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Research Article

Design, synthesis, and acute anti-inflammatory assessment of new ketoprofen analogs having 4-thiazolidinone nucleus

Monther F. Mahdi[1], Noor H. Naser[2], Sanad M. Alfadhel*[2], Basma M. Abd Razik[1], Hasnah Osman[3]

[1]College of Pharmacy/Al- Mustansiriyah University, Baghdad, Iraq
[2]College of Pharmacy, University of Kufa, Najaf, Iraq
[3]School of Chemical Sciences, University Sains Malaysia, Minden, 11800 Penang, Malaysia

ABSTRACT

Ketoprofen is well known non-selective non-steroidal anti-inflammatory drug possess different side effects, the major one is the gastric irritation and ulceration due to the presence of free carboxyl group in the chemical structure in addition to lack of selectivity in its action by the inhibition of COX-1 enzyme which is required for the production of cytoprotective prostaglandins. This study aims to reduce the effects by conversion the carboxylic group into a thiazolidinone ring to reduce the direct side effects and increasing its selectivity toward COX-2 enzyme through increased bulkiness by using GABA as spacer, in which the Preferential inhibition of COX-2 is thought to be due to the additional space in the COX-2 hydrophobic channel, as well as to the presence of a side pocket in the channel. The preliminary anti-inflammatory study has revealed that V_e & V_f having better anti-inflammatory activity than ketoprofen. Number of docking results of synthesized compounds have surpassed the docking score of celecoxib as control. Incorporation of 4-Thiazolidinone pharmacophore into a ketoprofen maintained or enhanced it is anti-inflammatory activity.

Keywords: 4-Thiazolidinone; Anti-inflammatory activity; COX-2; ketoprofen.

INTRODUCTION

Pain & Pain remedies have deep-rooted evolutionary & archeological relationship (Weyrich et al. 2017). One of pain signaling components is cyclo-oxygenase (COX) enzyme, a highly-conserved enzyme (Ja et al. 2004) reflects its essentiality for survival that is substantiated by the results of mice knockouts (Harris 2003). The evolution of COX enzyme has resulted in two different expression patterns in Human, constitutive and inducible that is encoded by separate single copy genes on different chromosomes resulting in three isozymes (Ja et al. 2004)(Zarghi & Arfaei 2011)(Rouzer & Marnett 2009). Both types of expression have assigned different physiological and pathological roles with different predominant COX isozymes in which they considered the first enzymes in arachidonic acid breakdown cascade. A variety of prostaglandins (PGs) evoking wide range of physiological responses such as inflammation that might be manifested clinically as swelling, redness and pain (Ashley et al. 2012). COX-1 enzyme is constitutively expressed and it has been assigned as housekeeping and possess anti-inflammatory roles as it synthesize PGE2 which promotes gastric mucus production (Abdellatif et al. 2016), while its inhibition by NSAIDs has been linked to their adverse effects(Neal 2016). COX-2 enzyme expression is inducible by many triggers such as lipopolysaccharide (LPS), interlukin-1 (IL-1) and tumor necrosis factor-alpha (TNF) (Rumzhum & Ammit 2016) and it has been assigned to has pro-inflammatory functions (Ricciotti & FitzGerald 2012), hence its inhibition by NSAIDs would therefore decrease pain and inflammation. It has been discovered that there is also a constitutive expression (tissue specific expression) of COX-2 (Euchenhofer et al. 2001). Therefore, selective COX-2 inhibitors would theoretically lessen the associated adverse effects of non-selective COX-1/COX-2 inhibitors. The chemical development of new NSAIDs have introduced new possible uses for cancer, neurodegenerative diseases (Bacchi et al. 2012), diabetes mellitus type 2 (Wellen & Hotamisligil 2005) , asthma (Murdoch & Lloyd 2010), and irritable bowel syndrome (Sinagra et al. 2016) alongside the classical therapeutic uses as anti-inflammatory, analgesics and antipyretics (Rang et al. 2012). The withdrawal of many selective COX-2 inhibitors class members led to few choices for decision makers for the patient (Block 2011). One of the possible approaches of developing new selective COX-2 is the repurposing of existing nonselective COX inhibitors scaffolds (Oprea & Mestres 2012). 4-thiazolidinone has been nicknamed as “magic moiety”(Gupta 2016) for its wildly reported biological activates such as anti-inflammatory

* Corresponding Author
Email: phsanad@gmail.com
Contact: +964-7812750371
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(Ashok.D.Taranalli 2010), analgesic (Jain et al. 2012), anticancer (Kamel et al. 2010), antimicrobial (Mahdi et al. 2017), antiviral (Tripathi et al. 2014), antipsychotic(Hrib et al. 1996) and antiparasitic (Abhinit et al. 2009). Therefore, a group of 4-thiazolidinone pharmacophore derivatives incorporated in the carboxylate group of a ketoprofen using GABA as spacer were designed, synthesized, studied by docking and preliminary evaluated as anti-inflammatory agents with expected inhibitory selectivity towards COX-2 enzyme.

