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The Synthesis of New Phenytoin Derivative and the Study of Its Inhibition Activity to Cyclooxygenase-2 (COX-2)

Usama H. Ramadhan

Department of Pharmaceutical Chemistry, College of Pharmacy, University of Basrah, Basrah, Iraq.

e-mail: usama_ramadhan@yahoo.com

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Abstract

Inflammation is the complex biological response to a protective attempt to remove the injurious stimuli, most strongly implicated are prostaglandins (PGs), leukotrienes (LTs), histamine, bradykinin, platelet activity factor (PAF) and interleukins. The Phenytoin is a drug used for the treatment of antiepileptic. Phenytoin acts to suppress the abnormal brain activity seen in seizure by reducing electrical conductance among brain cells by stabilizing the inactive state of voltage gated sodium channels, Phenytoin (5,5-diphenylimidazolidine-2,4-dione) were prepared from benzil and urea with sodium hydroxide in absolute ethanol. The derivative of phenytoin was prepared from a histidine acidifies with hydrochloric acid in absolute ethanol and phenytoin to give phenytoin-3-histdine IUPAC name is (S)-3-(2-amino-3-(1H-imidazol-4-yl) propanoyl)-5,5-diphenylimidazolidine-2,4-dione.

The identifications were performed by measuring the melting point, the Fourier transform infra red (FT-IR) spectra and elemental analysis (CHN). The Carrageenan induced inflammation model was used to determine the anti-inflammatory activity. The Inflammations were induced by sub-plantar injection of homogenous suspension of (1%) carrageenan in water. The Phenytoin derivative (with histidine) has significant ($p<0.001$) anti-inflammatory activity. The presence of imidazole ring in the compound (as in phenytoin and histidine) increased the activity. So it resembles some of the non-steroidal anti-inflammatory drugs in its structure. It was predicted that the phenytoin derivative will act as an anti-inflammatory agent, through the inhibition of biosynthesis of prostaglandins and inhibitor for cyclooxygenase-2 enzyme (COX-2).

Key words: Anti-inflammatory agent, Phenytoin, Phenytoin derivative, COX-2 inhibitor.

