DOI: https://doi.org/10.31351/vol28iss1pp138-146

Synthesis, Characterization and Acute Anti-inflammatory Evaluation of New Mefenamic Acid Derivatives Having 4-Thiazolidinone Nucleus

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Abstract

Mefenamic acid (MA) is one of the non-steroidal anti-inflammatory drugs, it is widely used probably due to having both anti-inflammatory and analgesic activity, the main side effects of mefenamic acid include gastrointestinal tract (GIT) disturbance mainly diarrhea, peptic ulceration, and gastric bleeding. The analgesic effects of NSAIDs are probably linked to COX-2 inhibition, while COX-1 inhibition is the major cause of this classic adverse effects. Introduction of thiazolidinone may lead to the increase in the bulkiness leads to the preferential inhibition of COX-2 rather than COX-1 enzyme. The study aimed to synthesize derivatives of mefenamic acid with more potency and to decrease the drug's potential side effects, new series of 4-thiazolidinone derivatives of mefenamic acid were synthesized IVa-g. The synthetic procedures for target compounds and their intermediates are designed to be as follows: acylation of secondary amine of mefenamic acid by chloroacetylchloride to produce compound (I), then reaction between compound (I) and hydrazine hydrate to form hydrazine derivative of mefenamic acid (compound II). After that, Schiff base formation by addition of seven benzaldehyde derivatives and finally, cyclization in presence of thioglycolic acid to form 4-thiazolidinone heterocyclic ring. The characterization of the titled compounds has been established on the basis of their spectral FTIR, ^{1}HNMR data, and by measurements of their physical properties. In vivo acute anti-inflammatory effect of the synthesized compounds was evaluated in rats using egg-white induced edema model of inflammation. The tested compounds and the reference drug produced significant reduction of paw edema with respect to the effect of dimethyl sulfoxide 10%v/v (control group). Compound IVe showed more potent effect than mefenamic acid at 240-300 min, while at time 300 min, compounds IVa and IVd exhibit more potent anti-inflammatory effect than mefenamic acid (50mg/kg, i.p.) as they reduced paw edema significantly more than mefenamic acid at mentioned intervals ($p<0.05$) . On the other hand compound IVc exhibited lower anti-inflammatory effect.

Keywords: Mefenamic acid , 4-thiazolidinone, Anti-inflammatory

تصنيع وتشخيص والتقييم المضاد للالتهاب الحاد لمشتقات جديدة لحمض الميفينامك مع نواة ٤-ثيازوليدينون

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الخلاصة

ان حمض الميفيناميك واسع الاستخدام لخواصه المسكنة والمضادة للالتهاب وهو احد افراد الادوية المضادة للالتهاب الغير ستيرويدية. من اهم الاثار الجانبية لاستخدام حمض الميفيناميك هي اضظرابات الجهاز الهضمي وبشكل رئيسي الاسهال والقرحة الهضمية ونزيف المعدة. حيث ان التاثير المضاد للالتهاب للادوية المضادة للالتهاب الغير ستيرويدية ناتج عن كبح انزيم كوكس-٢ بينما الاثار الجانبية هي نتاج عن تثبيط انزيم كوكس-١. ان اضافة مجموعة الثيازوليدينون ربما يؤدي الى زيادة الحجم و بالتالي ترجيح تثبيط انزيم كوكس-٢ على كوكس-١. الهدف من هذه الدراسة هو تصنيع مشتقات لحمض الميفيناميك بفعالية اعلى و اعراض جانبية اقل. حيث تم تصنيع مشتقات ذات نواة ٤-ثيازوليدينون لحمض الميفيناميك، ان طرق تصنيع المركبات النهائية والمركبات الوسطية تم تصميمها كالتالي:

اضافة مجموعة اسيل الى الامين الثنائية لحمض الميفيناميك بمفاعلته مع مركب الكلورواستيل كلورايد لانتاج المركب(I)، ثم مفاعلة المركب (I) مع مركب هيدرازين هيدريت لتكوين المركب الوسطي الثاني،بعد ذلك تم تصنيع قواعد شف باضافة سبعة مشتقات للبنزلديهايد و اخيرا تكوين المركب الحلقي بوجود مركب حمض الثيوجليكولك لتصنيع حلقة ٤- ثيازوليدينون.

تم تشخيص المركبات المحضرة باستعمال مطياف الاشعة تحت الحمراء وتحليل االرنين النووي المغناطيسي للبروتون و كذلك قياس الخصائص الفيزيائية للمركبات.