MATERIALS & METHODS

Reagents and chemicals

All reagents and solvents are of analytical grade, and were supplied from (AKSci USA, Sigma Aldrich Germany and ketoprofen from Lishui Nanming China).

Instrumentation and chromatographic conditions

Electro thermal melting point apparatus and open capillary tubes were used to determine the melting points and are uncorrected. Thin layer chromatography was run DC-Kartan SI Alumina gel 0.2 mm, for checking the purity of the products as well as monitoring the progress of the reaction. Chromatograms were eluted by using two different solvent systems: A: Hexane: Ethyl Acetate (25:75). B: Chloroform: Methanol (85:15). Compounds were revealed upon irradiation with UV light. IR spectra were recorded on a FT-IR, Shimadzu 8100s spectrometer.^{1}HNMR spectra were recorded on Bruker 300 MHz-Avanc III.

Typical procedure for the reactions

The synthesis of target compounds (I-V_{a-g}) was achieved following procedures illustrated in Scheme 1. The carboxyl group of GABA was esterified in presence of thionyl chloride to give compound (I) that reacted with ketoprofen to give compound (II) which then reacted with hydrazine hydrate to give hydrazide derivative (III) in which the primary amine group was reacted with different aldehydes (listed in table 1) to give a series of Schiff bases derivatives (IV_{a-g}). The cyclization was induced in presence of thiomercaptoacetic acid to yield 4-thiozolidinone nucleus.

Synthesis of methyl-4-aminobutyrate hydrochloride (I) (Li & Sha 2008)**:**

GABA (9.7 mmol, 1g) was dissolved in methanol (10 ml) cooled to (0°C) then (9.7 mmol, 0.7 ml) thionyl chloride was added gradually to the solution and left at room temperature for (45 min.) then refluxed for 12 hrs. On completion of the reaction, the solvent was evaporated under reduced pressure and the residue was collected and washed with methanol several times until complete removal of thionyl chloride and purified by recrystallization from methanol: diethyl ether. FT-IR (cm^{-1}): 1743 (C=O ester) and 1180 (C-O-C ester). Melting point(°C): 120. R_f: A=0.80, B= 0.52.

Synthesis of methyl 4-(2-(3-benzoylphenyl) propanamido) butanoate (II) (Hadjipavlou-litina 2010)

A mixture of Ketoprofen (8.5mmol, 2.16g), and GABA methyl ester (8.5 mmol,1g) was dissolved in dichloromethane:DMF (5:1) 30 ml, then TEA (17 mmol, 1.2 ml) was added with continuous stirring. The reaction mixture was refluxed for 5 hours, when off white precipitate was formed which was removed by adding cold water to form two immiscible layers, the organic layer was removed by separatory funnel and dried using anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure to give an oily product that re-crystallized several times with ethyl acetate: petroleum ether to give the oil product. FT-IR (cm^{-1}): 3433 (N-H amid); 1734 (C=O ester) and 1658 (C=O amide). ^{1}H NMR (300 MHz, DMSO) δH: 3.67 (3H, s, CH_3 ester); 1.33 (3H, d, CH_3 ketoprofen); 3.34 (1H, m, CH ketoprofen); 1.74-2.38 (4H, m, CH_2 GABA); 6.75 (1H, s, NH amide); 7.47-7.83 (9H, m, aromatic CH_2). R_f: A= 0.96, B= 0.68.

Synthesis of 2-(3-benzoylphenyl)-N-(4-hydrazinyl-4-oxobutyl) propanamide (III) (Ibrahim et al. 2013)

A mixture of compound (II) (4.6 mmol, 1.7 g), and 80% hydrazine hydrate (13.8 mmol, 0.45 ml), was dissolved in methanol (25 ml) and stirred for 24 hours using magnetic stirrer, then solvent was evaporated under reduced pressure to give oily product that was washed several times with diethyl ether. FT-IR (cm^{-1}) 3394 (N-H amid); 1718 (C=O ketone) and 1660 (C=O amide). ^{1}H NMR (300 MHz, DMSO) δH: 4.14 (2H, d, NH_2 hydrazide); 8.93 (1H, s, NH hydrazide); 1.33 (3H, d, CH_3 ketoprofen); 3.04-3.72 (1H, m, CH ketoprofen); 1.65-2.39 (4H, m, CH_2 GABA); 6.75 (1H, s, NH amide); 7.49-8.09 (9H, m, aromatic CH_2); 8.93 (1H, s, NH-N). R_f: A= 0.92, B= 0.63.