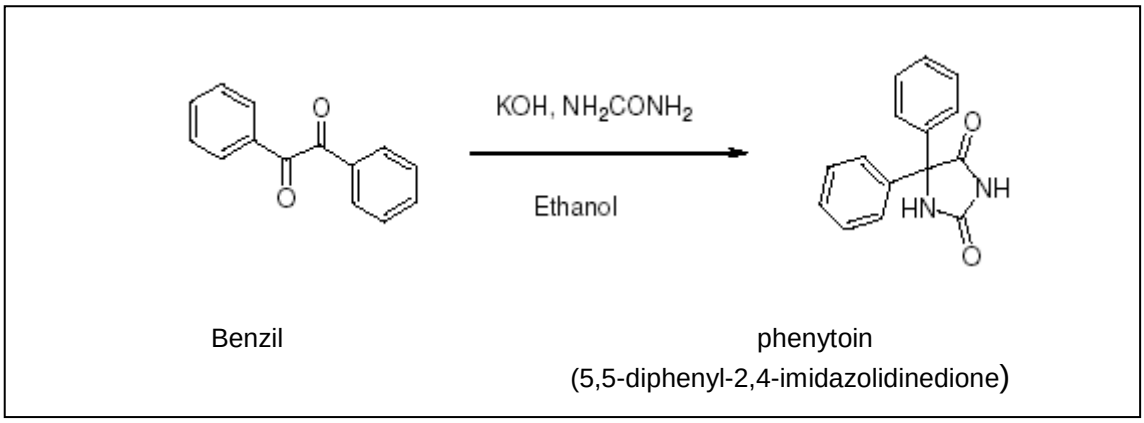
1. Introduction

Inflammation is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. Inflammation is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue. In the absence of inflammation, wounds and infections would never heal and progressive destruction of the tissue would compromise the survival of the organism. The chronic inflammation can also lead to a host of diseases, such as high fever, atherosclerosis and rheumatoid arthritis. So the inflammation is normally closely regulated by the body. The inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased synthesis of cyclooxygenases enzymes and cyclooxygenase-2 will appear into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue, most strongly implicated are prostaglandins (PGs), leukotrienes (LTs), histamine, bradykinin, platelet activity factor (PAF) and interleukins will synthesise according to the enzyme achievement. The prolonged inflammation known as chronic inflammation, leads to a progressive shift in the type of cells which are present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process [1-7]. The acute inflammation characterized by five cardinal signs redness, increased heat, swelling, pain and the loss of function. The redness and heat are due to increased blood flow at body core temperature to the inflamed site, swelling is caused by the accumulation of fluid, pain is due to the release of chemicals that stimulate nerve endings and the loss of function has multiple causes [3-7]. The phenytoin (5,5-diphenyl-2,4-imidazolidinedione) is a commonly used antiepileptic drug. The phenytoin acts to suppress the abnormal brain activity seen in seizure by reducing electrical conductance among brain cells by stabilizing the inactive state of voltage gated sodium channels. Aside of seizures, it is an option in the treatment of trigeminal neuralgia as well as certain cardiac arrhythmias [8]. The phenytoin therapy has many side effects like nystagmus, ataxia, slurred speech, mental confusion, dizziness, insomnia, headache, toxic hepatitis, liver damage and immunoglobulin abnormalities may occur [9, 10].

2. Materials and Methods

2.1 Preparation of Phenytoin

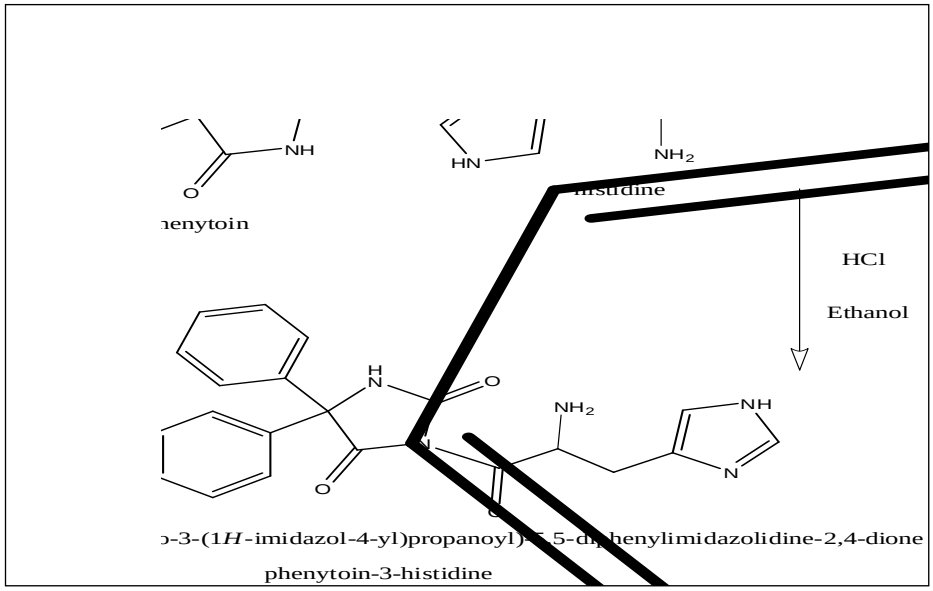
In (500 ml) round-bottomed flask (100 ml) of absolute ethanol, benzil (4.4 gm, 0.021 mol), urea (2.19 gm, 0.03 mol) and (2.3 gm) of sodium hydroxide pellets were refluxed for (6 hours). The flask was placed in an ice bath which resulted in the formation of a solid material which were filtered. To the filtrate, concentrated hydrochloric acid was added until the white solid material were obtained. The precipitate were recrystallised from (95%) ethanol yield 3.5 gm (66.3%), its melting point was found to be (293 °C) literature 293 °C [11-12]. The chemical equation of the reaction shows in Scheme 1.



