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Graphical Abstract

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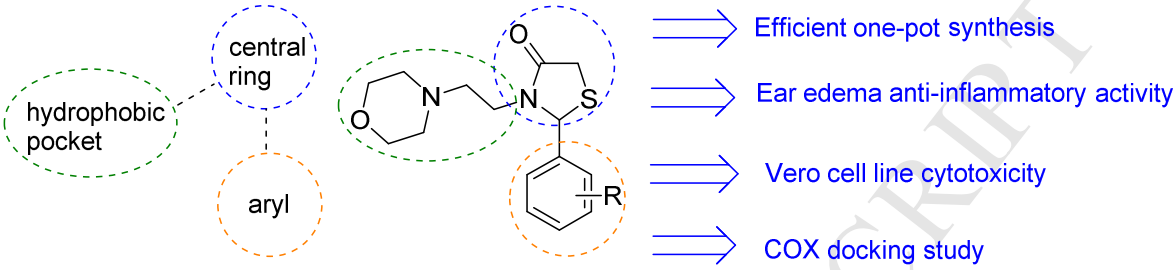

2-Aryl-3-(2-morpholinoethyl)thiazolidin-4-ones: Synthesis, anti-inflammatory *in vivo,* cytotoxicity *in vitro* and molecular docking studies.

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ABSTRACT

Seven new 4-thiazolidinones bearing the morpholino moiety were easily synthesized by one-pot reactions of 4-(2-aminoethyl)morpholine (2-morpholinoethylamine), arenealdehydes and mercaptoacetic acid refluxing toluene for 19 hours with moderate to good yields (45-97%). These novel compounds were fully identified and characterized by NMR spectroscopy and mass spectrometry. Thiazolidin-4-ones *in vivo* anti-inflammatory activities were determined using a croton oil-induced ear edema model of inflammation in BALB C mice. The best results were found for compounds **4c** (49.20 mmol/Kg)**, 4d** (49.20 mmol/Kg) and **4f** (52.48 mmol/Kg), which showed the ability to decrease the ear edema in mice by 50%, 48% and 54%, respectively, when compared to the standard drug indomethacin. In addition, the *in vitro* cytotoxicity activity of thiazolidin-4-ones against Vero cells was also performed and four compounds (**4a**, **4c**, **4d** and **4f**) showed no toxic effect at 500 µg/mL. A docking simulation of compounds into the 1Q4G (COX-1) and 4PH9 (COX-2) enzymes binding site was conducted. This preliminary result will guide us in for further studies to improve the anti-inflammatory activity.

Keywords: thiazolidin-4-ones, morpholine, anti-inflammatory activity, ear edema, cytotoxicity, molecular docking.

1. Introduction

The skin and mucous are an important protection of the immune response to external stimuli by mechanical and chemical agents, pathogens and autoimmune response [1, 2]. The inflammatory response is characterized at clinical level by four classic signs: heat; redness; swelling; and pain. An exaggerated inflammatory response can cause a physiological imbalance of the tissue and / or organ [3]. Anti-inflammatory drugs are widely used to treat inflammatory acute and chronic conditions. The clinical use of non-steroidal anti-inflammatory (NSAIDs) drugs is associated with significant toxicity particularly in the gastrointestinal tract and kidney [4]. Medicinal and organic chemistry have shown a great interest to the design and production of new bioactive compounds without these side effects [5].

In this context, the heterocyclic 4-thiazolidinone ring is an important moiety in medicinal chemistry. Alone or when incorporated into different templates, it has been reported to show potent biological activities [6] including *in vivo* anti-inflammatory activities in models of acute inflammation such as induced paw edema and ear edema assays [7]. Hu et al 2013 [8] also reported the significant inhibitory activity of thiazolidinones against LPS-induced TNF-α and IL-6 expression in RAW 264.7 macrophages. Based on knowledge regarding anti-inflammation mechanism results from the inhibition of cyclooxygenases (COX-1/COX-2) [9] and lipoxygenase (LOX) enzymes [2, 10], thiazolidinones may provide a promising approach for the treatment of multi-factorial disease states such as inflammation.

Compounds with morpholine scaffolds also feature broad biological activities as antioxidants, anti-antimicrobials, antidepressants, anti-diabetics and anti-inflammatories [11]. Literature reports that the modification of the classic anti-inflammatory ibuprofen [12] and indomethacin [13] with addition of the morpholine group increases the selective COX-2 inhibition.

Our research group has been working with the chemical and biological properties of heterocyclic thiazolidin-4-ones for the past few years. Recently, we published thiazolidin-4-ones with antifungal [14] and antioxidant [15] activity. In this work, we aim to report the synthesis,

anti-inflammatory activity against ear edema and cytotoxic activity against Vero cell lines of thiazolidin-4-ones bearing the morpholine core. Docking studies with COX-1 and COX-2 were also reported. The compounds were designed according to a similar structure of coxibs as shown in **Figure 1**.