Synthesis of 2-(3-benzoylphenyl)-N-(4-hydrazinyl-4-oxobutyl) propanamide (IV_{a-g}) (El-faham et al. 2013)

A few drops of glacial acetic acid were added to a mixture of compound (III) (1.37 mmol, 0.525 g), and (1.37 mmol) of appropriate aldehyde that listed in table (1) in methanol (25ml). The reaction mixture was refluxed for 5 hours. Then the solvent was evaporated under reduced pressure, and the oily product was washed several times with diethyl ether.

(R,Z)-2-(3-benzoylphenyl)-N-(4-(2-benzylidenehydrazinyl)-4-oxobutyl) propanamide (IV_a):

FT-IR (cm^{-1}): 3435 (N-H amide); 1681 (C=O amide) and 1600 (C=O amide overlap with C=N). ^{1}H NMR (300 MHz, DMSO) δH: 6.75 (1H, s, NH amide); 8.83 (1H, s, NH-N) and 8.18 (1H, s, NH=CH-Ar). R_f: A= 0.76, B= 0.81.

(R,Z)-2-(3-benzoylphenyl)-N-(4-(2-(4-chlorobenzylidene) hydrazinyl)-4-oxobutyl) propanamide (IV_b):

FT-IR (cm^{-1}): 3406 (NH amide); 1664 (C=O amide); 1602 (C=O amide overlap with C=N) and 1087 (C-Cl). R_f: A= 0.60, B= 0.77.

(R,Z)-2-(3-benzoylphenyl)-N-(4-(2-(4-methylbenzylidene)hydrazinyl)-4-oxobutyl) propanamide (IV_c):

FT-IR (cm^{-1}): 3433 (NH amide); 1658 (C=O amide); 1606 (C=O amide overlap with C=N). R_f: A= 0.53, B= 0.60.

(R,Z)-2-(3-benzoylphenyl)-N-(4-(2-(4-(dimethylamino)benzylidene)hydrazinyl)-4-oxobutyl) propanamide (IV_d):

FT-IR (cm^{-1}): 3398 (NH amide); 1674 (C=O amide); 1608 (C=O amide overlap with C=N) and 1388 ($N(CH_3)_2$). R_f: A= 0.55, B= 0.61.

(R,Z)-2-(3-benzoylphenyl)-N-(4-(2-(4-hydroxybenzylidene)hydrazinyl)-4-oxobutyl) propanamide (IV_e):

FT-IR (cm^{-1}): 3020-3363 (Broad OH &NH amide band); 1685 (C=O amide) and 1616 (C=O amide overlap with C=N). R_f: A= 0.51, B= 0.65.

(R,Z)-2-(3-benzoylphenyl)-N-(4-(2-(4-methoxybenzylidene)hydrazinyl)-4-oxobutyl) propanamide (IV_f):

FT-IR (cm^{-1}): 3419 (NH amide); 1674 (C=O amidic); 1604 (C=O amide overlap with C=N) and 1251 (OCH_3). R_f: A= 0.50, B= 0.63.

(R,Z)-2-(3-benzoylphenyl)-N-(4-(2-(4-nitrobenzylidene)hydrazinyl)-4-oxobutyl) propanamide (IV_g):

FT-IR (cm^{-1}): 3421 (NH amide); 1666 (C=O amide); 1626 (C=O amide overlap with C=N); 1523 (NO_2 asymmetric); 1346 (NO_2 symmetric). R_f: A= 0.54, B= 0.69.

Synthesis of 2-(3-benzoylphenyl)-N-(4-hydrazinyl-4-oxobutyl) propanamide (V_{a-g}) (Wardell et al. 2010)

Each one of compounds (IV a-g) (1mmol) was dissolved in mercaptoacetic acid (6 ml) separately and heated to 60°C with continuous stirring for 3 hours.

Ethyl acetate (5ml) was added to the reaction mixture; the organic layer was washed with saturated sodium bicarbonate (3x20ml) and water (10ml), dried with anhydrous magnesium sulfate, and concentrated to give an oil. The oil washed with ether to give the final compounds.

4-((3-(3-benzoylphenyl)-2-oxobutyl)amino)-N-(4-oxo-2-phenylthiazolidin-3-yl) butanamide (V_a) :

FT-IR (cm^{-1}): 3435 (NH amide); 1712 (C=O ketone) and 1652 (C=O amide). ^{1}H NMR (300 MHz, DMSO) δH: 3.64-3.83 (4H, m, CH_2 thiazolidinone, CH_2 GABA); 5.75 (1H, s, S-CH-Ph); 6.75- 7.82 (14H, m, aromatic CH); 6.58 (1H, s, NH amide) and 8.1 (1H, s, NH-N). R_f: A= 0.98, B= 0.74.

