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Synthesis, Characterization and Preliminary Anti-inflammatory Evaluation of New Fenoprofen Hydrazone Derivatives

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Abstract

Non-steroidal anti-inflammatory drugs (NSAIDs) have become important as an analgesic, antipyretic and anti-inflammatory medications throughout the world. In 2006, NSAIDs market in the US alone was valued at $3.2 billion, and it was probably to reach $4.6 billion by 2013. NSAID use can cause a range of serious adverse effects including gastrointestinal complications, cardiovascular problems, renal failure and hypersensitivity reactions. In order to reduce the side effects and improve the anti-inflammatory activity, new derivatives of Fenoprofen were synthesized which contain hydrazones moiety. The compounds then evaluated for their anti-inflammatory activity by means of egg white induced paw edema method. All the synthesized target compounds were characterized by FT-IR spectroscopy, ^{1}HNMR analysis. The synthesis of the target compounds(H1-H4) was accomplished by multistep reaction procedures. The synthesized target compounds showed an activity in reducing paw edema thickness and their anti-inflammatory effect was comparable to that of the standard (Fenoprofen) except for compound H3 which shows anti-inflammatory activity higher than Fenoprofen.

Keywords: Fenoprofen hydrozone derivatives, Anti-inflammatory, Paw edema method.

تصنيع وتشخيص وتقييم اولي مضاد للالتهاب لمشتقات جديدة للفينوبروفين هايدروزون

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الخلاصة

مضادات الالتهاب غير الستيروئيدية هي واحدة من أكثر مجموعات الأدوية المستخدمة على نطاق واسع والتي تستخدم لعلاج الالتهاب والألم. وهي مسؤولة عن ما يقرب من ٥-١٠٪ من جميع الأدوية الموصوفة كل عام. يمكن أن يسبب استخدام مضادات الالتهاب غير الستيروئيدية مجموعة من الآثار الضارة الخطيرة بما في ذلك مضاعفات الجهاز الهضمي ومشاكل القلب والأوعية الدموية والفشل الكلوي وتفاعلات فرط الحساسية. من أجل تقليل الآثار الجانبية وتحسين النشاط المضاد للالتهابات ، تم تصنيع مشتقات جديدة من فينوبروفين تحتوي على جزء من الهيدرازون وتم تقييم فعاليتها المضادة للالتهاب باستخدام طريقة وذمة المخلب المستحث ببياض البيض. جميع المركبات المحضرة شخصت بمطياف الاشعة تحت الحمراء وجهاز الرنين النووي المغناطيسي. تم تحضير المركبات باتباع طرق تفاعلات متعددة الخطوات, حيث وجدت المركبات المحضرة ان لها فعالية في تقليل الذي اظهر تأثيرا مضادا H3 ورم المخلب وان تأثيرها كان مشابه للفينوبروفين عدا المركب للالتهاب اعلى من الفينوبروفين.

الكلمات المفتاحية: مشتقات الفينوبروفين هايدروزون, مضادة للالتهاب, طريقة وذمة المخلب .

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most widely used groups of medication used to treat inflammation and pain. They are accountable for nearly 5-10% of all medicines prescribes each year [(1)]. NSAIDs activity is caused by inhibition of cyclooxygenases (COX-1) and (COX-2) enzymes, which are contributed in prostaglandin synthesis, causing their analgesic, anti-inflammatory, and antipyretic effects [(2)]. This fall in prostaglandin synthesis is related to the occurrence of several undesirable effects accompanied with the use of NSAIDs, particularly gastrointestinal (GI) irritation and ulceration [(3)]. NSAIDs can result in GI tract damages in two different ways: the acidic moiety irritates the gastric mucosa directly, furthermore, inhibition of COX-I that reduce the levels of protecting prostaglandins [(4)]. NSAIDs carboxylic acid group can be substituted with other groups while these agents still exert a powerful anti-inflammatory activity [(5)]. Fenoprofen, 2-(3-phenoxy phenyl) propionic acid, or 2-methyl-2-(3-phenoxy benzene) acetic acid (Figure1) is a non-steroidal anti-inflammatory, analgesic and antipyretic drug belonging to groups of NSAIDs commonly referred to as 2-aryl propionic acids [(6)]. Like other NSAIDs, Fenoprofen is a cyclo-oxygenase (Cox-1 and Cox-2) inhibitor that blocks the formation of prostaglandins that are important in pain and inflammatory pathways. Current indications include mild-to-moderate acute pain as well as chronic joint pain due to osteoarthritis and rheumatoid arthritis. Fenoprofen is generally well-tolerated, but it has the side effects of NSAIDs especially gastrointestinal upset and ulceration [(7)].