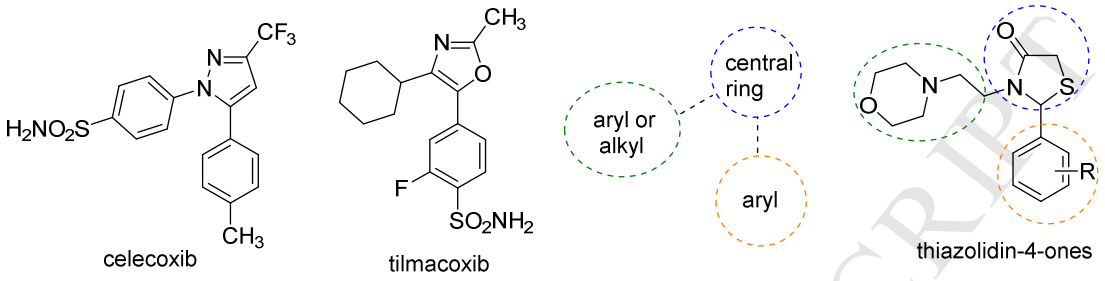


Figure 1. Design for proposed thiazolidin-4-ones

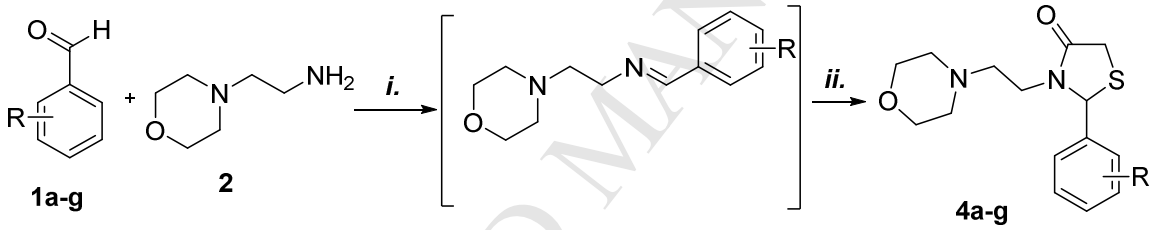
2. Results and discussion

4-Thiazolidinone derivatives have attracted continuing interest over the years because of their diverse biological activities, recently reviewed by Tripathi in 2014 [6a]. A literature survey reveals that several thiazolidin-4-ones have been prepared based on different synthetic routes and the most common procedure involves a three component reaction of an aldehyde or ketone, a primary amine and mercaptoacetic acid, either in a one (multicomponent or one-pot) or two-step process [6].

Initially, reaction conditions were studied for the formation of thiazolidinone **4c** by a conventional heating methodology. For this purpose, all reactions were monitored by TLC and/or GC analysis. According to papers that we recently published, the kind of amine (aromatic or aliphatic) influences the reaction time and the multicomponent or one-pot reaction influences the yields: Aliphatic amines generally required less time to complete formation of thiazolidin-4-ones than aromatic amines [14a, 16]; and the one-pot reaction is important to avoid the formation of undesired by-product oxathiolan-5-one [17]. We reported recently that thiazolidin-4-ones from aliphatic amines were obtained in 2 to 5 hours [14a, 15a, 18]. Therefore, we expected a similar reaction time to the thiazinanones synthesis when the aliphatic amine 2-

aminoethylmorpholine was used. However, to our surprise, 19 hours were needed to complete thiazolidinone **4c** formation.

So, the 4-thiazolidinone derivatives **4a-g** were synthesized from the reaction of 2-aminoethylmorpholine **2** (1 mmol) and corresponding arenealdehyde (**1a-f**) (1 mmol) in toluene reflux for 3 hours to complete formation in situ of the imine. Mercaptoacetic acid (**3**) (2 mmol) was then added and the mixture was continuously refluxed for more than 16 hours to intramolecular cyclization (Scheme 1). The thiazolidin-4-ones **4a-g** were obtained at moderate to excellent yields after purification by washing with hot hexane (45-97%). The structures of unpublished thiazolidin-4-ones **4a-g** were identified and characterized by ^{1}H and ^{13}C NMR, with the assistance of 2D spectra (HMBC and HSQC) and by HRMS (see experimental section).



R= 4-F, 4-Cl, 2-NO_2, 3-NO_2, 4-OH, 4-OCH_3, 4-CH_3

Scheme 1. General synthesis of thiazolidin-4-ones: Reaction conditions: (***i***) Toluene, 110°C, 3h; (***ii***) $HSCH_2COOH$, 110°C, 16h.

To investigate the anti-inflammatory activity, the phorbol ester 12-*o*-tetradecanoylphorbol-13-acetato (TPA) was used. It was present in croton oil (Croton tiglium L.), a potent phlogistic agent and tumor promoter. Croton oil promotes an intense inflammatory and hyperproliferative response, resembling some skin diseases [19, 20]. Topical application of croton oil/TPA promotes an acute inflammatory reaction characterized by vasodilation, polymorphonuclear leukocyte infiltration into the tissue and edema formation [3a, 21]. Ear thickness was measured 6 hours after the administration of the compounds. Indomethacin was used as a standard drug [3a].

Table 1 shows the result for the anti-inflammatory activity of thiazolidin-4-ones **4a-g**. All compounds reduce the ear inflammation at 166 mg/Kg (39-58%). The best activity was found for compounds **4c** and **4d**, both with electron-withdrawing substituent nitro, and for compound **4f**, with electron-donating substituent methoxy, which showed to be able to decrease the ear edema in mice, by 50%, 48% and 54%, respectively. For this reason, it was difficult to determine a structure-activity relationship for the aryl group substituent attached to thiazolidinones. This result suggests aryl has little electronic influence of the activity. These results were similar to those found for the standard drug Indomethacin that reduced the ear inflammation by 58%.

It should be noted that the thiazolidin-4-ones **4a-g** at doses up to the highest concentration tested (333 mg/Kg), did not show the classical symptoms of inflammation, such as heat, redness and swelling when compared to induced control with a phlogistic agent (acute inflammation). Nowadays, it is difficult to treat inflammatory skin diseases because they are chronic and require a lengthy treatment which could result in the development of drug resistance. The most common treatments used are based on corticosteroids and recently, treatments such as monoclonal antibodies against immunoglobulin E have also been used [21].