Scheme 1. The chemical equation of phenytoin preparation.

2.2 Preparation of phenytoin-3-histidine

In (250 ml) round-bottomed flask (20 ml) of absolute ethanol, (1 gm, 0.004 mol) of phenytoin, (1.25 gm, 0.008 mol) of histidine and (3 drops) of concentrated HCl were refluxed for (3 hours). The white precipitate were formed, filtrated and washed by ethanol then by acetone, recrystallised with methanol the melting point was (248 °C) decomposition [13, 14] yield 1 gm (62.5%). The chemical equation of the reaction is shown in scheme (2).



Scheme 2. The preparation of phenytoin 3-histidine.

2.3 Pharmacology part

2.3.1 Animals

The Wister albino mice (20-25 gm), 2 months age, housed in temperature (21-25 °C) and had free access of foods and water. The mice were divided into groups of eight in each experiment (n=8, 4 males and 4 females).

2.3.2 Anti-inflammatory activity the carrageenan induced inflammation test

For the determination of the effect of our prepared drugs on acute inflammation, the carrageenan induced inflammation model was used. Inflammations were induced in left hand paw by sub-plantar injection (20 µl) in micro-syringe of homogenous suspension of (1%) carrageenan in water. Mice were orally given compound in a single dose (100 mg/kg) at (1.5 hour) before the induction of paw inflammation. Paws oedema size was measured (by using electronic digital micrometer) in every mouse. The control group was given diclofenac (25 mg/kg) orally and this group is considered positive control, while the blank group received distilled water (0.2 ml) orally too and this group is considered negative control [15-17].

2.4 Statistical analysis

Data were expressed as mean ± S.E. of eigth values and analysed by student't' test for differences among controls and treated groups. The values of $P < 0.001$ and $P<0.01$ were considered statistically significant [18].

3. Result and Discussion

The identification of compounds were performed by measuring the melting point, the FT-IR spectra and the elemental analysis (CHN). The melting point of phenytoin was (293 °C) literatures (293 °C), this indicates the purity of the prepared phenytoin. The FT-IR spectrum was shows in Figure 1.

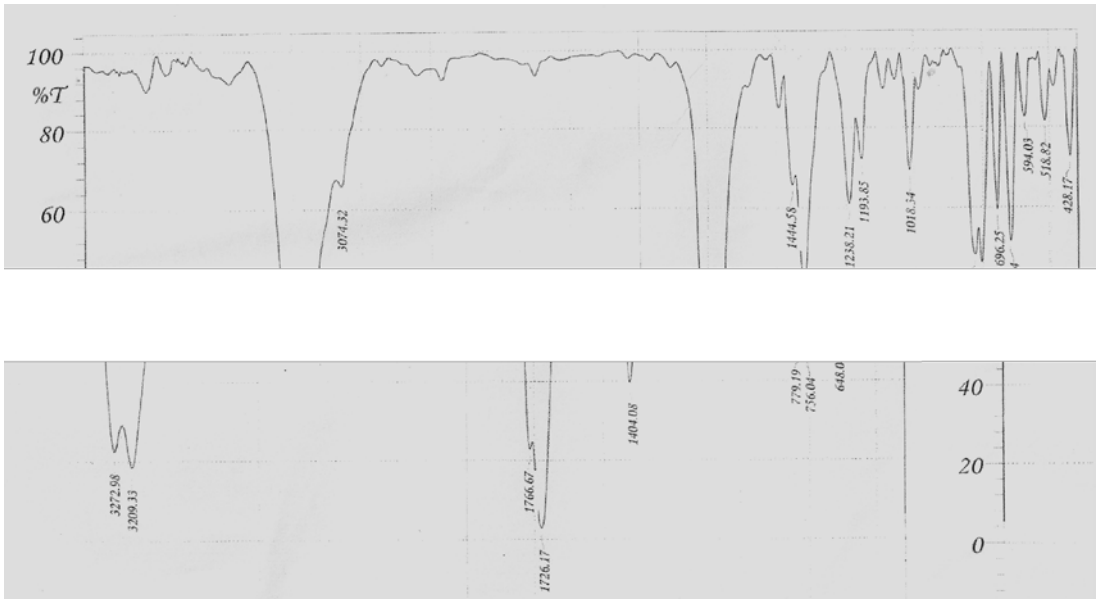


Figure 1. The FT-IR spectra of phenytoin.