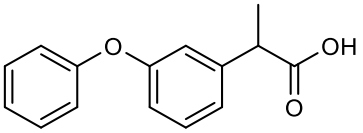

Figure 1. Chemical structure of Fenoprofen

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The class of organic compounds which have the structure R1R2C=NNH2 called hydrazones. Hydrazones can be synthesized through the reaction of hydrazide or hydrazine with aldehydes and ketones [8]. Hydrazones have shown that they exert a wide variety of biological activities antimicrobial, analgesic, anti-inflammatory, antidepressant, anticancer, antitubercular, and antifungal [9]. Some of the hydrazone derivatives were developed to overcome gastrointestinal disruption and toxicity [10]. In an attempt to synthesize new Fenoprofen analogs with improved anti-inflammatory activity and more selective toward the COX-2 enzyme which results in decreasing gastrointestinal side effects, new Fenoprofen analogs containing hydrazone moiety were synthesized and assesses their anti-inflammatory activity.

Experimental*:*

Fenoprofen was bought from Hyper chem. Company (China). Solvent and other reagents that used through reaction were bought from commercial sources. The purity of products and monitoring of the reactions were done by thin-layer chromatography TLC(GF254, merk- Germany) under UV light (254nm) two solvent system was used A:toluene: ethyl acetate: ethanol (3:2:1) and B: ethyl acetate: petroleum ether(1:1). Melting points were incorrected and detected by using Stuart SMP3 melting point apparatus in open capillary tubes.

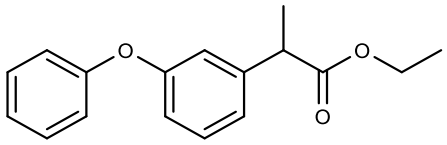
IR spectra were done by thin-film technique (υ, cm-1) in the university of Baghdad/college of pharmacy, (Shimadzu FTIR spectrophotometer, Japan) [11,12]. ^{1}HNMR were done in the university of Tehran/central laboratory using Brucker ultra shield model 300 MHz using DMSO as a solvent [13,14].

Synthesis of Ethyl 2-(3-phenoxyphenyl) propanoate (compound A)

Fenoprofen (R, S racemic mixture) (9.9 mmol, 2.4g) was dissolved in ethanol (20ml) and cooled to 0ºC. Thionyl chloride (2.15 ml, 29.7 mmol) was added slowly over 15 minutes, the mixture then stirred for 30 min. until it reaches room temperature. After that, it refluxed for 24 hr. at (80Cº) with stirring. Followed by stirring overnight at room temperature. At the end of the reaction (checked by TLC (B)), the solution allows to reach room temperature, after that, approximately 50 mL of distilled water was added to the solution. Saturated sodium bicarbonate solution (10% w/v) was then added for the neutralization of excess of acid. The product was extracted by dichloromethane (DCM) 20 ml for 3 times, then evaporate DCM under reduced pressure to get oil [15].

yield = 85%, R_f = 0.92(B)

IR (*v* cm^{-1})**:** 3066: Aromatic (C-H) str., 2981: (C-H) asym. str. of CH_3 and CH_2, 2873: (C-H) sym. str. of CH_3 and CH_2, 1732(C=O) str. of ester,1180.44(C-O-C) str. Of ester. **^{1}H NMR**: 1.12 (3H, t, -CH_2<u>CH_3</u> of ester), 1.37(3H, d, -C<u>H</u>$_3$), 3.76(1H, q, - C<u>H</u>), 4.06(3H, b.s. -C<u>H</u>$_2$$CH_3$ of ester), 6.89-7.43 (9H,m, Ar-<u>H</u>).