In addition (**Table 1**), the compounds showed low *in vitro* toxic effects on the Vero cell line, especially thiazolidin-4-ones **4a**, **4d** and **4f** that maintain the cell viability of 89, 92 and 89% at the highest concentration tested (1000 µg/mL). Compounds **4b**, **4e** and **4g** showed toxicity at 500 µ/mL, reducing cell viability by more than 20%.

Table 1. Anti-inflammatory (*in vivo*) and cytotoxicity activities (*in vitro*) of thiazolidin-4-ones **4a-g**.

Comp.	R	M.W.[a]	Ear edema		Cytotoxicity				
			Dose[b] (mmol/Kg)	Anti-infl. activity[c]	Cell viability (µg/mL) (%)				
					62.5	125	250	500	1000
4a	4-F	310.39	53.48	39	100	100	100	91	89
4b	4-Cl	326.84	50.79	44	87	87	87	76	74
4c	2-NO_2	337.39	49.20	50	98	98	98	91	83

4d	3-NO_2	337.39	49.20	48	100	100	100	92	92
4e	4-OH	308.04	53.88	45	83	83	83	79	73
4f	4-OCH_3	322.42	51.48	54	100	100	100	90	89
4g	4-CH_3	306.42	54.17	42	87	87	80	76	73
Indomethacin		357.77	9.22	58					

[a] g/moL molecular weight; [b] 166 mg/Kg; [c] % at 6h of anti-inflammatory activity.

The importance of these anti-inflammatory activities led us to perform a study of molecular docking in cyclooxigenase-1 (COX-1) and cyclooxigenase-2 (COX-2) enzymes to verify a comparison with a standard celecoxib drug. Results are shown in **Table 2** for COX-1 and **Table 3** for COX-2.

Table 2. Interactions of ibuprofen, indometacin, celecoxib and thiazolidin-4-ones **4a-g** with COX-1.

Compound	Dock score (Kcal/mol)	N of interactions	Distance (Å)	aa	
Ibuprofen	-8.1	3	2.9	Arg120	COOH
			3.1	Arg120	COOH
			2.8	Tyr355	COOH
Indometacin	-7.8	3	4.0	Arg120	N
			3.7	Tyr355	OCH_3
			3.1	Tyr355	COOH
Celecoxib	-6.6	3	3.7	His513	NH_2
			2.7	Tyr355	SO_2
			3.1	Tyr355	SO_2
4a	-6.5	2	3.2	Arg120	O morph
			3.8	Arg120	N morph
4b	-6.4	2	3.2	Arg120	N morph
			3.8	Arg120	N morph
4c	-6.6	2	3.1	Arg120	NO_2
			3.8	Tyr355	NO_2
4d	-6.6	2	3.1	Arg120	N morph
			3.8	Arg120	N morph
4e	-6.0	0	-	-	-
4f	-6.3	2	3.1	Arg120	O morph.
			3.8	Arg120	N morph.
4g	-6.7	1	3.3	Arg120	N morph.

Table 3. Interactions of ibuprofen, indometacin, celecoxib and thiazolidin-4-ones **4a-g** with COX-2.

Compound	Dock score (Kcal/mol)	N of interactions	Distance (Å)	aa	
Ibuprofen	-7.6	2	2.4	Tyr355	-C(O)OH

			3.0	Arg120	-C(O)OH
Indometacin	-7.7	2	3.1	Ser353	OCH_3
			3.7	His90	OCH_3
Celecoxib	-10.6	6	3.7	His90	NH_2
			3.4	Arg513	SO
			2.1	Gln192	NH_2
			2.7	Leu352	NH_2
			2.2	Ser353	NH_2
			3.4	Tyr355	NN
4a	-6.3	2	3.0	Arg120	O morph.
			3.3	Arg120	O thiaz.
4b	-6.4	2	3.0	Arg120	O morph.
			3.3	Arg120	O thiaz.
4c	-6.7	1	3.6	Arg120	NO_2
4d	-7.6	6	2.9	Tyr355	NO_2
			3.9	Arg120	NO_2
			3.4	His90	O morph.
			3.7	Ser353	O and N morph.
			3.7	Met523	O thiaz.
			3.6	Ser530	S thiaz.
4e	-6.8	3	3.7	His90	OH
			3.3	Arg120	Tyr355
			2.8	O morph.	OH
4f	-6.9	3	3.2	His90	O morph.
			3.3	Ser353	O and N morph.
			3.8	Ser530	S of thiaz.
4g	-6.6	2	3.0	Arg120	O morph.
			3.3	Arg120	O thiaz.

Celecoxib shows six interactions with COX-2 (**Figure 2**): a) four hydrogen bonds are formed between NH_2 with amino acids His90, Gln192, Leu352 and Ser353 at distances of 3.7, 2.1, 2.7 and 2.2 Å, respectively; b) hydrogen bonds between SO with Arg513 at 3.4 Å and; c) N of pyrazole ring with Tyr355 at 3.4 Å. In COX-1, the celecoxib shows only two hydrogen bonds between NH_2 with His513 (3.7Å) and one hydrogen bond of SO_2 with Tyr355 (2.7Å) (see supplementary information).