4-((3-(3-benzoylphenyl)-2-oxobutyl)amino)-N-(2-(4-chlorophenyl)-4-oxothiazolidin-3-yl) butanamide (V_b):

FT-IR (cm^{-1}): 3128 (NH amide); 1730 (C=O ketone); 1690 (C=O amide) and 1087 (C-Cl). ^{1}H NMR (300 MHz, DMSO) δH: 3.63-3.76 (4H, m, CH_2 thiazolidinone, CH_2 GABA); 5.96 (1H, s, S-CH-Ph); 6.75- 7.82 (13H, m, aromatic CH); 6.75 (1H, s, NH amide) and 8.01 (1H, s, NH-N). R_f: A= 0.90, B= 0.67.

4-((3-(3-benzoylphenyl)-2-oxobutyl)amino)-N-(4-oxo-2-(p-tolyl)thiazolidin-3-yl) butanamide (V_c):

FT-IR (cm^{-1}): 3437 (NH amide); 1710 (C=O ketone) and 1685^{-1}637 (C=O amide). ^{1}H NMR (300 MHz, DMSO) δH: 3.64-3.83 (4H, m, CH_2 thiazolidinone, CH_2 GABA); 6.00 (1H, s, S-CH-Ph); 6.75- 7.83 (13H, m, aromatic CH_2); 6.75 (1H, s, NH amide) and 7.95 (1H, s, NH-N). R_f: A= 0.94, B= 0.67.

4-(2-(3-benzoylphenyl)propanamido)-N-(2-(4-(dimethylamino)phenyl)-4-oxothiazolidin-3-yl) butanamide (V_d):

FT-IR (cm^{-1}): 3431 (NH amide); 1710 (C=O ketone); 1669 (C=O amide); and 1384 ($C-N(CH_3)_2$). ^{1}H NMR (300 MHz, DMSO) δH: 3.65-3.83 (5H, m, CH_2 thiazolidinone, CH_2 GABA); 6.15 (1H, s, S-CH-Ph); 6.66-7.83 (14H, m, aromatic CH_2); 6.58 (1H, s, NH amide) and 7.89 (1H, s, NH-N). R_f: A= 0.81, B= 0.63.

4-(2-(3-benzoylphenyl)propanamido)-N-(2-(4-hydroxyphenyl)-4-oxothiazolidin-3-yl) butanamide (V_e):

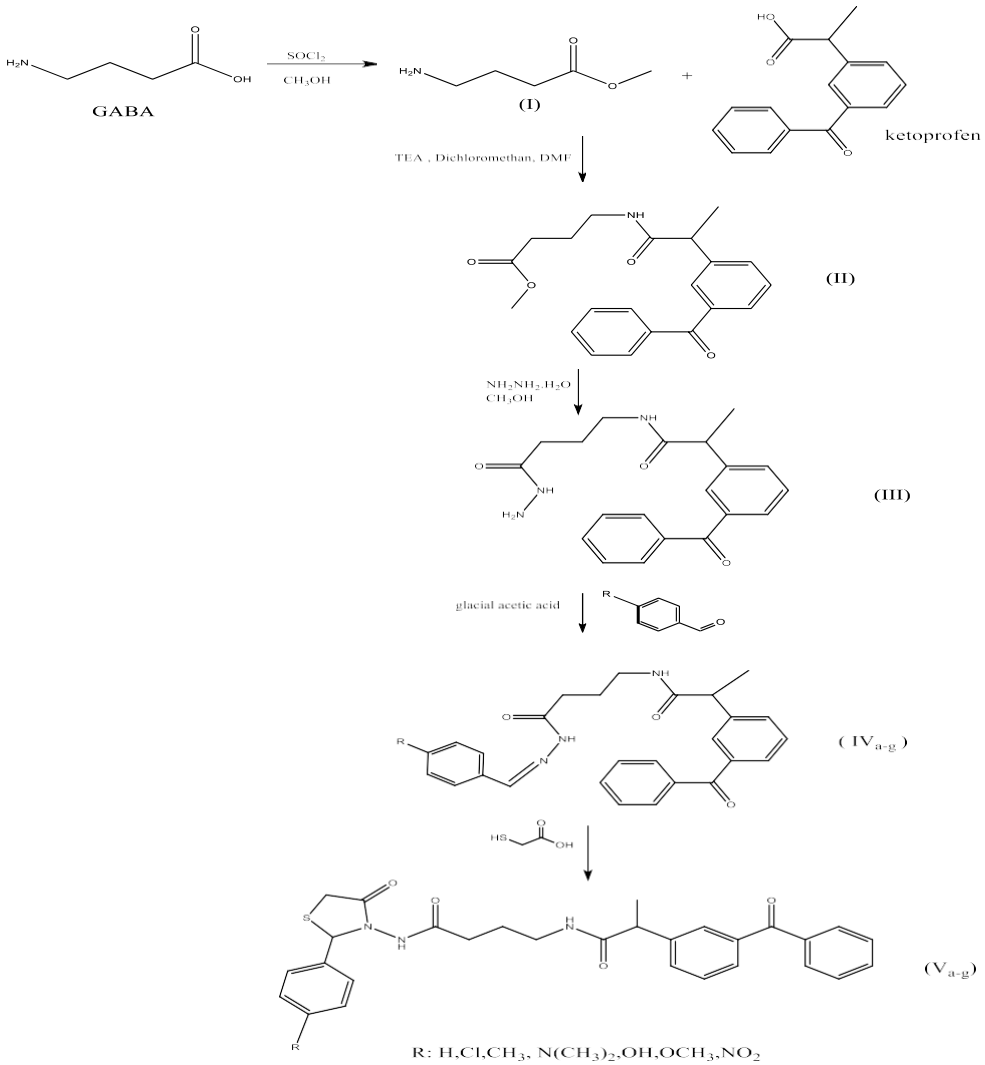
FT-IR (cm^{-1}): 3030-3398 (OH & NH amide); 1707 (C=O ketone) and 1656 (C=O amide). ^{1}H NMR (300 MHz, DMSO) δH: 3.43-3.83 (4H, m, CH_2 thiazolidinone, CH_2 GABA); 6.25 (1H, s, S-CH-Ph); 6.73- 7.83 (14H, m, aromatic CH); 6.58 (1H, s, NH amide); 8.17 (1H, t, OH phenolic) and 7.94 (1H, s, NH-N). R_f: A= 0.90, B= 0.67.