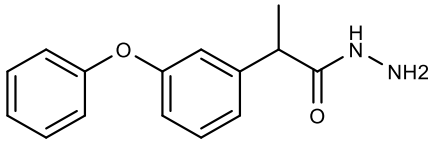


Synthesis of 2-(3-phenoxyphenyl) propanehydrazide (compound B)

Compound (A) (10 mmol,2.42 g) dissolved in 10 ml ethanol, followed by addition of the hydrazine hydrate (80%) (3 ml,60 mmol), the reaction mixture was refluxed for 8 at (80Cº) hours, then left overnight with continuous stirring, then solvent evaporation under reduced pressure to give an oily product. The product then was washed with diethyl ether five times [16].

yield = 86%, R_f= 0.46(B)

IR (v cm^{-1}): 3317,3217 (NH) str. Of hydrazide NH2, 3035 Aromatic (C-H) str.,1612 (c=o) str. Of carbonyl amide, 1566(N-H) bending. **^{1}H NMR**: 1.3(3H, d, -C<u>H</u>3) 3.5(1H, q, - C<u>H</u>), 4.2(2H,s. NH-N<u>H</u>2), 6.80-7.39(9H,m,Ar-<u>H</u>), 9.18 (1H, s, <u>NH</u>-NH2).

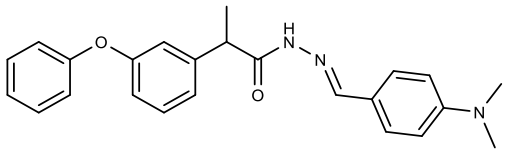


Synthesis of final target compounds **(Compounds H1-H4):**

Ethanolic solution (5 ml) of one of the following aldehydes: 4-(dimethylamino) benzaldehyde (2mmol,0.298g), 4-nitro benzaldehyde (2mmol, 0.302g), 4-chlorobenzaldehyde (2mmol,0.281g) and 4-bromobenzaldehyde (2mmol, 0.37) were prepared, followed by the addition of three drops of glacial acetic acid and the solution was stirred for 30 min. Compound B (2mmol, 0.512 g) was dissolved in 10 ml of absolute ethanol then added to the solution above, then refluxed for 4 hr. at (80 Cº), followed by stirring overnight at room temperature. The precipitate which is form was filtered and recrystallized from ethanol [17].

(H1) N'-(4-(dimethylamino) benzylidene)-2-(3-phenoxyphenyl) propanehydrazide

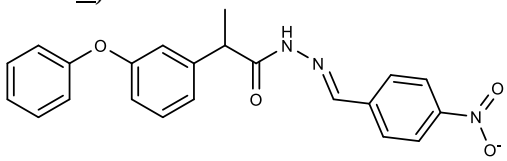
Yellowish powder, **Yield:** 82%, **M.P.:** (158-160°C) **R_f**=0.82(A), IR (*v* cm^{-1})**:** 3163 (NH) str. of hydrazone, 3078Aromatic (C-H) str., 2943: (C-H) asym. str. of CH_3 and CH, 2893,2850: (C-H) sym. str. of CH_3 and CH, 1662: (C=O) str. of amidic.1577: (C=N) str**. ^{1}H NMR:** 1.39 (3H, d, -C<u>H</u>$_3$),2.89(6H, s, -N-2C<u>H</u>$_3$), 3.63(1H, q, -C<u>H</u>), 6.68-7.48 (13H,m, Ar-<u>H</u>), 8.04(1H, s, N=C 11.2(1H, s, -CO-N<u>H</u>).



(H2) N'-(4-Nitrobenzylidene)-2-(3 phenoxy phenyl) propanehydrazide:

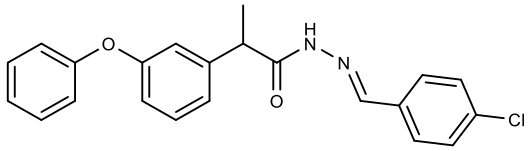
White powder, **Yield:** 80%, **M.P.:** (157-159°C), **Rf**=0.84(A), **IR** (*v* cm-1)**:**3178 (NH) str. Of hydrazone, 3066Aromatic (C-H) str., 2951: (C-H) asymm. str. of CH_3 and CH, 2893,2854: (C-H) symm. str. of CH_3 and CH, 1666: (C=O) str. of amidic.1581: (C=N) str1519 a sym. (NO_2) str, 1338: sym. str. of (NO_2).

^{1}H NMR: 1.39 (3H, d, -C<u>H</u>$_3$), (3.72, q, -C<u>H</u>), 6.83-7.9 (13H, m, Ar-<u>H</u>), 8.3(1H, s, N=C<u>H</u>), 11.8(1H, 2s, -CO-N<u>H</u>).