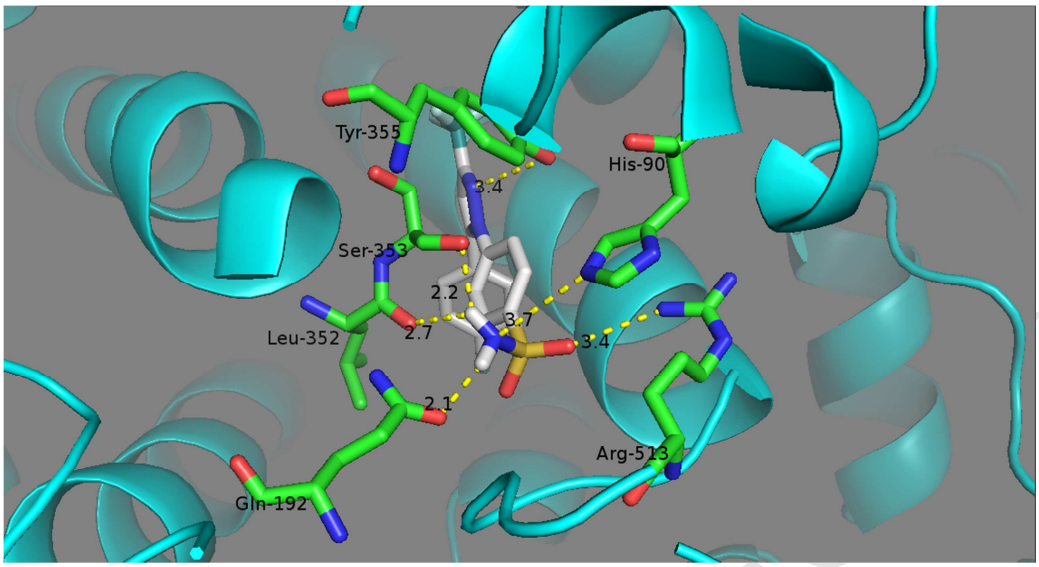


Fig. 2. Molecular docking of celecoxib binding site of COX-2 (dock score -10.6 kcal/mol) containing six hydrogen bonds as yellow dotted lines.

According to the docking analyses of thiazolidin-4-ones **4a-g**, the nature and position of the substituent in the phenyl ring induce different interactions with COX-2 (**Figure 3**).

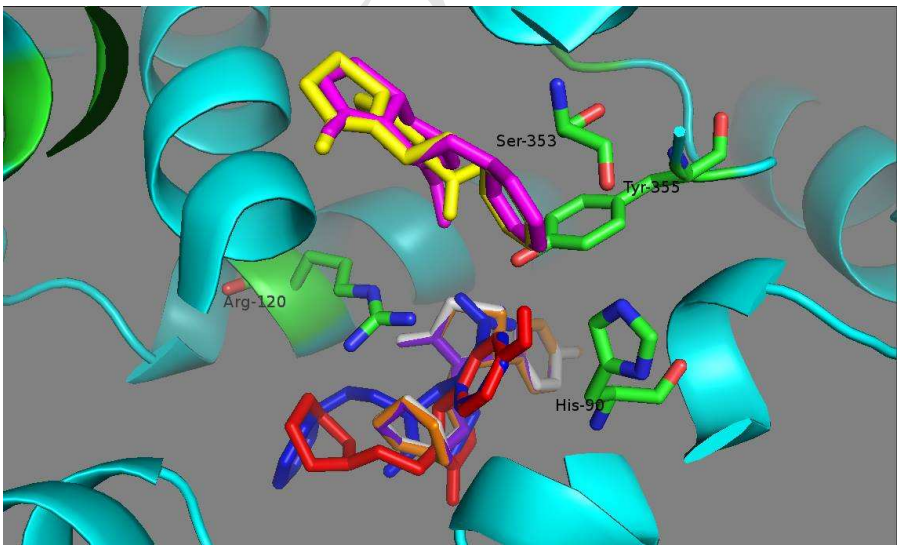


Fig. 3. Molecular docking of thiazolidin-4-ones **4a-g** binding COX-2. **4a** (White); **4b** (orange); **4c** (blue); **4d** (yellow); **4e** (red); **4f** (pink); **4g** (purple).

Thiazolidin-4-ones **4d**, **4e** and **4f** exhibit the greatest number of interactions with COX-2 amino acids. Compound **4d**, with nitro group at *m*-position, shows six hydrogen bonds

(**Figure 4**): a) the O of morpholine ring with His90 (3.4 Å), as NH_2 in celecoxib; b) the O and N of the morpholine ring with Ser353 (3.2 and 3.7 Å), as NH_2 in celecoxib; c) the NO_2 group with Tyr355 (2.9 Å), as NN of pyrazole in celecoxib; d) the NO_2 with Arg120 (3.9 Å) and; e) the S of the thiazolidinone moiety with Ser530 (3.5 Å). This compound (**4d**) exhibits only two interactions with COX-1 (see supplementary information) through two hydrogen bonds between the N of morpholine and Arg120 with a distance of 3.1 and 3.8 Å.

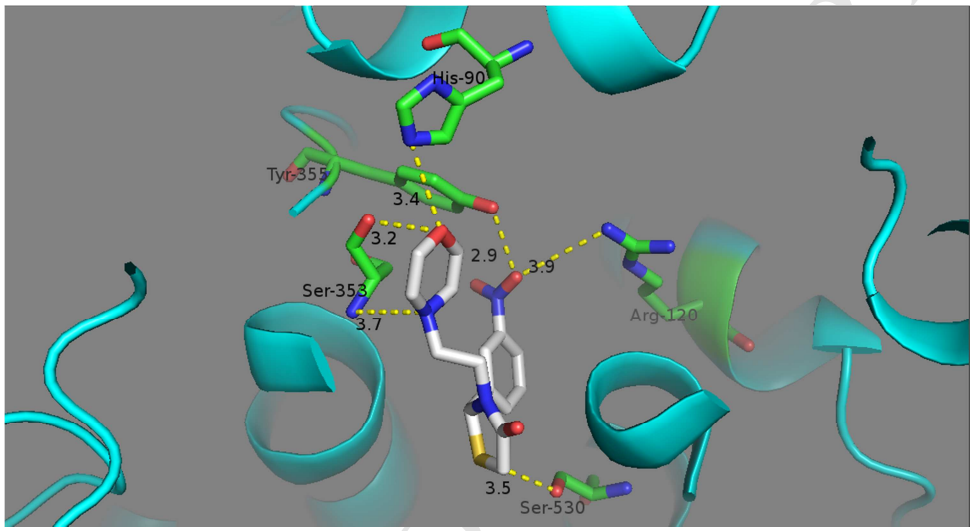


Fig. 4. Molecular docking of **4d** binding site of COX-2 with dock score -7.6 kcal/mol exhibits six hydrogen bonds illustrated as yellow dotted lines.

