20) دقيقة في المذيب (DMF) .

لقد تم تشخيص المركب باستخدام (FTIR) وجهاز (HNMR) وقد اظهر المركب فعالية تثبيطية لانزيم الفوسفاتيز القاعدي وقد كان نوع التثبيط غير تنافسي بقيم $Km=0.36\times10^{-2}$ M و V_{max} is 10.0 U/L .

Introduction:

Pyrimidines are a class of heterocyclic compounds with a wide range of application. These compounds received considerable a tention for their important application in many fields[1]. A novel organic derivative of pyrmidine namely 1,3-di(prop-2-ynyl)pyriimide-2,4(1H,3H)-dione was a hompted to be prepared since in the area of cancer chemotherapy there are a long standing desire to prepare selective derivatives with anti tumor activities[2], because of the wide spread applications of pyrimidine derivatives as N-heterocyclic carbenes[3].

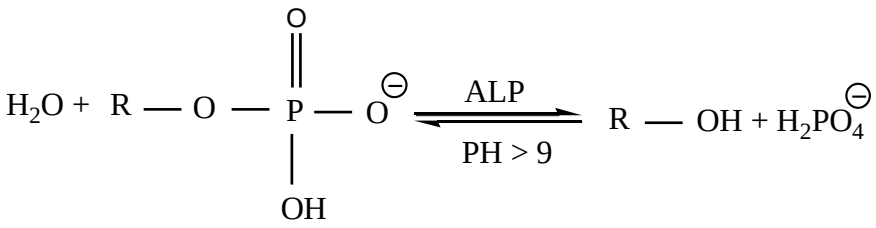
Pyrimidines themselves are part of nucleosides which are part of natural nucleosides, the monomeric units of RNA and DNA[4]

Nucleoside analogues show importance in several established chemotherapy (anticancer, antiviral and anti bacterial) and other attractive fields like immunomedecal, immunomodulation or regulation of gene expression which could constitute new therapeutic approaches[5]

In general , pyrimidine alkyl prepared by treatment of pyrimidine with alkyl halide in DMF as solvent[6]. The classical method is time consuming and high temperature application. Thus Microwave irradiation is a better technique as source of energy for biochemical reactions, the hesitancy of its onset, compared to organic synthesis, is most likely due to the high temperatures associated with microwave, assisted trans formations. Many of the biochemical molecules are temperature- sensitive. Now, with current technology, temperatures as low as 35-40ºC can be maintained by precise power input[7].

The synthesis of pyrimidine derivatives assisted in the development of the other a pentic drugs have been taking much interest in the benefit from Microwave irradiation[8].

Alkaline phosphatase is a group of nonspecific phosphatases catalyze the reaction shown below.[9]



Alkaline phosphatase (ALP) is present practically in all mammaline tissues, specially at or in the cell membrane, the activity of the enzyme raised in many diseases including different types of tumors[10].

The aim of the present study is to synthesize " 1-(pro-2ynyl) pyrimidine-2,4(1H,3H)-dione" by the applications of microwave radiation to the organic reactions, also to study the effect of the above compound on alkaline phosphatase activity in sera of breast cancer patients.

Experimental Section:

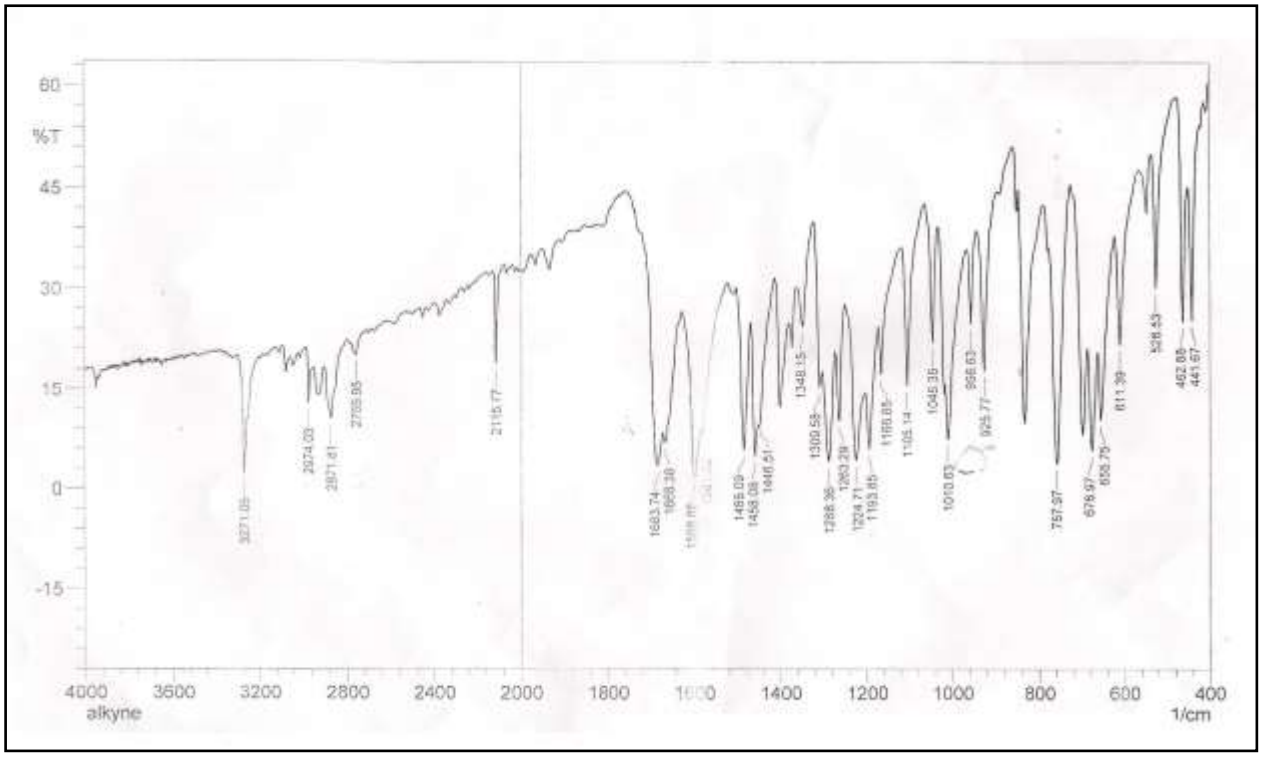
General methods:

Microwave (BIOTAJ), in fared were recorded IRP restige-21(F.T.I.R). Alkaline phosphates activity were performed using Biomerieux ready kit (France).Elemental analysis (C.H.N) was carried out with Perken Elmo B_240 Elemental analyzer of Al-Mousel University. Melting point determined with :Stuart melting point Appartus and were uncorrected.

Synthesis of 1,3-di(prop-2-ynyl)pyriimide-2,4(1H,3H)-dione . Uracil (1.0mmol), finely powdered potassium carbonate (1.5 mmol), 3-chloroprop-1-yne (1.2 mmol), and DMF(2 ml) were placed into a (10 ml)process vial equipped with a Stirring bar. The vial was sealed by capping with a Teflon septum fitted in aluminum then irradiated with microwave for (20min) at (150ºC). after cooling the reaction mixture was transferred to a round bottom flask and the solvent was evaporated in vacuo. The residue was partitioned between ethyl acetate (10 ml) and water (10 ml). the organic phase was washed twice with (10 ml) of water and the combined aqueous phases are re-extracted twice with (10ml) of ethyl acetate. The combined organic phases were dried over sodium sulfate and concentrated vacua to yield the alkylated uracil (sold).

FT-IR (3271) cm^{-1} for (C-H) Str Alkyne ,(2974)cm^{-1}. C-H) Alkene Str,. (2759) cm^{-1} (C-H) Alkane Str., (2115) cm^{-1} (C≡C), (1683 – 1666) cm^{-1}(C=O) Str. , (1193) cm^{-1} for Str (C-N) , (1508) cm^{-1} (C=C) olefinic Str ,

Figure (1):FTIR of the compound.