The following bands were appeared in the IR spectra of phenytoin (756.04 and 779.19 cm^{-1}) for benzene ring, (1404.08-1444.58 cm^{-1}) bands of (C=C) bonds, (1726.17 and 1766.67 cm^{-1}) bands of (C=O) bonds, (3074.32 cm^{1}) band of (C-H) aromatic bonds and (3209.33-3272.98 cm^{-1}) bands of (N-H) bond.

The derivative phenytoin-3-histidine IUPAC name is (S)-3-(2-amino-3-(1H-

imidazol-4-yl) propanoyl)-5,5-diphenylimidazolidine-2,4-dione during the heating of the compound decomposed completely at (248 °C) which is not equal to the phenytoin melting point (293 °C) and not equal to the histidine melting point (282 °C), this indicated the formation of new compound (phenytoin derivative). The sharp decomposition range indicates the purity of this derivative. Also the resulting compound was not dissolved in ethanol or acetone or water, while phenytoin dissolved in ethanol. The IR spectrum of the derivative compound is shown in the Figure 2.

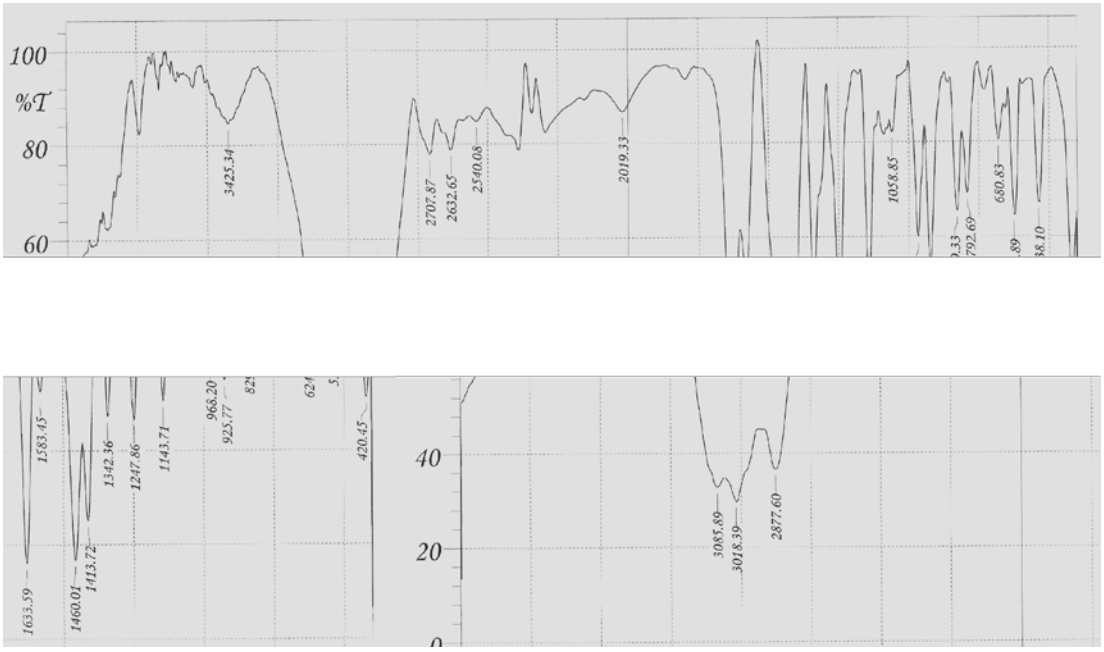


Figure 2. The FT-IR spectra of phenytoin-3-histidine.