Changing the NO_2 group from the meta position (**4d**) to the orto position (**4c**) in the phenyl ring, changes the binding mode of thiazolidin-4-one **4c**. Compound **4c** shows only one hydrogen bond between the NO_2 group and Arg120 at 3.6 Å (**Table 3**). The COX-1 docking of compound **4c** shows two hydrogen bonds between the NO_2 and Arg120 and Tyr355 at 3.1 and 3.8 Å, respectively (**Table 2**).

The thiazolidin-4-one **4f** with substituent 4-OCH_3, exhibits COX-2 dock score -6.9 Kcal/mol, containing hydrogen bonds between O and N of morpholine with Ser353, O of thiazolidinone with Met523 (3.7 Å) and S of thiazolidinone with Ser530 (3.8 Å) (**Table 3**). This compound also shows one hydrogen bond between the N of morpholine and Arg120 (3.3 and 3.8 Å) (**Table 2**).

The thiazolidinone **4e** (R=4-OH) shows the COX-2 docking score -6.8 kcal/mol and reveals hydrophilic interactions (H-bonds) between the hydroxyl group and Tyr355 and His90 at 2.8 and 3.7 Å. The hydrogen bond between the O of morpholine and Arg120 at 3.3 Å of distance is also observed (**Table 3**). This compound does not show interaction (less than 4 Å) with amino acids of the COX-1 enzyme.

The compounds **4a, 4b** and **4g**, with substituents 4-F, 4-Cl and 4-CH_3, show dock scores -6.3, -6.4 and -6.6 Kcal/mol, respectively. According to docking, these compounds overlapped (**Figure 3**) and show the same type of interaction with COX-2 enzyme: hydrophilic interaction between Arg120 and O of the morpholine ring and O of thiazolidinone at 3.0 and 3.3 Å of distance (**Table 3**). These compounds also show interaction with the COX-1 enzyme through hydrogen bonds between N of morpholine and Arg120 (**Table 2**).

These *in silico* results suggest that some thiazolidin-4-ones may be COX-2 selective, especially thiazolidinone **4d**, however, enzyme studies were necessary to confirm this hypothesis.

3. Conclusion

In summary, seven novel thiazolidin-4-ones were easily synthesized by one-pot multicomponent reaction in good yields. In addition, two thiazolidin-4-ones (**4d** and **4f**) showed *in vivo* anti-inflammatory activity, reducing the ear edema inflammation up to 50% with no cytotoxic effect at 1000 μg/mL. The molecular docking showed that compound **4d** has six hydrogen bonds with six different amino acids, three of them like celecoxib had. These important findings will guide us to further chemical modifications for the development of new compounds to be employed as biologically useful agents.

4. Experimental

4.1. Chemistry

The reactions were monitored by thin layer chromatography (TLC, using silica gel 60F253 aluminum sheets; visualization by ultraviolet light 254 nm; (hexane / ethyl acetate 3:1) and/or by a Shimadzu Gas Chromatograph GC-2010, Column L.D., 0,25 nm; Column length, 30 m. Column Head Pressure, 14 psi, program: T°= 50 °C; t° = 2.0 min; rate 16.0 °C/min; Tf = 250 °C; tf = 10.0 min; Injector = 250 °C; Detector = 270 °C. Melting points were determined using open capillaries on a Fisatom model 430 apparatus and are uncorrected. GC–MS analyses were performed on a GC 2010-plus GC–MS-QP2010SE System AOC-20i – auto injector. ^{1}H and ^{13}C NMR spectra were recorded using a Bruker DRX 400 spectrometer (^{1}H at 400.14 and 500 MHz and ^{13}C at 100.61 MHz) or a Bruker Avance 500 spectrometer (^{1}H at 500.13 MHz and ^{13}C at 125.75 MHz) or a Bruker Ac-200F spectrometer (^{1}H at 200.13 MHz and ^{13}C at 50.32 MHz) in $CDCl_3$ containing TMS as in internal standard. For compound **4a**, the ^{13}C NMR was recorded at a Bruker DRX 400 spectrometer (^{13}C at 100.61 MHz). Infrared spectra (IR) were obtained in ATR on an IR Agilent Absorption Spectrometer, model CARY 630 FTIR. The elemental analyses (C, H, N) were used for all compounds on a CHNS 2400 Perkin Elmer Analyses equipment. Analyses were consistent within ± 0.4 theoretical values.

*4.1.1. General synthesis of 2-aryl-3-(2-morpholinoethyl)thiazolidin-4-ones **4a-g***

To a flask containing toluene (70 mL) attached to a Dean–Stark trap, 2-morpholinoethylamine **1** (1 mmol) and corresponding arenealdehyde **2a-f** (1 mmol) were added. The mixture was refluxed for 3 hours, the mercaptoacetic acid **3** (2 mmol) was added and the mixture was refluxed for 13 hours more. The organic layer was washed with a saturated solution of $NaHCO_3$ (3 x 30 mL), dried with $MgSO_4$ and concentrated to give the products. When necessary, the crude products were washed with hot hexane to furnish the pure thiazolidin-4-ones.