4-(2-(3-benzoylphenyl)propanamido)-N-(2-(4-methoxyphenyl)-4-oxothiazolidin-3-yl) butanamide (V_f):

FT-IR (cm^{-1}): 3431 (NH amide); 1714 (C=O ketone); 1610 (C=O amide) and 1255 ($C-O-CH_3$). ^{1}H NMR (300 MHz, DMSO) δH: 3.62-3.83 (4H, m, CH_2 thiazolidinone, CH_2 GABA); 5.75 (1H, s, S-CH-Ph); 6.73- 7.83 (13H, m, aromatic CH); 6.57 (1H, s, NH amide); and 7.94 (1H, s, NH-N). R_f: A= 0.95, B=0.61.

4-(2-(3-benzoylphenyl)propanamido)-N-(2-(4-nitrophenyl)-4-oxothiazolidin-3-yl) butanamide (V_g):

FT-IR (cm^{-1}): 3425 (NH amide); 1722 (C=O ketone); 1671-1631 (C=O amide) and 1517 (NO_2 asymmetric) and 1369 (NO_2 symmetric). ^{1}H NMR (300 MHz, DMSO) δH: 3.64-3.83 (4H, m, CH_2 thiazolidinone, CH_2 GABA); 6.13 (1H, s, S-CH-Ph); 6.75-7.97 (14H, m, aromatic CH); 6.62 (1H, s, NH amide); and 8.10 (1H, s, NH-N). R_f: A= 0.90, B=0.66.



Scheme 1: steps of chemical synthesis of target compounds

Table 1: Compounds with their molecular weight and dose

Compound	Molecular weight	Dose mg/ kg
Ketoprofen	254.28	5.00
V_a	529.66	10.41
V_b	564.1	11.09
V_c	543.68	10.69
V_d	572.72	11.26
V_e	545.75	10.73
V_f	559.68	11.01
V_g	574.65	11.30

Preliminary Pharmacological Studies

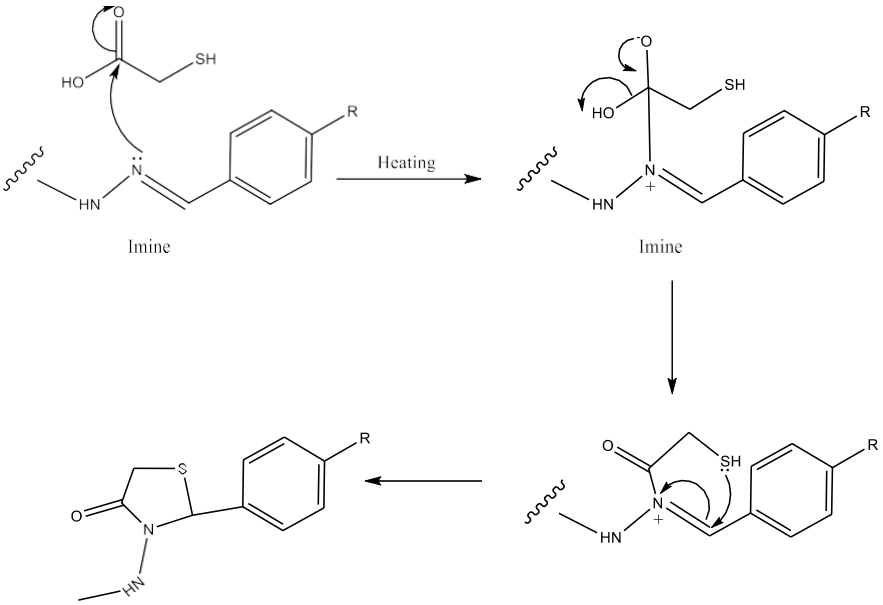
In vivo acute anti-inflammatory effects of the final compounds (V_{a-g}) were evaluated in egg-white induced paw edema, to compare their anti-inflammatory activity with ketoprofen (standard). The decrease of paw thickness is the basis of screening of the newly synthesized compounds for their anti-inflammatory activity. The protocol of assessment was approved by ethical committee of college of pharmacy / Kufa university (ACE file number 2 at 10-4-2017).