The band of (N-H) at (3272.98 cm^{-1}) in phenytoin spectra was disappeared in the phenytoin-3-histidine spectra and appearance a new band at (3018.39 cm^{-1}) for (C-H) aromatic bond, appearance another new band at (2877.60 cm^{1}) for (C-H) aliphatic bond, this indicate the binding between phenytoin and histidine to form new compound which is phenytoin-3-histidine. In addition to the migration of carbonyl groups bands from (1766.67 and 1762.17 cm^{-1}) to (1633.59 and 1583.45 cm^{-1}) respectively. The appearance of a new band at (1460.01 cm^{-1}) of (C-N) bond and this indicates the formation of phenytoin-3-histidine compound (Scheme 1) [19, 20]. The elemental analyses were performed to the compounds to confirm their basic chemical compositions and the results were presented in Table 1. The measured percentages of CHN analysis shows a reasonable good agreement with calculated results.

Table 1. The elemental analysis of the compounds.

Compounds	Molecular formula	M.wt	Elemental analysis %			
				C	H	N
Phenytoin	$C_{15}H_{12}N_2O_2$	252.27	Calculated	71.42	4.79	11.10
			Found	70.93	4.21	11.81
Phenytoin-3-histidine	$C_{21}H_{19}N_5O_3$	389.41	Calculated	64.77	4.92	17.98
			Found	64.54	4.23	18.11

The results of carragenan induced inflammation tests on mice shown in Table 2 and Figure 3 and 4. The new compound was more active than diclofenac which used as standard drugs, with high significant effects ($p < 0.001$) at 1.5 and 3 hour.

Table 2. The Changes of paw size in carrageenan induced inflammation tests.

Compounds	**Changes of paw size in mm x 10^{-3}**		
	0 h	**1.5 h**	**3 h**
Blank	0.05±0.01	1.24±0.2	1.125±0.12
Diclofenac	0.01±0.003	0.82±0.14*	0.585±0.05*
Phenytoin	0.02±0.005	1.05±0.08	0.93±0.1
Phenytoin-3-histidine	0.0±0.0	0.384±0.03**	0.224±0.04**

*=p<0.01; **=p<0.001

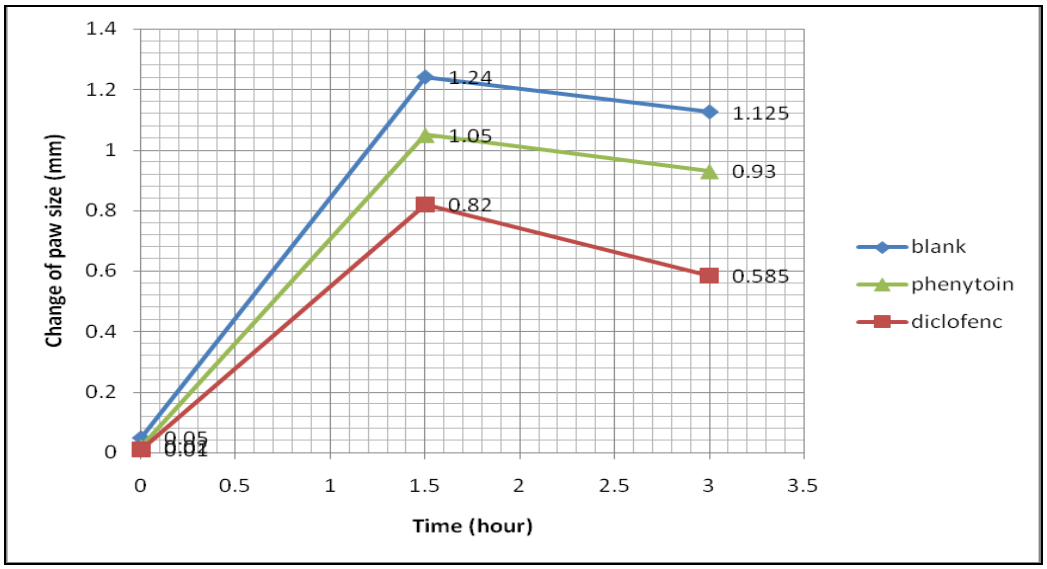


Figure 3. The results of anti-inflammatory activity of phenytoin.