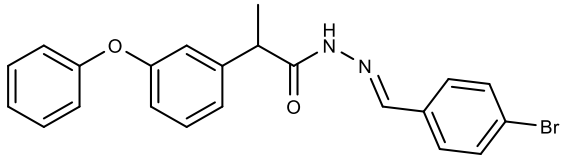
(H3) N'-(4-chlorobenzylidene)-2-(3-phenoxyphenyl) propanehydrazide:

Off-white powder, **Yield**: 75%, **M.P.:** (155-156°C) Rf=0.84(A), **IR** (*v* cm-1)**:** 3178 (NH) str. of hydrazone, 3066Aromatic (C-H) str., 2947: (C-H) asym. str. of CH_3 and CH, 2904,2858: (C-H) sym. str. of CH_3 and CH, 1662: (C=O) str. Of amide.1577: (C=N) str**.. ^{1}H NMR:** 1.37 (3H, d, -C<u>H</u>3),3.68 (1H, q, -C<u>H</u>), 6.81-7.7 (13H,m, Ar-<u>H</u>), 8.18(1H, s, N=C<u>H</u>), 11.36,11.6(1H, 2s, -CO-N<u>H</u>).



(H4) N'-(4-bromobenzylidene)-2-(3-phenoxyphenyl) propanehydrazide:

White powder, **Yield:** 70%, **M.P.**: (162-164°C) **R_f**=0.78(A), **IR** (*v* cm^{-1})**:** 3178 (NH) str. of hydrazone, 3066Aromatic (C-H) str., 2947: (C-H) asym. str. of CH_3 and CH, 2900,2873: (C-H) symm. str. of CH_3 and CH_2, 1662: (C=O) str. of amidic.1577: (C=N)str**.. ^{1}H NMR:** 1.37 (3H d, -C<u>H</u>$_3$),3.68 (1H, q, -C<u>H</u>), 6.81-7.84 (13H, m, Ar-<u>H</u>), 8.17(1H, s, N=C<u>H</u>), , 11.6(1H,2s, -CO-N<u>H</u>).



Evaluation of the anti-inflammatory activity

The anti-inflammatory effects of the synthesized products (H1-H4) were evaluated using an egg-white induced paw edema model. Measuring the reduction of paw thickness as the basis for the assessment of the anti-inflammatory activity of the anticipated compounds [18].

Albino rats of both sexes which have the weight of (190 ± 10 g) were delivered by the animal house of the National Center for Drug Control and Research, were accommodated in the animal house of the College of Pharmacy, University of Baghdad, under standardized environments for 10 days for adaptation. Animals were fed commercial chaw and had free access to water. Animals were divided into six groups (each group was containing 6 rats):

Group 1: which consists of six rats that worked as a control group; and gives the solvent (dimethyl sulfoxide) [19].

Group 2: consists of six rats treated with Fenoprofen, which is the reference(standard) drug in a dose of 20 mg/kg [20], dissolved in the (DMSO).

Group 3-6: consist of six rats/group treated with the target compounds H1-H4, and given the dose that is equivalent by weight to 20 mg/kg of Fenoprofen and also dissolved in the (DMSO). The procedure was done by the administration of an intra-peritoneal (i.p.) injection of each of the Fenoprofen, control and final products (H1-H4), individually to the six animal groups. Thirty minutes after that subcutaneous injection (S.C.) of 0.05 mL of undiluted egg-white was injected into the plantar side of the left hind paw of the rats of every group. Vernia was used to measure paw thickness at six-time periods (0, 30, 60, 120, 180, and 240 min.), where zero time was the time at which the products, standard, and control were administered intraperitoneally [21].