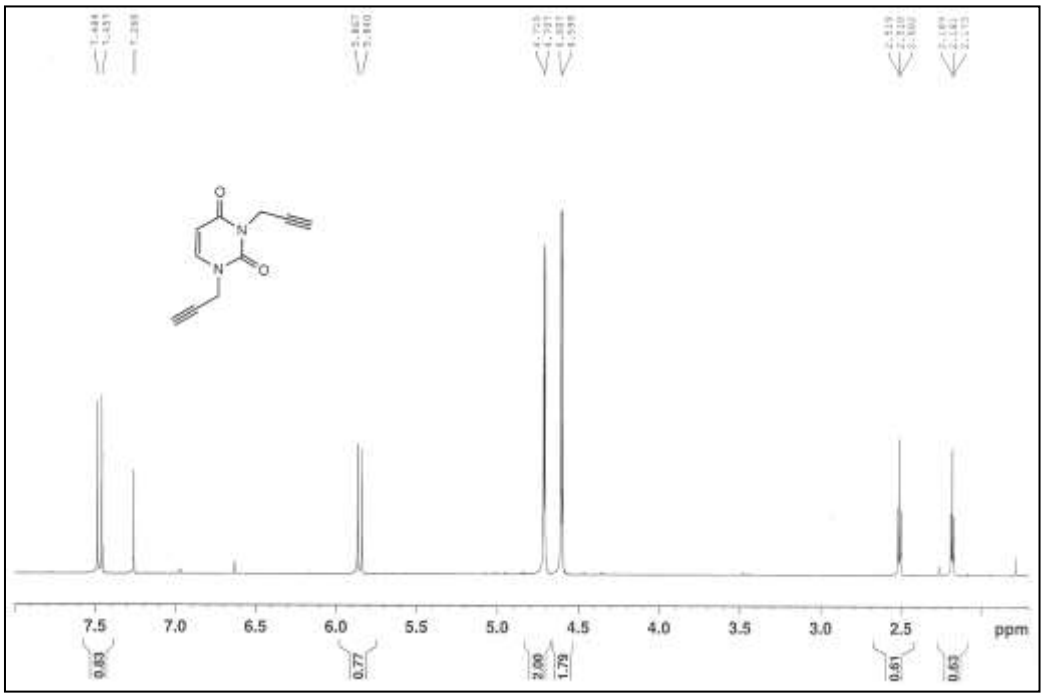
The CHN elemental analysis of the compound: C10H8N2O2
C % : 63,79, H% : 4.23 , N% : 14,88 (found)

Melting point :(291-293) ^{0}C

The yield is 82% .