*4.1.1.1. Selected data for 2-(4-fluorophenyl)-3-(2-morpholinoethyl)thiazolidin-4-one **4a***

Yield 60% of the yellow solid, m.p. 90-93 °C. ^{1}H NMR δ (200 MHz, ppm, J_{H-H} = Hz); 7.30 (d, 2H, ^{3}J=8.6); 7.08 (t, 2H, ^{3}J=8.6); 5.85 (s, 1H, H2); 3.80 (dt, 1H, ^{2}J=12.4,^{3}J=6.1); 3.79 (dd, 1H,

^{2}J=14.4, ^{4}J=1.7, H5a); 3.70 (d, 1H, ^{2}J=14.7, H5b); 3.67 (t, 4H, ^{3}J=4.6); 2.78 (dt, 1H, ^{2}J=12.6, ^{3}J=6.3); 2.55-2.31 (m, 6H). ^{13}C NMR δ (50 MHz, ppm); 171.2 (C4); 137.8 (d, $^{1}J_{C-F}$=242.6); 128.9, (d, $^{3}J_{C-F}$=8.2); 11.,4 (d, $^{2}J_{C-F}$=21.9); 66.8; 63.3 (C2); 55.8; 53.8; 39.3; 32.9 (C5). IR (ATR) V max/cm-^{1}: 2950 (C-H sp^{2}); 2819 (C-H sp^{3}); 1653 (C=O); 1115 (C-O). GC-MS m/z (%): 310 (M^{+}, 2); 281 (5); 207 (12); 153 (2.5); 139 (3); 113 (7); 100 (100); 56 (12.5); 44 (32.5). HRMS-[ESI] for $C_{15}H_{19}FN_2O_2S$ (M+ H)$^{+}$: 307.1475; found 307.1475. Anal. Calc. for $C_{15}H_{19}FN_2O_2S$; C, 58.04; H, 6.17; N, 9.03. Found. C. 57.49; H, 5.98; N, 8.86.

4.1.1.2. Selected data for 2-(4-clorophenyl)-3-(2-morpholinoethyl)thiazolidin-4-one ***4b***

Yield 76% of the white solid, m.p. 50-53 °C, ^{1}H NMR δ (200 MHz, ppm, J_{H-H} = Hz); 7.37 (d, 2H, ^{3}J=8.5); 7.24 (t, 2H, ^{3}J=8.5); 5.84 (s, 1H, H2); 3.82 (dt, 1H, ^{2}J=14.3, ^{3}J=6.1); 3.75 (dd, 1H, ^{2}J=15.0, ^{4}J=1.7, H5a); 3.73 (dt, 1H, ^{2}J=14.4,^{3}J=6.4); 3.71 (t, 4H, ^{3}J=4.6); 3.70 (d, ^{2}J=14.4, H5b); 2.52 (dt, 1H, ^{2}J=12.8, ^{3}J=6.4); 2.42 (t, 4H, ^{3}J=4.3); 2.32 (dt, 1H, ^{2}J=12.9, ^{3}J=5.9). ^{13}C NMR δ (50 MHz, ppm); 171.2 (C4); 138.2; 134.9, 129.3; 128.3; 66.6 (2C); 60.2 (C2); 56.2; 53.6 (2C); 39.6; 32.8 (C5). IR (ATR) V max/cm-^{1}: 2948 (C-H sp^{2}); 2855 (C-H sp^{3}); 1670 (C=O); 1171 (C-O). GC-MS m/z (%): 326 (M^{+}, 2); 207 (5); 155 (5); 113 (3); 98 (100); 96 (4); 69 (5). $C_{15}H_{19}ClN_2O_2S$ (M+ H)$^{+}$: 327.0929; found 327.0931. Anal. Calc. for, $C_{15}H_{19}ClN_2O_2S$: C, 55.12; H, 5.86; N, 8.57. Found: C, 55.09; H, 5.87; N, 8.38.

4.1.1.3. Selected data for 3-(2-morpholinoethyl)-2-(2-nitrophenyl)thiazolidin-4-one ***4c***

Yield 45% of the white solid, m.p. 104-106 °C, ^{1}H NMR δ (200 MHz, ppm, J_{H-H} = Hz); 8.11 (d, 2H, ^{3}J=8.1); 7.72 (t, 2H, ^{3}J=7,7); 7.52 (t, 1H, ^{3}J=8.3, ^{4}J=1.2); 7.32 (t, 1H, ^{3}J=7.9), 6.60 (s, 1H, H2); 3.97 (dt, 1H, ^{2}J=14.3, ^{3}J=5.3); 3.74 (dd, 1H, ^{2}J=15.4, ^{4}J=1.8, H5a); 3.61 (d, 1H, ^{2}J=15.6); 3.76 (dt, 1H, ^{2}J=13.7, ^{3}J=6.9); 3.58 (q, 4H, ^{3}J=3.9); 2.81 (dt, 1H, ^{2}J=14.3, ^{3}J=5.0); 2.57 (dt, 1H, ^{2}J=12.4, ^{3}J=5.1); 2.44 (dt, 1H, ^{2}J=12.4, ^{3}J=5.1); 2.44 (t, 4H, ^{3}J=4.3).^{13}C NMR δ (50 MHz, ppm); 172.3 (C4); 136.7; 134.3, 129.1, 126.1; 115.9; 66.9 (2C); 63.2 (C2); 58.8; 53.6 (2C); 39,4; 32,7 (C5). IR (ATR) V max/cm-^{1}: 2970 (C-H sp^{2}); 2929 (C-H sp^{3}); 1653 (C=O); 1521 and 1346 (NO_2); 1115 (C-O). GC-MS m/z (%): 281 (M^{+}-56, 2); 220 (5); 207 (14); 113 (5); 100 (100); 70 (5); 56 (10). HRMS- [ESI] for $C_{15}H_{19}N_3O_4S$ (M+ H)$^{+}$: 338.1169 found: 338.1175. Anal. Calc. for $C_{15}H_{19}N_3O_4S$: C, 53.40; H, 5.68; N, 12.45. Found: C. 53.07; H, 5.75; N, 12.16.

