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Synthesis, characterization and preliminary anti-inflammatory evaluation of 5-benzylidine thiazolidine-4-one derivatives.

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Abstract

A series of four compounds which contain thiazolidine-4-one ring were synthesized and preliminarily assessed as anti-inflammatory agents with anticipated selectivity against COX-2 enzyme. The synthesized compounds structures have been recognised according to their spectral FT-IR and ^{1}H NMR data. GOLD software was used to perform molecular docking study. The crystallographic structure of the molecular target cyclo-oxygenase enzyme-2 (COX-2) was taken from PDB database however , the celecoxib and naproxen were selected as a positive controls. The docking results of the newly synthesized compounds shown that they can enter the substrate-binding area of the active site .The compound (Ia) showed the highest PLP fitness ,while compound (Ic) appeared to have the lowest binding ability compared to celecoxib which may be due to the presence of para nitro group that deactivates the ring system. Whereas *In vivo* acute anti-inflammatory effects of the synthesized compounds were evaluated in rats using egg-white induced edema model of inflammation. The tested compounds and the reference drug produced significant decrease in the paw edema with respect to the effect of propylene glycol 50%v/v (control group). Compounds Ia,b,d showed potent anti-inflammatory effect than naproxen (50mg/kg, i.p.) at 180 min., while compound **Ic** exhibited lower anti-inflammatory effect.

Keywords : 4-Thiazolidinone , Anti-inflammatory activity, Naproxen.

INTRODUCTION

Inflammation is a key response of the immune system to tissue damage and infection, also it is a multi-factorial, protective attempt and considered as an important aspect in rheumatoid arthritis, osteoarthritis, Alzheimer's disease and obesity related diseases.[1] Inflammation can be useful as an acute, transient immune response to harmful conditions . Traumatic tissue injury or an invading pathogen can play a good example here. This response also smooths the repair, turnover, and adaptation of many tissues.[2] Nonsteroidal anti-inflammatory drugs (NSAIDs) are drugs with activity against inflammatory symptoms but without any glucocorticoid action. Arachidonic acid is delivered from the cell membrane phospholipids by phospholipase (PL) A2. It metabolizes to various physiologically active substances such as prostaglandins (PGs) by the rate limiting enzyme COX. Human COX-1 and COX-2 are the homodimers of 576 and 581 amino acids, respectively. In 1971, Vane discovered that inhibition of cyclooxygenase (COX) was the major mechanism of action for aspirin and some other NSAIDs.[3]. So the anti-inflammatory effects of NSAIDs are related to the inhibition of prostaglandin synthesis by blocking the activity of cyclooxygenase enzyme (COX) [4].

These Prostaglandins (PGs) are hormone-like bioactive substances mediating autocrine and paracrine signalling over the short distances and are involved in many physiological and pathological processes and those that are produced by COX-2 play a major role in inflammatory reactions and are responsible for the characteristic inflammatory symptoms (redness, pain, edema, fever and loss of function). The inducible isoform has also been implicated in pathological processes such as various cancer types (colorectal, breast), Alzheimer and Parkinson's diseases.[5].

There are numerous biologically active hetrocycles molecules bearing nitrogen, sulphur and oxygen, always drawn the attention over the years mainly because of their biological importance [6]. Thiazolidinones are thiazolidine derivatives contain sulfur atom which take the position 1, an atom of nitrogen at position 3, and a carbonyl group at position 2, 4, or 5. The 4-thiazolidinone is very versatile and possesses almost all types of biological activities like anti-bacterial [7], anti-tubercular [8], anti-inflammatory [9], anti-convulsant [10], anti-cancer and antiviral agents, especially as anti-HIV agents [11]. the classic NSAIDs are composed of a hydrophobic aromatic nucleus and an attached acidic group where the aromatic portion lies in the hydrophobic channel of COX-1, while the acidic group is H-bonded to the side chain hydroxyl group of Tyr355 and guanidinium group of Arg120 as shown in figure 1 . Except that of aspirin, all the other NSAIDs inhibit COX-1 in a similar way by working as competitive inhibitors, whereas aspirin and its analogues acetylate the active-site Ser530 to act as suicide inhibitors of COX enzymes . in contrast to the classical NSAIDs a new class of enzyme inhibitors is lacking a carboxylic group, thus interacting with COX-2 isoenzyme by different orientation within the enzyme active site without formation of a salt bridge in the hydrophobic channel of it. Most of these new classes possess central heterocyclic ring with 2,3,5 tri aryl substitution and the most important heterocyclic ring is thiazolidinone-4-one which act as an ideal pharmacophore for any compound to act as a selective COX-2 inhibitor.The direction of the present work is to synthesis thiazolidine-4-one pharmacophore containing compounds and may