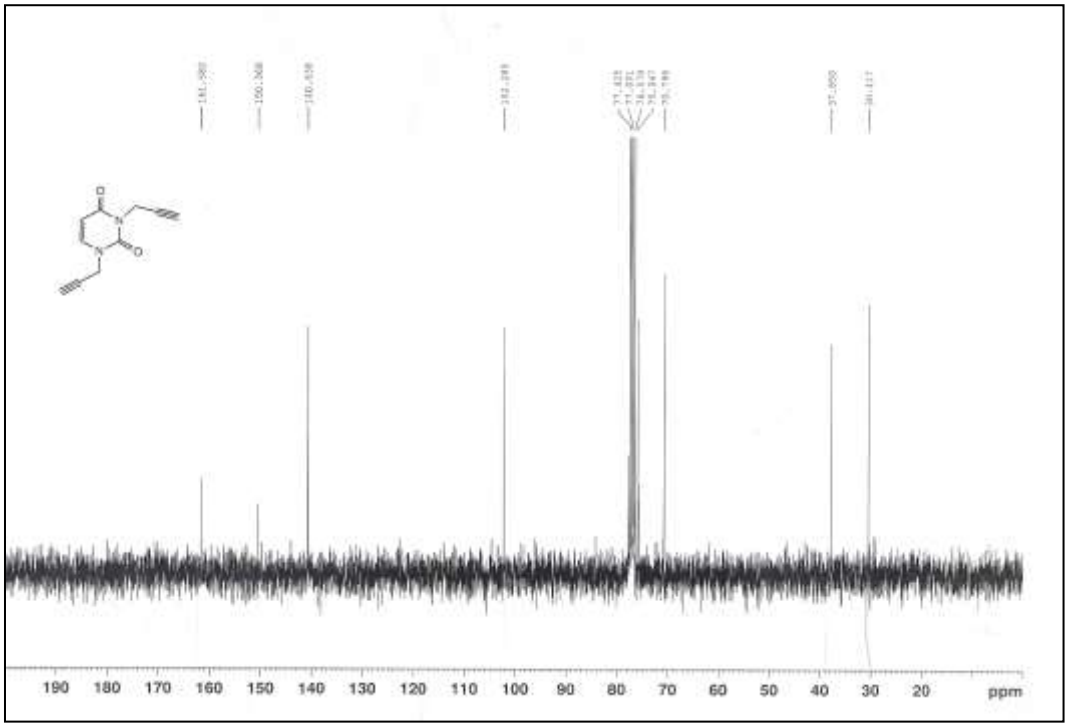
The ^{1}HNMR spectrum in(Fig 2) of the compound showed H- actlyene at δ 2.2 ppm and δ 2.5 ppm , H aliphatic at δ 4.5 ppm , δ 4.7 ppm and we showed H- ethylene near carbonyl group at δ 5.9 ppm and H – ethylene near amine group at δ 7.5 ppm .

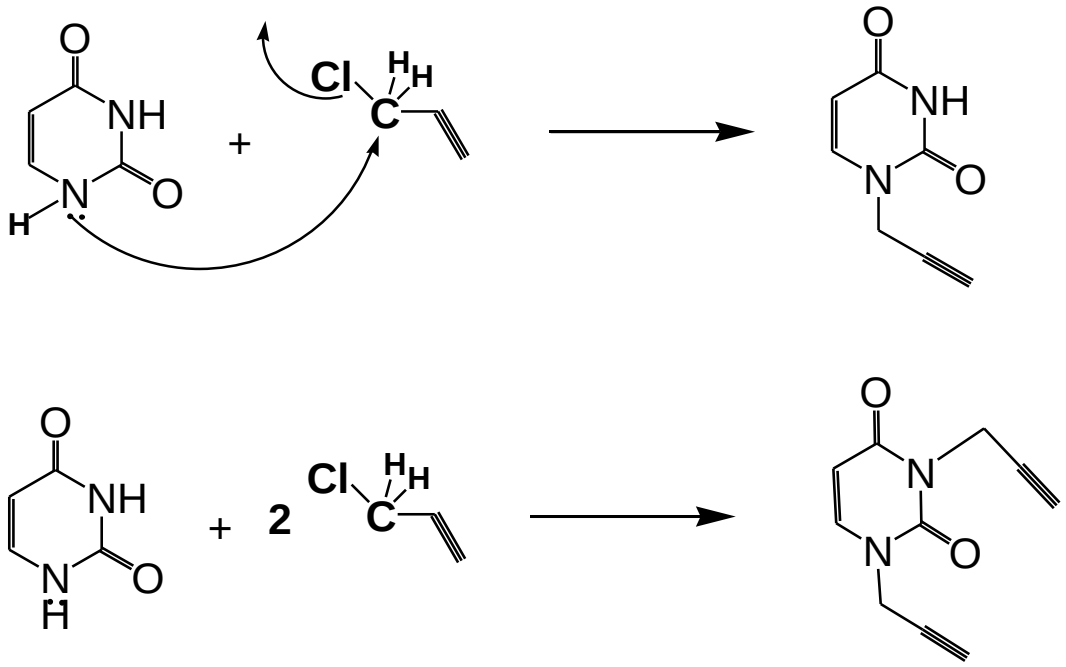
Figure(2): HNMR of the compound.