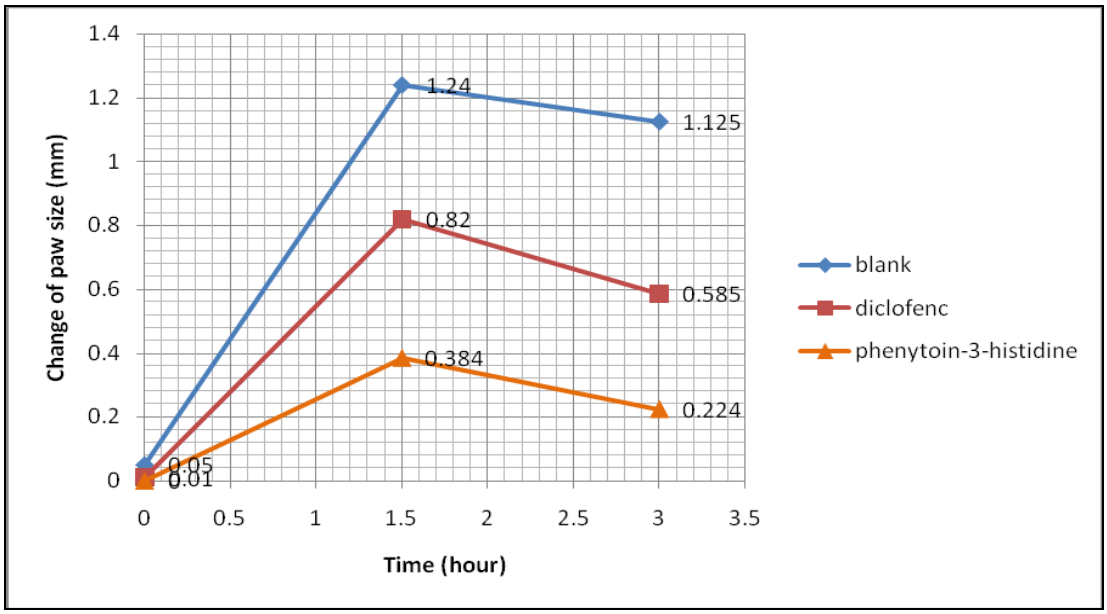
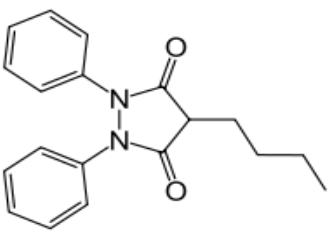
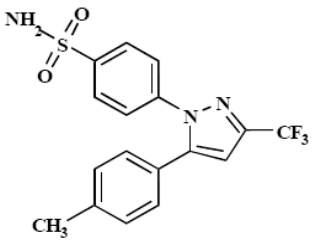


Figure 4. The results of anti-inflammatory activity of phenytoin-3-histidine.

The histidine has nontoxic effect because it is an amino acid compound and contains the imidazole ring that increases the anti-inflammatory activity. The phenytoin also contains an imidazole ring, it also resembles the structure of the non-steroidal anti-inflammatory drugs, it resembles the salicylate derivatives by containing (C=O) group and phenyl rings, resembles the para-aminophenol derivatives and propionic acid derivatives. The heteroaryl acetic acid derivatives and enolic acid derivatives are containing aromatic rings, heterocyclic ring and (C=O) group. Phenytoin most closely related in its structure to pyrazolon derivatives like (phenylbutazon) and tricyclic series like (celecoxib) [21, 22] may inhibit the COX-2 enzyme by irreversible binding with active site of COX-2 enzyme, subsequently the embarrassment of prostaglandins synthesis is followed by the inhibition of the inflammation. So the new compound can be used instead of tricyclic compounds because it's active compound and less difficult to prepare.