represent potent anti-inflammatory agents and exhibit expected selectivity towards COX-2 enzyme.

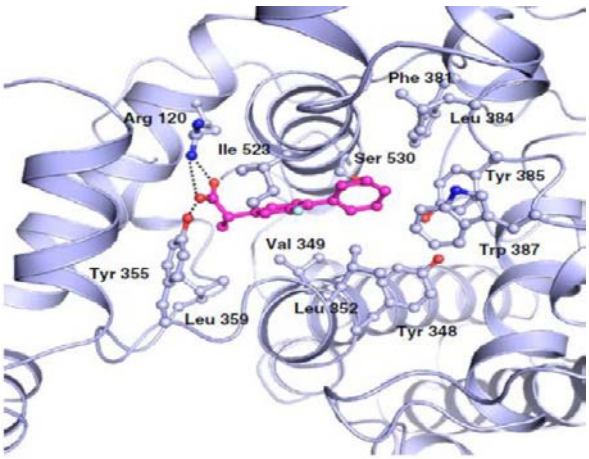


Figure 1 ribbon diagram of COX-1 (blue) in complex with flurbiprofen

2. MATERIALS AND METHODS

2.1 General

All reagents and anhydrous solvents were of analar type, were purchased from (Himedia, GCC India, AK science, USA and BDH, England). Naproxen was a product of the General Company for Pharmaceutical Industries and Medical Appliances, Samarra, Iraq.

Electro thermal melting point apparatus and open capillary tubes were used to determine the melting points and are uncorrected. Thin layer chromatography was run on TLC silica gel (60) F254, Merck (Germany), for checking the purity of the products as well as monitoring the development of the reaction. Chromatograms were eluted by using two dissimilar solvent systems: A: Methanol: Acetic acid: Ether: Benzene B: Chloroform: Methanol. Compounds were revealed upon irradiation with UV light. IR spectra were recorded on a FTIR,Bruker 8100s spectrometer.[1]HNMR spectra were recorded on Bruker 300 MHz.

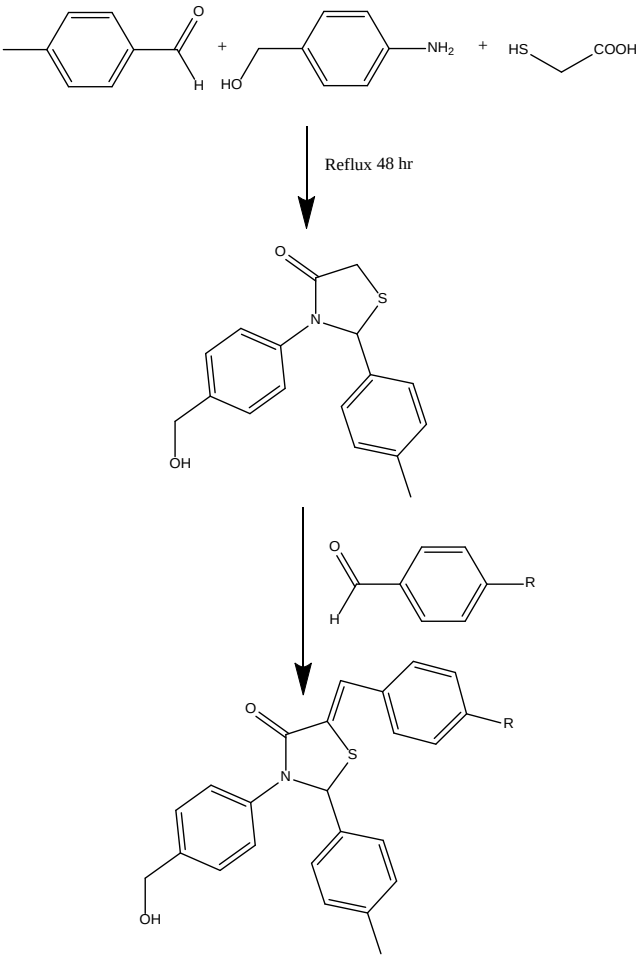
2.2 Typical procedure for the reactions

The synthesis of target compounds (**I_{a-d}**) was achieved following procedures illustrated in Scheme 1.