Anti-inflammatory Evaluation Study

Albino male rats were supplied by the animal house of the College of Medicine, AL-Nahrain University, and were housed in the animal house of college of pharmacy, Kufa University, under standardized conditions. Animals were fed commercial chaw and had free access to water ad *libitum*. Animals were divided into nine groups (each group consist of 6 rats) as follow:

Group A: six rats served as control; and treated with the vehicle (propylene glycol 50% v/v).

Group B: six rats treated with ketoprofen as reference substance in a dose of 5mg/ kg (Weisbroth et al. 2015). dissolved in propylene glycol 50% (v/v).



Scheme 2: Mechanism of thiazolidinone synthesis

Table 2: The anti-inflammatory effect of compounds Va-g on paw edema in rats

	compounds	Time (min)						
		0	30	60	120	180	240	300
	control	3.37±0.10	4.41±0.08	5.17±0.09	5.92±0.07	6.00±0.15	5.65±0.04	4.32±0.15
	ketoprofen	3.33±0.12	4.47±0.05	4.43±0.06*^{a}	4.37±0.14*^{a}	4.29±0.12*^{a}	4.21±0.08*^{a}	3.66±0.09*^{a}
	V_a	3.34±0.09	4.41±0.12	4.40±0.05*^{a}	4.32±0.06*^{a}	4.06±0.04*^{b}	3.98±0.10*^{b}	3.55±0.12*^{b}
	V_b	3.37±0.07	4.38±0.06	4.38±0.04*^{a}	4.35±0.05*^{a}	4.16±0.12*^{c}	4.01±0.14*^{b}	3.59±0.13*^{b}
	V_c	3.31±0.10	4.50±0.12	4.48±0.05*^{a}	4.41±0.10*^{a}	4.33±0.07*^{a}	4.25±0.04*^{a}	3.65±0.17*^{a}
	V_d	3.36±0.12	4.51±0.04	4.48±0.09*^{a}	4.39±0.12*^{a}	4.31±0.05*^{a}	4.24±0.08*^{a}	3.70±0.10*^{a}
	V_e	3.32±0.23	4.39±0.16	4.35±0.21*^{b}	4.30±0.07*	4.07±0.11*^{b}	3.82±0.23*^{c}	3.56±0.23*^{b}
	V_f	3.32±0.05	4.37±0.10	4.31±0.12*^{b}	4.29±0.04*	4.20±0.07*^{c}	4.09±0.12*^{d}	3.51±0.04*^{b}
	V_g	3.35±0.12	4.50±0.07	4.48±0.04*^{a}	4.38±0.12*	4.30±0.04*^{a}	4.25±0.08*^{a}	3.53±0.10*^{b}

*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 ketoprofen and propylene glycol. Time (30) is the time of injection of egg white (induction of paw edema). Non-identical superscripts (a, b, c, and d) among different groups are considered significantly different (p<0.05).