تم تقييم الفعالية المضادة للالتهاب للمركبات المصنعة في الجسم الحي باستعمال نموذج الالتهاب المحدث بواسطة حقن بياض البيض تحت الجلد في الجرذان. جميع المركبات المحضرة اضافة الى حمض الميفيناميك ابدوا فعالية ملحوظة في تقليل التورم المحدث في كف الجرذ بالمقارنة مع تاثير المذيب المستعمل (ثنائي مثيل اوكسيد الكبريت ١٠%)

ابدى المركبIVe فعالية اقوى من حمض الميفيناميك في الدقيقة ٢٤٠ والدقيقة ٣٠٠ و كذلك المركبات IV a,d ابدت فعالية اقوى في الدقيقة 300 مقارنة بحمض الميفيناميك (٥٠ ملغ/كغ) بالحقن تحت الغشاء البريتوني للجرذ ، بينما ابدى مركب IVc فعالية اقل كمضاد للالتهاب مقارنة بحمض المفيناميك.

الكلمات المفتاحية: حمض الميفيناميك، ٤-ثيازوليدينون، مضادات الالتهاب.

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Received: 30 / 12 /2018
Accepted: 26 / 3/2019

Introduction

NSAIDs have become important as an analgesic, antipyretic, and anti-inflammatory medications throughout the world. The main mechanism of NSAIDs action is inhibition both isoforms of the enzyme cyclooxygenase COX-1 and COX-2 which catalyzes the synthesis of PGH2 from arachidonic acid [1,2]. Since the gastrointestinal (GI) toxicity, as well as renal toxicity, were identified as the main adverse effect of the chronic use of the known (NSAIDs), which were mediated by COX-1 enzyme inhibition, attempt was made to develop COX-2 selective inhibitors (coxibs), which possess the therapeutic effect devoid of gastrointestinal (GI) and renal toxicity. [1-6]

3-Dimensional structures analysis for the two isomers of COX enzyme demonstrated differences in the hydrophobic channel structure, as well as a rather larger orifice and the presence of an additional pocket lying away from the catalytic site, this makes the selective COX-2 inhibitors to require longer time to fit the COX-2 active site, but when they bind the enzyme, their bond may become permanent. They also may become competitive COX-1 inhibitors when given at high doses [7].

Well-known NSAIDs modification into more selective COX-2 inhibitors is considered an interesting maneuver. Yet, no general line is followed as a procedure for this purpose [8, 9].

Thiazolidinones are thiazolidine derivatives possessing at position 1 a sulfur atom, at position 3 a nitrogen atom and at position 2, 4, or 5 a carbonyl group .

Derivatives of this compound constitute members of the widely investigated agents, and their presence was first documented in penicillin. Similarly, 1,3-thiazolidin-4-ones are heterocyclic nucleus that contain a sulfur atom and nitrogen at position 1 and 3, respectively and a carbonyl group at position 4, it has been studied extensively in recent years [10].The 4- thiazolidinone skeleton is very flexible and has included in a variety of clinically important medications such as some anti-tubercular, antimicrobial, anti-inflammatory and antiviral especially anti-HIV agents [11].Zarghi et al. reported a novel series of 2,3-diaryl-1,3-thiazolidine-4-ones showing COX-2 inhibition properties which was more selective than celecoxib [9].

The aim of this work is synthesize and preliminary anti-inflammatory evaluation of 4-thiazolidinone derivatives of mefenamic acid in order to obtain a more potent mefenamic acid analogues.

Materials and Methods

Chemicals used during the synthesis were supplied by hyper-chem (China). Completion of reactions and the purity of compounds were monitored by thin-layer chromatography (TLC), using silica gel GF254 (type 60) pre-coated aluminum sheets, Merck (Germany) exposed to UV-254 nm light, five solvent systems were used which include: ethyl acetate:hexane(4:6) [12,13] , ethyl acetate:hexane (6:4) , ethyl acetate: hexane (3:7),ethyl acetate: petroleum ether(5:5) and ethyl acetate: petroleum ether(4:6). Melting points were detected by using Stuart SMP3 melting point apparatus in open capillary tubes, and are uncorrected. The infrared spectra were performed in thin film techginqe, (υ, cm^{-1}), on Shimadzu FTIR spectrophotometer, (Japan). ^{1}HNMR spectra were obtained on BRUKER model Ultra shield 300 MHz spectrophotometer, and BRUKER model Ultra shield 400 MHz spectrophotometer, using deuterated DMSO-d_6 as solvents and TMS as an internal standard, at Al-albayt University, Amman-Jordan.

Chemical synthesis

Synthesis of 2-(2-chloro-N-(2,3-dimethylphenyl) acetamido)benzoic acid (I) [14,15]

Mefenamic acid (2.0 g, 8.29 mmol) was dissolved in dichloromethane (DCM) 20 ml and in the presence of triethylamine (1.4 mL, 9.97 mmol) the mixture were stirred at 0 ºC for10 min. Then Chloroacetylchloride (1.4 mL,17.6 mmol) was added dropwise. After that stirring at 0 ºC for 15 min and at room temperature for one hour ,The end of reaction was detected by TLC , After end of reaction aqueous K_2CO_3(2 eq.)(2.5 g) was added to the mixture and the mixture were stirred for 30 min was washed with Dichloromethane and water. The aqueous layer was extracted with 3 × 30 mL of CH_2Cl_2. The organic layers were collected and concentrated. The remaining residue was purified by recrystallization from benzene.