2.2.1. Synthesis of[3-(4-(hydroxymethyl) phenyl)-2-(p-tolyl)thiazolidine-4-one] (I):

To a stirred solution of para amino benzyl alcohol (8 mmol , 0.98 g) in dry toulene (50 mL), 2-mercaptoacetic acid (16 mmol , 1.1 mL) and the para tolualdehyde (8 mmol , 0.94mL) were added. The reaction mixture was refluxed for (48 hrs.) and then neutralized by a solution of $NaHCO_3$. After removal of the solvent under reduced pressure, the oily residue was converted to powder by management with a mixture of ethanol and diethyl ether to give the dry compounds then the residue was purified by recrystallization from ethanol.[12].

Yield: 90%. M.p.: 110 °C. R_f. A= 0.68, B= 0.46. FTIR: 3361 cm^{-1}(OH alcohol);1669 cm^{-1}(C=O); and 1571 cm^{-1}(C=C aromatic). ^{1}H NMR (300 MHz, DMSO-*d*6, δ, ppm): 2.25 (3H,s,CH_3); 3.95 (2H,s,CH_2 thiazolidinone); 4.49(2H,s,CH_2 side chain), 5.31(1H,s,OH alcohol) ; 6.15 (1H,s,CH thiazolidine); 6.86-7.44(8H,m,Ar-H).



R= H , OH , NO_2 , CH_3

Scheme 1 Synthesis of intermediate and target compounds (I_{a-d})

2.2.2. General procedure for the synthesis of (Z)-5-(arylidene)-3-(4 (hydroxymethyl)phenyl)-2-(p-tolyl)thiazolidin-4-one (I_{a-d}).

A solution of appropriate aromatic aldehyde (0.5 mmol) mixed with a solution of compound (I) (0.5 mmol ,0.15 g) in the presence of piperidine (1drop) as a catalyst in MeOH (10 mL) was stirred at room temperature overnight. Then the elimination of solvent was accomplished under reduced pressure to get the final compounds then they are recrysllized from ethanol.[13]

(Z)-5-benzylidene-3-(4-(hydroxymethyl)phenyl)-2-(p-tolyl)thiazolidin-4-one(I_a):

Yield: 60%. M.p.: 189°C. R_f. A=0.9, B=0.53. FTIR:3337 cm^{-1} (OH), 1672 cm^{-1} (C=O), 1573 cm^{-1} (C=C) and 1215 cm^{-1} (C-S) .^{1}H NMR (300 MHz, CDCl3: DMSO-d6) : 2.38 (3H, s, CH_3); 4.43(2H, s, CH_2 side chain); 5.33 (1H,s,OH); 6.11(1H,s,CH thiazolidinone); 6.42(2H,d,Ar-H); 6.77-7.26(6H,m,Ar-H) and 7.42-7.78 (6H,m,Ar-H and chalcone).

(Z)-5-(4-hydroxybenzylidene)-3-(4-(hydroxymethyl)phenyl)-2-(p-tolyl)thiazolidin-4-one(I_b):
Yield: 65% M.p.: 181°C. R_f. A= 0.88, B=0.57. FTIR: 3359 cm^{-1}(OH),1672 cm^{-1} (C=O),1570 cm^{-1} (C=C) and 1166 cm^{-1} (C-S). ^{1}H NMR (300 MHz, CDCl3: DMSO-d6) : 2.2(3H,s, CH_3); 4.44 (2H,s, CH_2 side chain); 5.29 (1H,s,OH); 5.9 (1H,s,CH thiazolidinone); 6.55-7.1(4H,m,Ar-H); 7.2-7.7(9H,m,Ar-H and chalcone) and 10.2 (1H,s,phenolic OH).