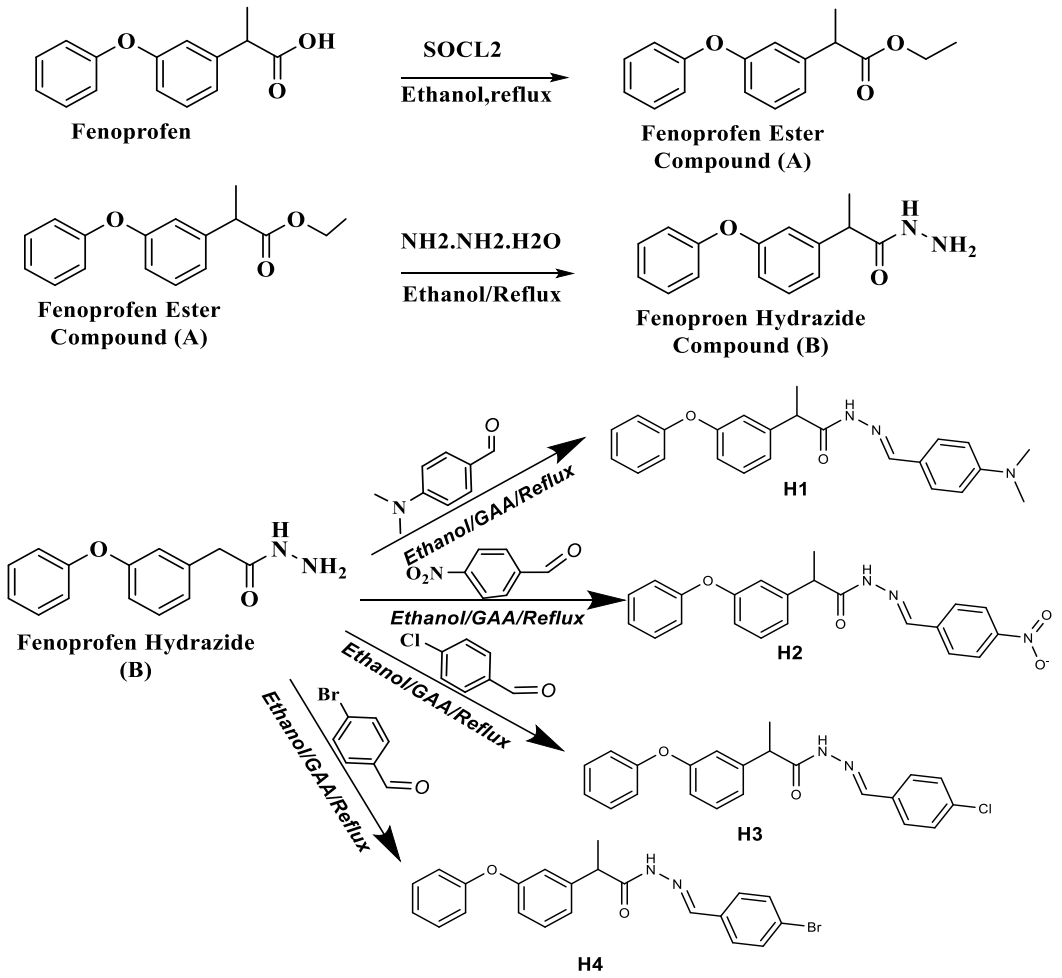
Statistical analysis

Data were reported using mean ± SEM. student T-test (Two sample assuming equal variances) then used to calculate statistical significance between means, while ANOVA test (Two factors without Replication) was used to compare between different groups. P-value < 0.05 considered statistically significant.

Result and Discussion

Chemistry

The pathway of synthesis for target Fenoprofen hydrazones derivatives(H1-H4) was illustrated below(scheme1). Fenoprofen ethyl ester (compound A) was synthesized by reaction of Fenoprofen with ethanol in presence of thionyl chloride. Fenoprofen hydrazide (compound B) was formed by refluxing of compound (A) with hydrazine hydrate in ethanol. The synthesis of final target compounds was involved the reaction of Fenoprofen hydrazide with different aldehydes in ethanol using glacial acetic acid as a catalyst



Scheme 1. Multi-step reactions for the synthesis of target compounds (H1-H4)

Comparative analysis

At time 0 and after 30 minutes, there are no significant differences between all groups. However, at time 60,120,180 and 240, there is a significant reduction of paw thickness for both Fenoprofen and target compounds (H1,H2,H3,H4) compared to control. Compounds (H1, H2, H4) show comparable activity to the standard while compound H3 demonstrate significantly higher activity than standard (Fenoprofen) at time 60,120 and 180 which indicate rapid onset and short duration. as shown in Table (1) and Figure (2) below:

Table 1. Effect of dimethyl sulfoxide (control), Fenoprofen(standard) and target compounds (H1-H4) on egg-white induced paw edema