The percent yield and physical data are given in Table (2)

FTIR: 2920 cm^{-1}(C-H stretching of CH_3); 1705 cm^{-1}(C=O of amide) and 1639 cm^{-1} (C=O of carboxylic acid). ^{1}HNMR (400 MHz: $DMSOd_6$) H: 1.91(3H, s, Ar- CH_3,ortho mefenamic); 2.09 (3H, s, Ar-CH_3 meta mefenamic); 4.89 (2H, s, CH_2 chloroacetyl group); 7.47-7.79 (7H, m, Ar- H)

Synthesis of 2-(N-(2,3-dimethylphenyl)-2-hydrazineylacetamido)benzoic acid (II). [16]

A mixture of compound (I) (0.98 g, 3.1 mmol) and hydrazine hydrate 80% (9.4 mmol) in ethanol (20 mL) was heated under reflux for six hours, then cooled and poured on to crushed ice (30 g), then the solid product which formed was filtered off, washed with water and recrystallized from ethanol to give compound (II)

The percent yield and physical data are given in Table (2).

FTIR: 3425 & 3325 cm^{-1} (NH of primary amine); 1735 cm^{-1} (C=O amide)& 1635 cm^{-1} (C=O of

carboxylic acid), ^{1}HNMR (400 MHz: $DMSOd_6$) H: 2.17(3H, s, Ar- CH_3,ortho mefenamic); 2.28 (3H, s, Ar-CH_3 meta mefenamic); 4.8 (2H, s, CO-CH_2-NH group); 6.49-7.77 (7H, m, Ar- H)

Synthesis of schiff base(2-(2-(2-benzylidenehydrazineyl)-N-(2,3-dimethyl phenyl)acetamido)benzoic acid)compound III a-g .[17]

A mixture of 1.9 mmol of Compound II and 1.9 mmol of the corresponding aldehyde derivatives that mentioned in table (1) in 20 ml of absolute ethanol was stirred at room temperature for 0.5 to 1h, with addition of two to three drops of hydrochloric acid as a catalyst. The end of the reaction was observed by TLC, and the benzyledine hydrazines were isolated by concentrating of the crude product at reduced pressure, the resulting precipitate was filtered, washed with 10 ml water and recrystallized from ethanol.

The percent yield and physical data are given in Table (2)

2-(N-(2,3-dimethylphenyl)-2-(2-(4-hydroxybenzylidene)hydrazineyl)acetamido)benzoic acid (IIIa)

FTIR: 3275 cm^{-1}(O-H Phenol& N-H hydrazine);1739 cm^{-1} (C=O amide); 1654 cm^{-1} (C=O of carboxylic acid) & 1604 cm^{-1} (C=N).

2-(N-(2,3-dimethylphenyl)-2-(2-(4-nitrobenzylidene)hydrazineyl)acetamido)benzoic acid (IIIb)

FTIR: 1739 cm^{-1} (C=O amide); 1662cm^{-1} (C=O of carboxylic acid); 1597 cm^{-1} (C=N); 1519 & 1342 cm^{-1} (asym. & sym. Stretching of NO_2). ^{1}HNMR (400 MHz: $DMSOd_6$) H: 4.9 (2H, s, CO-CH_2-NH); 6.83-8.43(11H, m, Ar-H) ; 9 (1H, s, CH=N); 12.1(1H, s, COOH)

2-(2-(2-(4-chlorobenzylidene)hydrazineyl)-N-(2,3-dimethylphenyl)acetamido)benzoic acid (IIIc)

FTIR: 3210 cm^{-1}(NH hydrazine); 1743 cm^{-1} (C=O amide); 1647 cm^{-1} (C=O of carboxylic acid) & 1597 cm^{-1} (C=N) and 1083 cm^{-1} (C-Cl).

2-(N-(2,3-dimethylphenyl)-2-(2-(4-fluorobenzylidene)hydrazineyl)acetamido)benzoic acid (IIId)

FTIR: 3210 cm^{-1}(NH hydrazine); 1743 cm^{-1} (C=O amide); 1647 cm^{-1} (C=O of carboxylic acid) and 1600 cm^{-1} (C=N)and 1095 cm^{-1} (C-F).

2-(2 - (2 - (4 - (dimethylamino) benzylidene) Hydrazineyl) -N-(2,3-dimethylphenyl)acetamido) benzoic acid (IIIe)

FTIR: 3210 cm^{-1}(NH hydrazine) ;1743 cm^{-1} (C=O amide); 1651 cm^{-1} (C=O of carboxylic acid) & 1600 cm^{-1} (C=N).