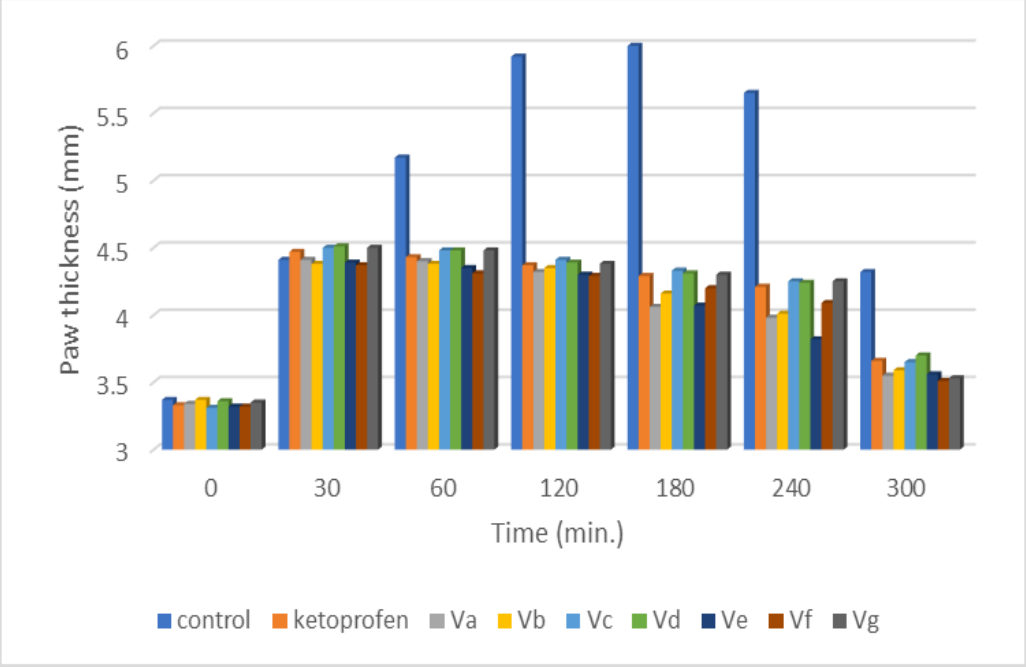
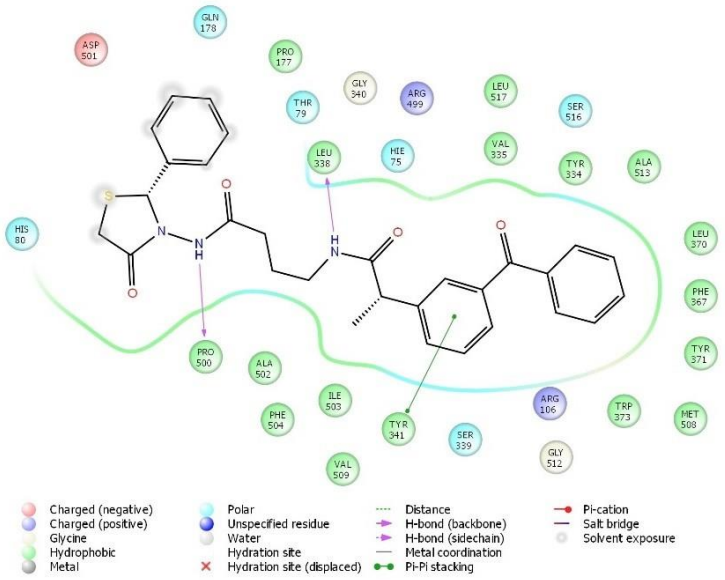


Figure 1: Effect of compounds Va-g in rats' paw edema

Table 3: Docking results and the corresponding compounds

Compound	Docking Score	Compound	Docking Score
Va (RR)	-9.831	Vg (SS)	-8.556
Ve (RS)	-9.724	Vf (SR)	-8.179
Va (SS)	-9.488	Vb (RR)	-7.509
Ve (RR)	-9.324	Vc (RS)	-5.765
Vb (SS)	-9.189	Ve (SS)	-5.440
Vf (RS)	-9.028	Vg (RR)	-3.876
Celecoxib	-8.890	Vc (RR)	-2.404
Va (RS)	-8.803	Vg (RS)	-2.123
Vb (RS)	-8.797	-	-
Ve (SR)	-8.729	-	-



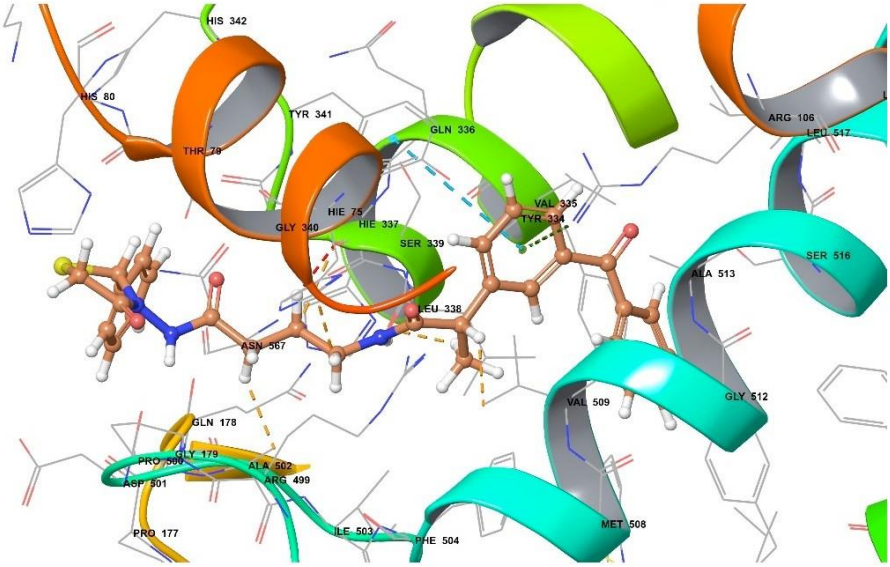


Figure 2: Compound Va interaction with COX-2 active site

Group C-i: six rats/group treated with the tested compounds (V a-g) in doses that determined in table (1). (dissolved in propylene glycol 50% v/v).