4.1.1.4. Selected data for 3-(2-morpholin-4-ethyl)-2-(3-nitrophenyl)thiazolidin-4-one ***4d***

Yield 93% of the white solid, m.p. 89-92 °C, ^{1}H NMR δ (500 MHz, ppm, J_{H-H} = Hz); 8.24 (dd, 1H, ^{3}J=8.1); 8.08 (d, 1H, ^{4}J=1.7); 7.58 (d, 1H, ^{3}J=7.7); 7.53 (t, 1H, ^{3}J=7.5); 5.90 (d, 1H, ^{4}J=1.2); 3.86 (dd, 1H, ^{2}J=14.5, ^{3}J=5.9); 3.80 (dt, 1H, ^{2}J=14.5, ^{3}J=5.9); 3.86 (dd, 1H, ^{2}J=15.3, ^{4}J=1.6); 3.77 (d, 1H, ^{2}J=15.5); 3.70 (t, 4H, ^{3}J=4.6); 2.69 (dt, 1H, ^{2}J=14.5, ^{3}J=6.6); 2.44 (dt, 2H, ^{2}J=13.0, ^{3}J=6.3); 2.32 (q, 4H, ^{3}J=5.7). ^{13}C NMR δ (125 MHz, ppm); 171.3 (C4); 148.7; 132.8; 130.4; 124.1; 122.2; 66.9 (2C); 62.9 (C2); 56.0; 53.7 (2C); 39.6; 32.8 (C5). IR (ATR) V max/cm-^{1}: 3050 (C-H sp^{2}); 2950 (C-H sp^{3}); 1650 (C=O); 1500 and 1300 (NO_2); 1100 (C-O). GC-MS m/z (%): 281 (M^{+}-56, 4%); 253 (3); 207 (14); 113 (3); 100 (100); 70 (8); 56 (14). HRMS- [ESI] for $C_{15}H_{19}N_3O_4S$ (M+ H)$^{+}$: 338.1169; found 338.1171. Anal. Calc. for $C_{15}H_{19}N_3O_4S$: C, 53.40; H, 5.68; N, 12.45. Found. C. 52.94; H, 5.46; N, 11.96.

4.1.1.5. Selected data for 2-(4-hidroxyphenyl)-3-(2-morpholinoethyl)thiazolidin-4-one ***4e***

Yield 65% of the white solid, m.p. 138-141 °C, ^{1}H NMR δ (400 MHz, ppm, J_{H-H} = Hz); 7.09 (dd, 3H, ^{3}J=6.1, ^{4}J=1.5); 6.72 (dd, 1H, ^{3}J=8.5, ^{4}J=1.6); 5.69 (s, 1H, H2); 3.76 (dt, 1H, ^{2}J=14.4, ^{3}J=6.0); 3.74 (dd, 1H, ^{2}J=15.7,^{4}J=1.8, H5a); 3.67 (d, 1H, ^{2}J=15.4, H5b); 3.61 (t, 4H, ^{3}J=4.9); 2.78 (dt, 1H, ^{2}J=14.4, ^{3}J=6.3); 2.42 (dt, 2H, ^{2}J=12.9, ^{3}J=6.6); 2.45-2.24 (m, 5H).^{13}C NMR δ (100 MHz, ppm); 171,4 (C4); 141.9; 135.1; 130.4; 129.3; 127.1; 125,1; 66.9 (2C); 63.3 (C2); 55.9; 53.6 (2C); 39.5; 32.8 (C5). IR (ATR) V max/cm-^{1}: 3186 (OH); 2959 (C-H sp^{2}); 2924 (C-H sp^{3}); 1633 (C=O); 1113 (C-O). GC-MS m/z (%): 308 (M^{+}, 2); 280 (2); 207 (3); 113 (6); 100 (100); 56 (7). HRMS- [ESI] for $C_{15}H_{20}N_2O_3S$ (M+ H)$^{+}$: 309.1267 found: 309.1272.

4.1.1.6. Selected data for 2-(4-methoxylphenyl)-3-(2-morpholinoethyl)thiazolidin-4-one ***4f***

Yield 96% of the yellow solid, m.p. 40-43 °C. ^{1}H NMR δ (500 MHz, ppm, J_{H-H} = Hz); 7.25 (d, 2H, ^{3}J=8.3); 6.90 (d, 2H, ^{3}J=8.7); 5.80 (s, 1H, H2); 3.87 (s, 1H, OCH_3); 2.81 (dt, 1H, ^{2}J=13.1, ^{3}J=8.7); 3.76 (dd, 1H, ^{2}J=15.9,^{4}J=1.6, H5a); 3.75 (d, 1H, ^{2}J=16.8, H5b); 3.68 (t, 4H, ^{3}J=4.6); 2.81 (dt, 1H, ^{2}J=12.7, ^{3}J=7.1); 2.50 (dt, 1H, ^{2}J=12.8, ^{3}J=6.4); 2.37 (dt, 2H, ^{2}J=12.8, ^{3}J=6.4); 2.40 (t, 4H, ^{3}J=5.7). ^{13}C NMR δ (125 MHz, ppm); 171.2 (C4); 160.2; 138.2; 134.9; 129.6; 128.3; 66.9 (2C); 63.8 (C2); 55.8; 53.8 (2C); 39.5; 32.8 (C5). IR (ATR) V max/cm-^{1}: 2949 (C-H sp^{2}); 2850 (C-H sp^{3}); 1665 (C=O); 1115 (C-O). GC-MS m/z (%): 322 (M^{+}, 5); 208 (2); 151 (3);

113 (17); 100 (100); 86 (10); 70 (7); 56 (12). HRMS- [ESI] for $C_{16}H_{22}N_2O_2S$ $(M+H)^+$: 307.1475 found: 307.1475.