Phenylbutazon



celecoxib

phenytoin-3-histidine

So, as it resembles the non-steroidal anti-inflammatory drugs in its structure, so it was predicted that phenytoin derivative will act as an anti-inflammatory agent by the same mechanism of the non-steroidal anti-inflammatory drugs, through the inhibition of the biosynthesis of prostaglandins. It inhibits the enzyme cyclooxygenase, which converts the arachidonic acid to prostaglandins [23]. Also the number of

carbon atoms for derivatives (C21) and for arachidonic acid (C20) makes it good predecessor in its place of substrate.

4. Conclusions

The NH-group of the phenytoin can react with the compound that contains carboxyl group to the replacement of the hydrogen atom to give substitute phenytoin. The phenytoin derivative with histidine (phenytoin-3-histidine) has an anti-inflammatory activity more than diclofenac. The presence of two imidazole rings in the compound (as in phenytoin and histidine) increased the activity of compound against inflammation, the new compound has ability to binding with an active site of COX-2 enzyme and inhibited it and can be used as an alternative of anti-inflammatory drugs especially tricyclic compounds.

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تحضير مشتق جديد للفنيتوين ودراسة فعاليته المثبطة لأنزيم السايكلواوكسجينيز-2 (COX-2).

أسامة حامد رمضان
فرع الكيمياء الصيدلانية، كلية الصيدلة، جامعة البصرة، البصرة، العراق

الخلاصة

يعرف الالتهاب على انه استجابة بيولوجية معقدة لمحاولة الخلايا حماية نفسها وإزالة المحفزات الخطرة مثل المواد الكيميائية والبكتيريا، اغلب التعقيدات الخطرة في الالتهابات تشمل زيادة تركيز البروستاكلاندين و اليكوتراين و الهستامين و محفز الصفيحات الدموية و الانترليولين. مركب الفنيتوين هو عبارة عن دواء يستخدم لمعالجة الصرع. الفنيتوين يعمل على تثبيط فعاليات الدماغ غير الطبيعية في الصرع عن طريق اختزال تداور الشحنات الكهربائية في خلايا الدماغ بوساطة استقرارية فولتية بوابات قنوات الصوديو م. الفنيتوين (5،5- ثنائي فنيل اميدازولدين- 2،4- ثنائي الكيتون) الذي حضر من تفاعل البنزل مع اليوريا مع هيدروكسيد الصوديوم في الايثانول المطلق. اما مشتقه فقد حضر من تفاعل الهستدين المحمض بحامض الهيدروكلوريك في الايثانول المطلق مع الفنيتوين ليعطي فنيتوين-3-هستدين الاسم العلمي
(S)-3-(2-amino-3-(1H-imidazol-4-yl)propanoyl)-5,5-diphenylimidazolidine-2,4-dione.

تم تشخيص المركبات المحضرة بوساطة قياس درجة الانصهار و طيف الاشعة تحت الحمراء و تحليل العناصر الدقيق. كما استخدم اختبار الالتهاب المحدث بوساطة الكاراجينان لتحديد الفعالية ضد الالتهابات، عن طريق حقن محلول متجانس من الكاراجينان 1% في الماء. اظهر مشتق الفنيتوين فعالية معنوية ضد الالتهاب ($p<0.001$). ان وجود مجموعة الاميدازول في المركب ادت الى ظهور الفعالية ضد الالتهاب، كما ان التركيب الكيميائي للمركب الجديد اصبح مشابها الى الادوية المضادة للالتهابات غير الستيرويدية التي تثبط السايكلواوكسيجينيز- 2، وبالتالي من المحتمل انه يعمل على تثبيط التحضير الحيوي للبروستاكلاندين عن طريق تثبيط انزيم السايكلواوكسيجينيز-2.