Calculations for Dose Determination:

$$\frac{\text{Dose of reference compound}}{\text{Mwt. of reference compound}} = \frac{\text{Dose of tested compound}}{\text{Mwt. of tested compound}}$$

Statistical Analysis

The data was expressed as the mean ± SEM and results were analyzed 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.

RESULTS AND DISCUSSIONS

Chemistry

The synthesis of methyl-4-aminobutyrate hydrochloride (I) was done by Fischer esterification method which have been confirmed by FT-IR by the disappearance of hydroxyl broad band of GABA and the shift of carbonyl absorption into higher frequency at 1743 and appearance of characteristic peak of C-O-C stretching at 1180.

The coupling of compound (I) with ketoprofen was done by direct amide bond formation using TEA as a catalyst to give compound (II) which confirmed by FT-IR by disappearance of broad band of hydroxyl group of ketoprofen and appearance of N-H stretching of amide bond instead at and appearance of higher absorption bond frequency at 1734 ester carbonyl group and lower absorption frequency at 1658 amide carbonyl group. ^{1}H-NMR was done and gave further confirmation for compound (II) synthesis.

Synthesis of hydrazide bond was done by reaction of compound (II) with hydrazine hydrate 80% by a ratio of (1:3 mmole) because hydrazine hydrate is serving as reactant and a catalyst. Synthesis of compound (III) was confirmed by FT-IR and ^{1}H-NMR.

Synthesis of compounds (IV_{a-g}) was done through Schiff base formation by the reaction of compound (III) with appropriate aldehydes in presence of acid catalyst. The imine bond formation has been confirmed by FT-IR & ^{1}H-NMR.

Final cyclization and 4-thiazolidine ring formation as illustrated in scheme 2 was done through solvent free synthesis by reacting compounds (IV_{a-g}) with thioglycolic acid to give compounds (V_{a-g}) and their structures were confirmed by FT-IR & ^{1}H-NMR.

Pharmacology

The anti-inflammatory activity of the tested compounds has been evaluated in comparison with their vehicle (control group) and ketoprofen (standard). Table (2) explains the effect of tested compounds (V_{a-g}) in comparison to control and ketoprofen. The intraperitoneal injection of tested compounds and the reference drug produced significant reduction of rat hind paw edema with respect to the effect of propylene glycol 50%v/v (control group). Compounds V_e & V_f exhibited superior anti-inflammatory effect when compared to ketoprofen as they reduce paw edema significantly more than ketoprofen throughout the time of the experiment ($p< 0.05$). Compound V_a & V_b produced comparable anti-inflammatory effect to ketoprofen at the first two hours of experiment but produced superior anti-inflammatory effect after the two hours till the end of the experiment as they reduce paw edema significantly more than ketoprofen ($p< 0.05$). Compounds V_c, V_d and V_g produced comparable anti-inflammatory effects when compared to ketoprofen at all the times of experiment as shown in Figure (1).

Docking study

The docking studies were performed using Glide module (Glide version 5.7; Schrodinger, LLC, 2011) installed on Maestro 9 (Maestro version 9.3, Schrodinger, LLC, 2012). The positive control (Celecoxib) show docking score at -8.89 with COX-2 enzyme. The studied group of compounds having two chiral centers giving rise for four stereoisomers (SS, RR, SR, RS) that may interact with the chiral environment of enzyme active site as shown in figures (2-7). The studied group consisted of seven target compounds giving rise for 28 possible molecules that may interact with active site.

Only 17 compounds have shown a docking ability to the enzyme according to the software settings with for this docking procedure. Six compounds out of 17 are showing higher affinity score than celecoxib (more negative score) as shown in table (3).

Generally, the in silico results (except V_d which has no docking ability according to software settings) are consisted with in vivo results in which all compounds are showing pharmacological action.

CONCLUSIONS

Acute anti-inflammatory study using egg white induced edema model of inflammation revealed that the incorporation of 4-thiazolidinone pharmacophore into a ketoprofen maintained or enhanced it is anti-inflammatory activity and these results were well substantiated by in silico docking study.

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