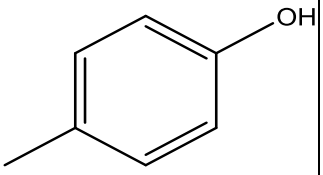
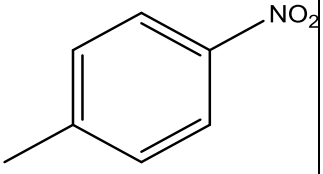
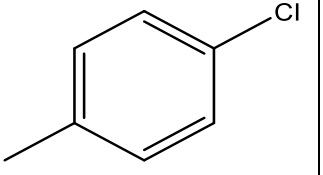
2-(2-(2-(3-chlorobenzylidene)hydrazineyl)-N-(2,3-dimethylphenyl)acetamido) benzoic acid (IIIf)

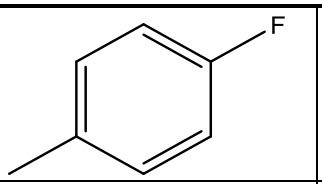
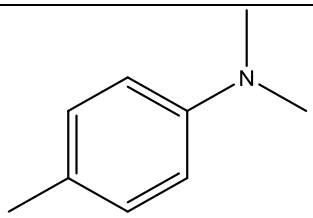
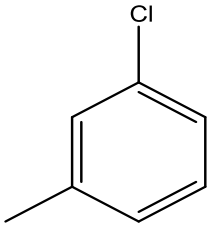
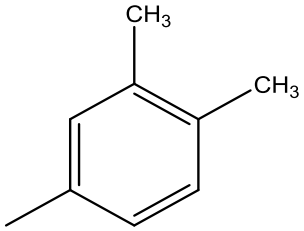
FTIR: 3210 cm^{-1}(NH hydrazine); 1743 cm^{-1} (C=O amide); 1647 cm^{-1} (C=O of carboxylic acid) & 1604 cm^{-1} (C=N) and 1080 cm^{-1} (C-Cl) .

2-(2-(2-(3,4-dimethylbenzylidene)hydrazineyl)-N-(2,3-dimethylphenyl)acetamido)benzoic acid (IIIg)

FTIR: 3210cm^{-1}(NH hydrazine); 1743 cm^{-1} (C=O amide ; 1651 cm^{-1} (C=O of carboxylic acid)& 1600 cm^{-1}(C=N).

Table (1) Aromatic aldehydes name and products no.

No.	Aromatic aldehyde's name	Product No.	**R**	Quantity(gm)
a	4-hydroxybenzaldehyde	IV a		0.231
b	4-nitrobenzaldehyde	IV b		0.286
c	4-chlorobenzaldehyde	IV c		0.266

d	4-fluorobenzaldehyde	IV d		0.235
e	4-dimethylaminobenzaldehyde	IV e		0.283
f	3-chlorobenzaldehyde	IV f		0.266
g	3,4-dimethylbenzaldehyde	IV g		0.254

Synthesis2-(N-(2,3-dimethylphenyl)-2-((4-oxo-2-phenylthiazolidin-3-yl)amino)acetamido)benzoic acid Compound IV a-g.[18]

A mixture of benzyledine hydrazine IIIa-g (1 mmol) and excess of thioglycolic acid a(0.071 mmol) (5 ml) was heated at 60 ^{o}C until reaction complete, as shown by TLC (about 6 h). Ethyl acetate (5 ml) was added, the organic layer was washed with water (1 × 10 ml), dried with $MgSO_4$, and concentrated to give an oily product, and the final compound was washed with diethyl ether
The percent yield and physical data are given in Table (2)

2-(N-(2,3-dimethylphenyl)-2-((2-(4-hydroxyphenyl) -4-oxothiazolidin-3-yl) amino)acetamido)benzoic acid (IVa)

FTIR: 3236 cm^{-1}(OH phenol & N-H hydrazine); 1782 cm^{-1}(C=O thiazolidinone); 1732 cm^{-1} (C=O amide); 1670 cm^{-1} (C=O of carboxylic acid) and 1238 cm^{-1} (C-O). ^{1}HNMR (300 MHz, : $DMSOd_6$) H: 3.8-3.9 (2H, d, CH_2 thiazolidinone);4.1 (1H,s,CH_2-NH-N);4.9 (2H,s,CO-CH_2-NH);6.1(1H,s,N-CH-S)(thiazolidinone); 6.7-7.79 (11H, m, Ar-H); and 10.1 (1H, s, Ar-OH).

2-(N-(2,3-dimethylphenyl)-2-((2-(4-nitrophenyl)-4-oxothiazolidin-3-yl)amino)acetamido)benzoic acid (IVb)

FTIR: 3236 cm^{-1} (N-H hydrazine); 1778 cm^{-1} (C=O thiazolidinone); 1728 cm^{-1} (C=O amide); 1681cm^{-1} (C=O of carboxylic acid) ; 1346 cm^{-1} (NO_2 stretching) and 1238cm-1(C-O). ^{1}HNMR (300 MHz, : $DMSOd_6$) H: 3.7-3.9 (2H, d, CH_2 thiazolidinone);4.1 (1H,s,CH_2-NH-N); 4.9 (2H,s,CO-CH_2-NH); 5.93(1H, s, N-CH -S)(thiazolidinone); 6.7-8.25 (11H, m, Ar-H) .