(Z)-3-(4-(hydroxymethyl)phenyl)-5-(4-nitrobenzylidene)-2-(p-tolyl)thiazolidin-4-one (I_c):
Yield: 57% M.p.: 173°C. R_f. A= 0.7, B=0.52 FTIR: 3274 cm^{-1} (OH), 1675 cm^{-1} (C=O), 1582 cm^{-1} (C=C), 1515 cm^{-1} (NO_2 asymmetric),1385 cm^{-1} (NO_2 symmetric) and 1170 cm^{-1} (C-S). ^{1}H NMR (300 MHz, CDCl3: DMSO-d6) : 2.1(3H,s, CH_3); 4.55 (2H,s, CH_2 side chain); 5.3 (1H,s,OH); 5.85 (1H,s,CH thiazolidinone); 6.95 (2H,d,Ar-H); 7.1-7.4(6H,m,Ar-H) and 7.8-8.1(5H,m,Ar-H and chalcone).
(Z)-3-(4-(hydroxymethyl)phenyl)-5-(4-methylbenzylidene)-2-(p-tolyl)thiazolidin-4-one (I_d)
Yield: 52% M.p.: 191°C. R_f. A= 0.91, B=0.64. FTIR: 3341 cm^{-1} (OH), 1674 cm^{-1} (C=O), 1572 cm^{-1} (C=C), and 1171 cm^{-1} (C-S). ^{1}H NMR (300 MHz, CDCl3: DMSO-d6) : 2.3(3H,s, CH_3);2.7(3H,s, CH_3); 4.43 (2H,s, CH_2 side chain); 5.42 (1H,s,OH); 5.9 (1H,s,CH thiazolidinone);6.95(4H,m,Ar-H)and 7.2-7.6 (9H,m,Ar-H and chalcone).

3. IN SILICO ANALYSIS AND MOLECULAR DOCKING STUDY:

The purpose of this study was to analyze the inhibitory action of the newly synthesized compounds to COX-2 isoenzyme by computational docking studies. The crystallographic structure of molecular target COX-2 isoenzyme was obtained from PDB database. Celecoxib a well-known brand of COX-2 inhibitor and naproxen as a classical NSAID were taken as the standards for comparative analysis. Computational docking analysis was performed using GOLD.
The PLP fitness indicated that the COX-2 protein was successfully docked with the newly synthesized compounds as shown in table 1. The docking of COX-2 target with the newly synthesized compounds using docking procedure revealed that all the computationally predicted lowest energy complexes of COX-2 are stabilized by intermolecular hydrogen bonds.

Table 1 PLP fitness value and H bonding of the synthesized compounds.

compound	PLP fitness value	No of H bonding
Celecoxib	91.25	2
naproxen	74.23	2
I_a	93.98	2
I_b	84.82	5
I_c	68.23	2
I_d	79.02	2

Docking results showed that the newly synthesized compounds can enter the substrate-binding region of the active site and the compound (I_a) had showed the highest PLP fitness, however compound (I_c) appear to have the lowest binding ability compared to celecoxib which may be due to presence of para nitro group which deactivate the ring system. Finally, the results demonstrated clearly that there is a good correlation between in vivo and in silico study.

4. PRELIMINARY PHARMACOLOGICAL STUDIES

4.1. Anti-inflammatory Evaluation Study

In vivo acute anti-inflammatory effects of the chemically synthesized compounds (I_{a-d}) were evaluated in egg white induced paw oedema. Their evaluation for their anti-inflammatory activity based on measuring the decreases of paw thickness.

Table 2. Compounds with their molecular weight and dose

compounds	Molecular weight	Dose mg/ kg
Naproxen	230.26	50
I_a	387.5	84
I_b	403.5	87
I_c	432.49	94
I_d	401.52	88

4.1.1. Methods

A.Animals:
Albino rats of either sex weighing (170 ± 10 gm) were housed in Baghdad university / college of science under standardized conditions for 10 days for acclimatization. Animals were fed commercial chaw and had free access to water.
Animals were brought to the laboratory, one hour before the experiment, and were divided into six groups (each group consist of 6 rats) as follows:
Group A: six rats served as a control and treated with the vehicle (propylene glycol 50% v/v).
Group B: six rats treated with naproxen as reference substance in a dose of 50mg/kg suspended in propylene glycol.[14]
Group C-F: six rats /group treated with the tested compounds (I_{a-d}) respectively in doses that determined below, also suspended in propylene glycol.