The CMR spectrum in Fig 3 of the compound showed C-aliphatic at 30 and 39 ppm , C- acetylene at 70 and 73 ppm . C – ethylene near carbonyl group at 102 ppm, C- ethylene near mine group at 141ppm and C- carbonyl between two amine group at 151 ppm and 162 ppm for C- carbonyl between amine group and ethylene group.

Figure(3): CMR of the compound.





Alkaline phosphatase activity color metric determination of alkaline phosphatase activity according to following reaction:

$$\text{phenyl phosphate} \xrightarrow[P^H 10]{\text{alkaline phosphatase}} \text{Phenol + Phosphate}$$

The liberated phenol is measured in the presence of 4- amino anti pyrine and potassium ferric amide the presence of sodium arsenate in the reagent stops the enzymatic reaction.

Determination of the type of inhibition:- was carried out using different concentration of substrate to get the Michaelis-Menten plot for alkaline phosphatase activity in breast cancer patients. The experiment was repeated using the same substrate concentrations, while a fir concentration of the organic compound was used which was 1×10^{-2}M.

The effect of DMSO which was used as diluents , was determined by adding a quantity equivalent to the sample and all steps completed as in the method of determination of alkaline phosphatase activity.

Results and discussion:

The conversion of Uracil to 1,3-di(prop-2-ynyl)pyriimide-2,4(1H,3H)-dione was confirmed by FTIR from the intensity of the banded at (1193) cm^{-1} for Str (C-N)
And . (3271) cm^{-1} for (C-H) Str Alkyne which is an excellent indication of the formation of the desired compound in addition to the newly appearance of (C-H) aliphatic band at (2759) cm^{-1} from Fig. (1)

The heating effect utilized in microwave assisted organic transformations is due in the main. To dielectric polarization, although conduction loses con also be important particularly at higher temperatures whilst the polar is ability of molecule (determined by the Debye equation) in the sum of number of contributions only dipolar and inter facial polarization are important to heating effects associated with microwave irradiation.

It is particularly convenient that qualitatively, the larger dielectric constant, the greater coupling with microwave. Thus solvents such as water, methanol, DMF- elhylacetate, acetone, chloroform, acetic acid and dichloro methane are all heated when irradiated with microwaves[12]

Fig.(2) showed Michaelis-Menten plot for serum alkaline phosphatase. The values for V_{max} was (4.4) U/L and the Km value was (2.0×10^{-2} M)

Fig.(3) showed that 1-(prop-2 ynyl) + pyrimidine- 2,4 (1H, 3H). dione is anon computative inhibitor for serum alkaline phosphatas for breast cancer patients according to Linwearver-Burk plot with km value of 0.36×10^{-2}M and V_{max} for the unihibited enzymes is (10.0)U/L and (6.3) U/L for the inhibited enzyme.

No data in the literature was found a bout the inhibitory effect of the prepared compound on serum alkaline phosphatase activity, yet varieties of substances have the ability to reduce or eliminate the catalytic activity of specific enzymes[13].

From the results of the present study a conclusion could be drawn that is the microwave irradiation is a better way for the reactions in many ways. Also the compound 1-(prop-2-ynyl) pyrimidine-2,4 (1H,3H)- dione showed a non compatative inhibitory effect on alkaline phosphatase activity from breast cancer patients.

Fig.(4) : Michaelis-Menten polt for serum a lkaline phosphatase of Brest cancer patients.