2-(2 - ((2 - (4 – chlorophenyl) – 4 –oxothiazolidin – 3 – yl) amino) – N - (2 , 3-dimethyl phenyl) acetamido) benzoic acid (IVc)

FTIR: 3236 cm^{-1} (N-H hydrazine); 1782 cm^{-1}(C=O thiazolidinone); 1728 cm^{-1} (C=O amide); 1670 cm^{-1} (C=O of carboxylic acid); 1087 cm^{-1} (C-Cl stretching) and 1280cm^{-1}(C-O). ^{1}HNMR (300 MHz, : $DMSOd_6$) H: 3.7-3.8 (2H, d, CH_2 thiazolidinone);4.1 (1H,s,CH_2-NH-N); 4.9 (2H,s,CO-CH_2-NH); 5.8 (1H, s, N-CH -S)(thiazolidinone); 6.7-7.79 (11H, m, Ar-H).

2- (N - (2 , 3 – dimethylphenyl) – 2 - ((2 - (4-fluorophenyl) – 4 – oxothiazolidin – 3 -yl) amino) acetamido) benzoic acid (IVd)

FTIR: 3251 cm^{-1} (N-H hydrazine); 1782 cm^{-1}(C=O thiazolidinone); 1724 cm^{-1} (C=O amide); 1670 cm^{-1} (C=O of carboxylic acid) and 1222 cm^{-1} (C-O). ^{1}HNMR (300 MHz, : $DMSOd_6$) H: 3.7-3.8 (2H, d, CH_2 thiazolidinone); 4.1 (1H,s,CH_2-NH-N); 4.9 (2H,s,CO-CH_2-NH); 5.8 (1H,s,N-CH-S)(thiazolidinone); 6.7-7.79 (11H, m, aromatic CH).

2- (2 - ((2 - (4 - (dimethylamino) phenyl) - 4-oxothiazolidin – 3 – yl) amino) – N - (2 , 3-dimethylphenyl)acetamido)benzoic acid (IVe)

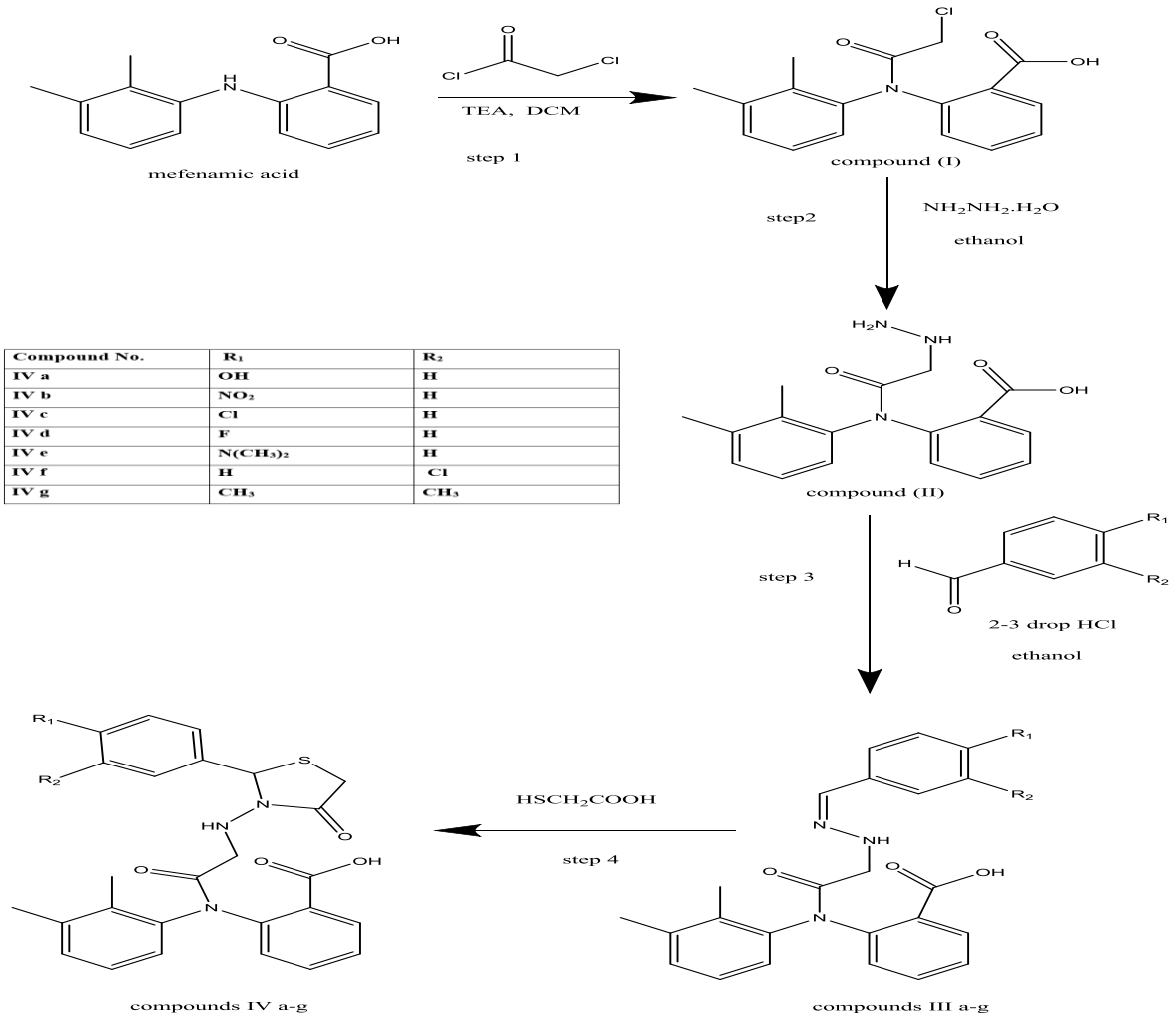
FTIR: 3251 cm^{-1} (N-H hydrazine); 1782 cm^{-1}(C=O thiazolidinone); 1728 cm^{-1} (C=O amide); 1670 cm^{-1} (C=O of carboxylic acid) and 1238cm^{-1}(C-O). ^{1}HNMR (300 MHz, : $DMSOd_6$) H: 3.7-3.8 (2H, d, CH_2 thiazolidinone); 4.1 (1H,s,CH_2-NH-N); 4.9 (2H,s,CO-CH_2-NH); 5.8(1H,s, N-CH-S)(thiazolidinone); 6.7-7.79 (11H, m, Ar-H).