B. Calculations for Dose Determination
M.Wt. of Naproxen = 230.26
50mg/kg /230.26 = Dose / M.Wt. of the tested compounds [15]

C. Experimental Design
The anti-inflammatory activity of the tested compounds was studied using the egg-white induced edema model.
The paw thickness was measured by vernea at seven time intervals (0, 30, 60, 120, 180, 240, and 300 min) after drug administration. Acute inflammation was produced by a subcutaneous injection of (0.05 ml) of undiluted egg-white into the plantar side of the left hind paw of the rats; 30 min after intra-peritoneal administration of the drugs or their vehicle.

4.1.2. Statistical Analysis

The data was expressed as the mean ± SEM and results were analysed for statistical significance using student *t* test (Two Sample Assuming Equal Variances) for comparison

between mean values. While comparisons between different groups were made using ANOVA: Two factors without replication. Probability (P) value of less than 0.05 was considered significant.

5. Results and Discussions

The anti-inflammatory activity of the tested compounds has been done in comparison with their vehicle (control group) and Naproxen.

The tested compounds and the reference drug produced significant reduction of paw edema with respect to the effect of propylene glycol 50%v/v (control group). All tested compounds significantly limited the inflammation in paw edema, the onset of compound **(I_a)** started at time 60 min. while the remaining compounds and Naproxen started at 120 min.

Compounds ($I_{a,b,d}$) show higher anti-inflammatory activity than Naproxen (50mg/kg, i.p.) at 180 min., while compound (I_c) exhibited lower anti-inflammatory effect. However, the duration of action of all tested compounds continued till the end of experiment with statistically significant (P<0.05) reduction in paw edema thickness.

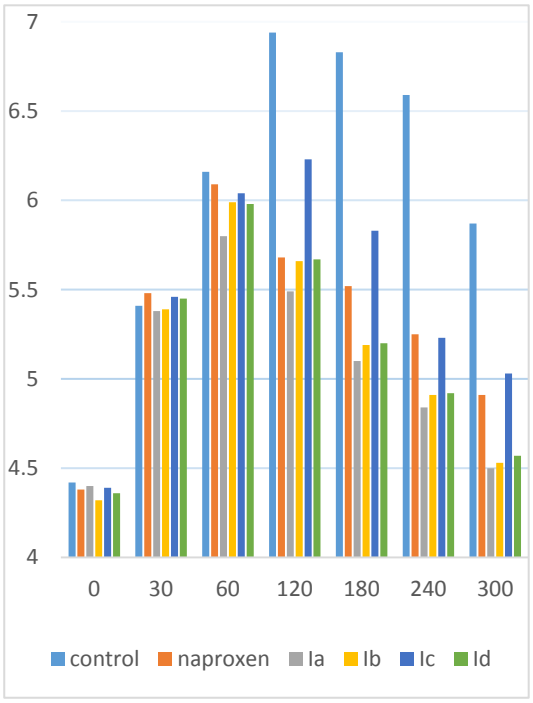


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Figure 2 Effect of Naproxen, propylene glycol and tested compounds (Ia-d) on egg-white induced paw oedema in rats.

Comparative Analysis

The comparison explains that at 0-30 min. there are no differences among all groups. Compounds ($I_{a,b,d,}$) at time 120-300 minutes show comparable effect to Naproxen; however at interval 180 minutes show significantly higher effect. Although; compound (I_c) significantly limited the increase in paw edema in comparison to control group, but it is significantly lesser effect than Naproxen and tested compounds ($I_{a,b,d,}$) at interval of 120-300 minutes.

Conclusions

Anti-inflammatory study using egg white induced edema model of inflammation revealed that the 4-thiazolidinone containing compounds have the same or higher activity compared to naproxen.

Acknowledgments

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