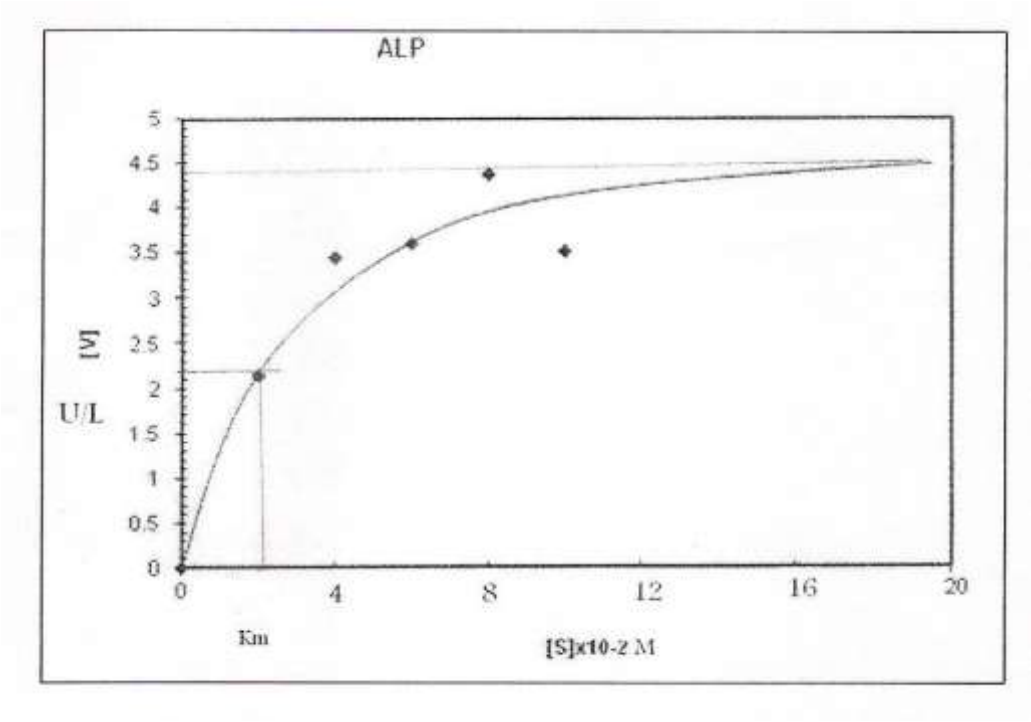
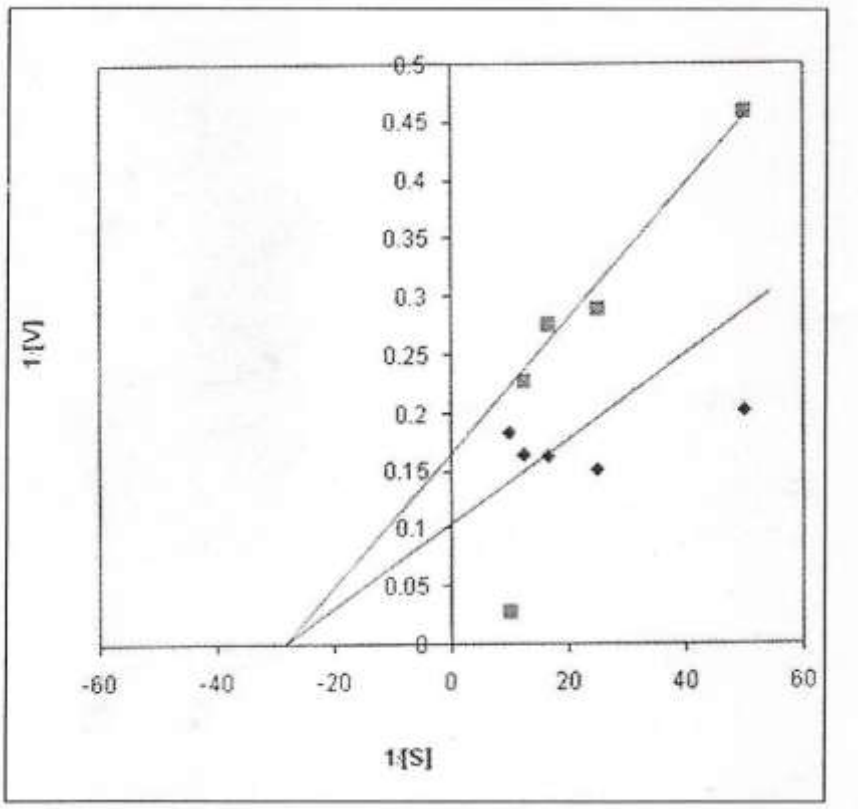


Fig.(5) Linweaver – Burk plot for alkaline phosphatase activity in the presence and the absence of the organic compound.



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