2- (2 - ((2 - (3 – chlorophenyl) – 4 – oxothiazolidin – 3 – yl) amino) – N - (2 ,3-dimethyl phenyl)acetamido)benzoic acid (IVf)

FTIR: 3232 cm^{-1}(N-H hydrazide); 1782 cm^{-1}(C=O thiazolidinone); 1724 cm^{-1} (C=O amide); 1670 cm^{-1} (C=O of carboxylic acid); 1076 cm^{-1} (C-Cl stretching) and 1242 cm^{-1}(C-O). ^{1}HNMR(300 MHz, : $DMSOd_6$) H: 3.7-3.8 (2H, d, CH_2 thiazolidinone); 4.1 (1H,s,CH_2-NH-N); 4.9 (2H,s,CO-CH_2-NH); 5.8 (1H, s, N-CH -S)(thiazolidinone); 6.7-7.79 (11H, m, Ar- CH).

2-(N - (2 , 3 – dimethylphenyl) – 2 - ((2-(3 , 4 – dimethylphenyl) – 4 - oxothiazolidin-3-yl)amino)acetamido)benzoic acid (IVg)

FTIR: 3251 cm^{-1}(N-H hydrazide); 1782 cm^{-1}(C=O thiazolidinone); 1728 cm^{-1} (C=O amide); 1670 cm^{-1} (C=O of carboxylic acid) and 1280 cm^{-1} (C-O). ^{1}HNMR (300 MHz, : $DMSOd_6$) H: 3.7-3.8 (2H, d, CH_2 thiazolidinone); 4.1 (1H,s,CH_2-NH-N); 4.9 (2H,s,CO-CH_2-NH); 5.7 (1H,s, N-CH-S)(thiazolidinone); 6.7-7.79 (10H, m, Ar-H).



Compound No.	R_1	R_2
IV a	OH	H
IV b	NO_2	H
IV c	Cl	H
IV d	F	H
IV e	$N(CH_3)_2$	H
IV f	H	Cl
IV g	CH_3	CH_3

Scheme 1.Synthesis of the target compounds (IV a-g)

Table (2) The characterization and physical parameters of the target compounds and their intermediates

No.	Molecular Formula	Molecular Weight	Description	% Yield	Melting Point °C
I	$C_{17}H_{16}ClNO_3$	317	Pale yellow crystals	81	156-159
II	$C_{17}H_{19}N_3O_3$	313	White powder	80	138-141
III a	$C_{24}H_{23}N_3O_4$	417	Orange crystals	69	164-166
III b	$C_{24}H_{22}N_4O_5$	466	Deep yellow crystals	67	170-172
III c	$C_{24}H_{22}ClN_3O_3$	435	Off white powder	45	155-158
III d	$C_{24}H_{22}FN_3O_3$	419	Yellow powder	63	110-112
III e	$C_{26}H_{28}N_4O_3$	444	orange powder	67	105-108
III f	$C_{24}H_{22}ClN_3O_3$	435	Pale yellow powder	85	102-105
III g	$C_{26}H_{27}N_3O_3$	451	Pale yellow powder	80	125-128
IV a	$C_{26}H_{25}N_3O_5S$	491	Orange oil	71	-
IV b	$C_{26}H_{24}N_4O_6S$	520	Yellow oil	60	-
IV c	$C_{26}H_{24}ClN_3O_4S$	510	Yellow to orange oil	57	-
IV d	$C_{26}H_{24}FN_3O_4S$	493	Yellow to orange oil	61	-
IV e	$C_{28}H_{30}N_4O_4S$	518	Brown oil	68	-
IV f	$C_{26}H_{24}ClN_3O_4S$	510	Yellow to orange oil	64	-
IV g	$C_{28}H_{29}N_3O_4S$	503	Yellow to orange oil	62	-
M.A	$C_{15}H_{15}NO_2$	241	White powder		230-233