COMPOUND	0	30	60	120	180	0
CONTROL	3.45±0.08	5.3±0.19	6.35±0.16	6.51±0.21	5.97±0.12	5.77±0.1
STANDARD	3.44±0.08	5.14±0.2	5.47±0.18*[a]	5.37±0.25*[a]	5.03±0.23*	4.79±0.24*[a]
H1	3.43±0.06	5.06±0.21	5.35±0.19*[a]	5.30±0.12*[a]	5.06±0.11*	4.55±0.17*[a]
H2	3.33±0.09	4.93±0.27	5.53±0.21*[a]	5±0.17*[a]	4.59±0.16*	4.63±0.15*[a]
H3	3.33±0.04	5.2±0.13	5.01±0.12*[b]	4.7±o.14*[b]	4.5±0.15*[b]	4.69±0.16*[a]
H4	3.43±0.07	4.9±0.14	5.79±0.07*[a]	5.37±0.14*	5.07±013*	4.7±0.19*[a]

#Non-identical superscripts (a, b) among different tested compounds are regarded significantly different ($p < 0.05$);*significantly different compared to control ($p < 0.05$). Data are expressed in mm paw thickness as mean ± SEM. n= number of rats. Time (0) is the time of i.p. injection of Fenoprofen, tested compounds and DMSO. Time (30) is the time of injection of egg white to induce edema.

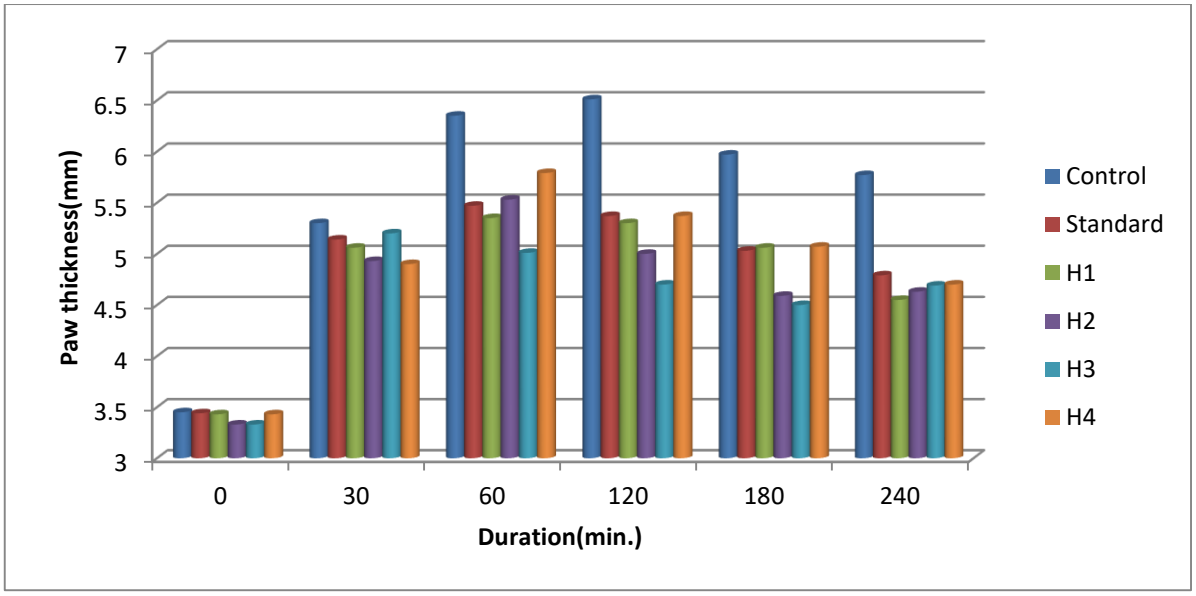


Figure 2. Effect of dimethyl sulfoxide, Fenoprofen and compounds (H1-H4) on egg-white induced paw edema in rats. Results are expressed as mean ± SEM (n = 6 for each group).

Conclusion

New Fenoprofen hydrazones were synthesized successfully. Their chemical structure was characterized using IR spectroscopy and ^{1}HNMR. The anti-inflammatory activity of the target compound (H1-H4) was evaluated using egg white induced edema method. All synthesized compounds show effect comparable to the standard (Fenoprofen), except compound H3 which shows effect superior to Fenoprofen in reducing paw edema in rats.

Acknowledgment

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