Preliminary pharmacological studies

Anti-inflammatory evaluation study

The inflammatory model that used to evaluate final compounds (IVa-g) for the *In-vivo* acute anti-inflammatory effects exploited egg-white induced rat paw edema, for comparison with anti-inflammatory activity of mefenamic acid. The decrease of paw thickness is the basis of screening of the newly synthesized compounds for their anti-inflammatory activity.

Methods

Animals

Albino rats of either sex weighing (250 ± 50 gm) were supplied by Biotechnology Research Center, AL-Nahrain University, and were housed under standardized conditions in the Biotechnology Research Center, AL-Nahrain University animal house. Commercial chaw was used to feed the animals and they had free access to water ad libitum. Animals were brought to the laboratory, one hour before the experiment; animals were divided into nine groups (six rats per group) as follow:

Group A: injected with the vehicle (dimethyl sulfoxide 10% v/v) and served as a control group.

Group B: injected with mefenamic acid as reference substance with a dose of 50mg/kg [19], dissolved in dimethyl sulfoxide 10% (v/v).

Group C-I: injected with the tested compounds (IVa-g) by doses that are determined below. (dissolved in dimethyl sulfoxide 10% v/v).

Calculations for dose determination [20]

Dose of reference compound /Mwt. of reference compound = dose of tested compound/ Mwt. of tested compound.

Table (3) Compounds with their molecular weight and dose

Compounds	Molecular weight	Dose mg / kg
Mefenamic acid	241	50
IV a	491	86.5
IV b	520	94
IV c	510	107
IV d	493	103
IV e	518	93
IV f	510	107
IV g	503	105

Experimental design

Egg albumin was used to induce rat paw edema as acute inflammatory model for studying of the final compounds activity[21]. 0.05mL of undiluted ovalbumin was subcutaneously injected into the rats' planter side of the hind paw; preceded by a half hour of intraperitoneal injection of the drugs or their vehicle.

Electronic LCD Digital vernier caliper gauge stainless steel ruler was used for measuring paw thickness at 7 time periods (0, 30, 60, 120, 180, 240, and 300 minutes) after the Compounds injection.

Statistical analysis

The mean ± SEM was used to report data of this work then student t-test (Two Sample Assuming Equal Variances) used to calculate data statistical significance between means. ANOVA (Two factors without Replication) is used to compare between different groups. P-value < 0.05 was assumed significant.

Results and Discussion

The anti-inflammatory activity of the tested compounds has been evaluated in comparison with their vehicle (control group) and mefenamic acid. Table (4) explains the effect of tested compounds (IVa-g) in comparison to control and mefenamic acid. The tested compounds and the reference drug produced significant reduction of paw edema with respect to the effect of dimethyl sulfoxide 10%v/v (control group). All tested compounds significantly limited the inflammation in paw edema, the onset of mefenamic acid and compounds IVa,c,e started at time 120 min but IVc became comparable to control at 180 min until the end of the study. While compound IVg started at 180 min and Compounds IVb,f started at 240 min. compound IVe exhibited more potent anti-inflammatory effect than mefenamic acid (50mg/kg, i.p.) at 240-300 min., while compounds IVa,d exhibited higher anti-inflammatory effect at time 300 min. However, the effect of all tested compound continued till the end of experiment with statistically significant ($P<0.05$) reduction in paw edema thickness as shown in figure (1).

Comparative analysis

The comparison explains that at 0-60 min., there are no differences among all groups. Compounds (IV a,d,e,f) at time 120-240 minutes show comparable effect to mefenamic acid except IV e which becomes more potent at 240-300 min. at time 300 min compounds IV a,d exhibit more potent effect than mefenamic acid, while IVf remains comparable until the end of the study. Although; compounds IVb,g significantly limited the increase in paw edema in comparison to control group, but they are significantly less effective than mefenamic acid and other tested compounds at interval of 120 minutes and after that they exhibit comparable effect to mefenamic acid until the end of the experiment.

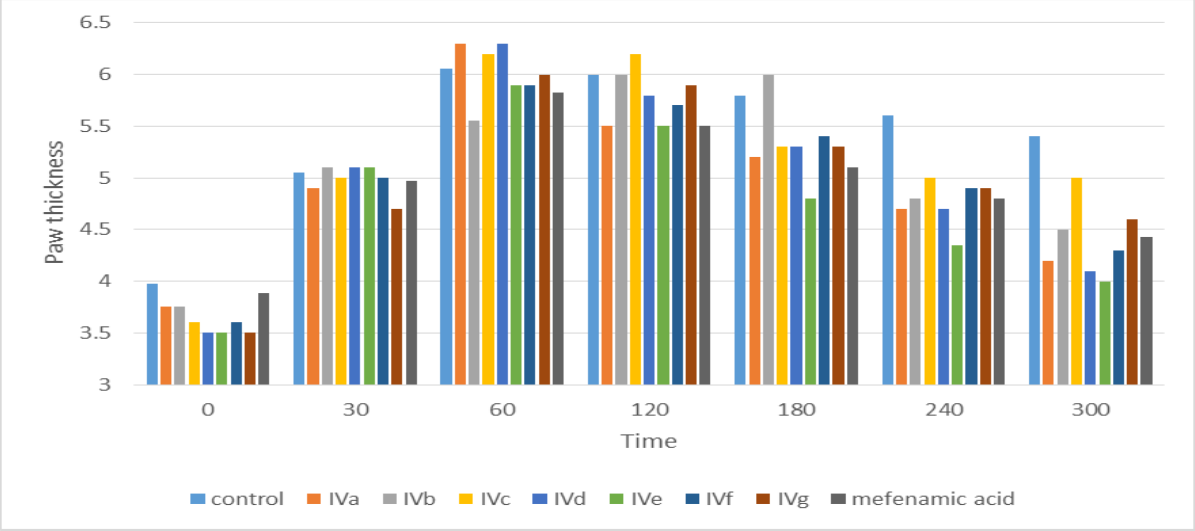


Figure (1) Effect of mefenamic acid, dimethyl sulfoxide, compounds IVa-g on egg-white induced paw edema in rats. Results are expressed as mean ± SEM (n = 6 for each group).

Table (4) The anti-inflammatory effect of control, mefenamic acid and compounds IVa-g on egg-white induced paw edema in rats:

	compounds	Time						
		0	**30**	**60**	**120**	**180**	**240**	**300**
Paw thickness (mm) ,n= 6	**Control**	3.98 ±0.1	5.05 ±0.2	6.06±0.1	6 ±0.1	5.8 ±0.1	5.6 ±0.1	5.4 ±0.1
	mefenamic	3.89 ±0.2	4.97 ±0.2	5.83 ±0.2	5.5 ±0.2*[a]	5.1 ±0.1*[a]	4.8 ±0.05*[a]	4.43 ±0.06*[a]
	IVa	3.75 ±0.2	4.9 ±0.1	6.3 ±0.1	5.5 ±0.14*[a]	5.2 ±0.15*[a]	4.7 ±0.1*[a]	4.2 ±0.08*[a]
	IVb	3.75 ±0.06	5.1 ±0.1	5.55 ±0.2	6 ±0.1	6 ± 0.1	4.8 ±0.1*[a]	4.5 ±0.1*[a]
	IVc	3.6 ±0.1	5 ±0.2	6.2 ±0.2	5.4 ±0.2*[a]	5.3 ±0.2	4.8 ±0.3	5 ±0.3
	IVd	3.5 ±0.04	5.1 ±0.2	6.3 ±0.14	5.8 ±0.2	5.3±0.14*[a]	4.7 ±0.1*[a]	4.1 ±0.04*[a]
	IVe	3.5 ±0.07	5.1 ±0.2	5.9 ±0.15	5.5 ±0.1*[a]	4.8 ±0.14*[a]	4.35 ±0.1*[a]	4 ±0.05*[b]
	IVf	3.6 ±0.1	5 ±0.1	5.9 ±0.25	5.7 ±0.2	5.4 ±0.2	4.9 ±0.2*[b]	4.3 ±0.1*[a]
	IVg	3.5 ±0.1	4.7 ±0.1	6 ±0.1	5.9 ±0.1	5.3 ±0.1*[a]	4.9 ±0.1*	4.6 ±0.1*[a]

*significantly different compared to control (P<0.05). Data are expressed in mm paw thickness as mean ± SEM. n= number of animals. Time (0) is the time of i.p. injection of mefenamic acid and dimethyl sulfoxide. Time (30) is the time of injection of egg white (induction of paw edema). Non-identical superscripts (a, b) among different groups are considered significantly different (p<0.05).

Conclusions

A new derivatives of mefenamic acid were successfully synthesized by conventional method and tested for anti-inflammatory activity, acute anti-inflammatory study using egg white induced edema model of inflammation revealed that the incorporation of 4-thiazolidinone moiety into a mefenamic acid maintained or enhanced its anti-inflammatory activity and also the anti-inflammatory study show that compounds (IVa , IVd , IVe) contain [OH,F,$N(CH_3)_2$] groups respectively which are electron donating groups showed superior anti- inflammatory activity to mefenamic acid probably due to formation of hydrogen bonding with the target receptor in the body .

Acknowledgments

The authors greatly thankful the College of Pharmacy, University of Baghdad, for their help and support.

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