4.1.1.7. Selected data for 2-(4-methylphenyl)-3-(2-morpholinoethyl)thiazolidin-4-one ***4g***

Yield 89% of the white solid, m.p. 105-107 °C. ^{1}H NMR δ (400 MHz, ppm, J_{H-H} = Hz); 7.21 (s, 4H); 5.86 (d, 1H, ^4J=1.2, H2); 3,83 (dt, 1H, ^2J=14.1, ^3J=6.2); 3.82 (dd, 1H, ^2J=15.6,^4J=2.0); 3.73 (d, 1H, ^2J=15.5); 3.67 (t, 4H, ^3J=4.6); 2.83 (dt, 1H, ^2J=14.3, ^3J=6.7); 2.51 (dt, 2H, ^2J=12.8, ^3J=6.4); 2.36 (m, 9H). ^{13}C NMR δ (100 MHz, ppm); 171.4 (C4); 139.2; 136.4; 129.8; 126.9; 67.0 (C2); 63.9; 55.9; 53.6; 39.4; 33.0 (C5); 21.4 (Me). IR (ATR) V max/cm-^{1}: 3166 (C-H sp^2); 1633 (C=O). GC-MS m/z (%): 306 (M^+, 5); 192 (2); 135 (5); 113 (15); 100 (100); 86 (12); 70 (6); 56 (12). HRMS- [ESI] for $C_{16}H_{22}N_2O_2S$ $(M+H)^+$: 307.1475 found: 307.1475. Anal. Calc. For $C_{16}H_{22}N_2O_2S$, C, 62.71; H, 7.23; N, 9.09. Found. C. 62.69; H, 7.43; N, 9.14.

4.2. Animals

Experiments were performed on BALB/c male mice (25-35 g). The animals were controlled at room temperature (22 ± 3 °C) under 8 h, with access to water and food *ad libitum.* The experimental protocols in this study were approved and followed in accordance with the guidelines of the Ethical Committee in Animal Experimentation of the Federal University of Pelotas (CEEA 7068), in accordance with international guidelines.

4.3. Determination of anti-inflammatory effect in ear edema

BALB/c mice weighing 30 g, in mean, of were divided into three animals in each group. Edema was induced of croton oil at a concentration of 2 μg/ear, dissolved in acetone. The compounds **4a, 4b, 4c, 4d, 4e, 4f** and **4g** at 33, 100, 166 and 333 mg/Kg concentrations and indomethacin at 33 mg/Kg concentration (a positive control). All the suspensions were prepared in the vehicle acetone (20%) and administered by topical application in a constant volume of 20 μl per ear mouse. The anti-inflammatory potency of thiazolidin-4-ones was compared with the used standard drug, Indomethacin [22].

The edema was expressed as an increase in ear thickness due to inflammation. Ear thickness was measured before and after inflammatory induction. A 6 mm section of ears was obtained and weighed analytical balance (BEL engineering Mark M214A). The swelling induced was assessed as the increase in weight of ear punch of treated groups over untreated one and it was called the edema index, according to the method reported by Tubaro et al 1985 [19].

Croton oil-induced ear inflammation, carry out all experiments between 9 a.m. and 5 p.m., in order to avoid the influence of circadian variations in corticosteroid levels in the inflammatory responses [23].

4.4. Inhibition of inflammation analysis

The percentage protection of edema (inhibition of inflammation) was calculated according to the formula percentage anti-inflammatory activity = 100 x (1 - V_t/V_c), where V_t and V_c are the volume of edema in test compounds and control groups edema, respectively [24]. Measure was obtained at 6 h after application of compounds [5b, 7b].

4.5. Cytotoxicity assay (MTT assay)

The Vero cells (African green monkey kidney cell line) were used to analyze toxicity of compounds. The culture medium used to culture and compounds diluted was the modified Eagle´s medium (E-MEM, Sigma-Aldrich, USA) supplemented with penicillin (Sigma Aldrich, USA), streptomycin (Vetec, Brazil), amphotericin B (Cristália, Brazil) and enrofloxacin (Bayer, Brazil). The Vero cells were cultured in 96-well plates (2 x 10^6 cells mL^{-1}) in E-MEM supplemented with 10% (v/v) fetal bovine serum (Gibco, USA) and were allowed to attach for 24 h and 48 h before treatment with compounds. Series dilutions were prepared, ranging between 62.5, 125, 250, 500 and 1000 μg/mL of a previous solution in E-MEM. After removing the test product, cells were washed with phosphate-buffered saline (PBS). Then, as described by Mosmann (1983) [25] and Scopel e Silva [26], 50 mL of MTT (3-(4,5-dimethylthiazol-2-yl)-

2,5-diphenyltetrazolium bromide) solution 1 mg/mL in PBS, was added to each to each well. After 4h of incubation at 37 °C in darkness, the supernatant was replaced to 100 mL of dimethylsulfoxide (DMSO, Synth®, purity 99.9%) to solubilize the formazan salt generated by the reaction between salt MTT and mitochondrial metabolism of viable cell. The plates were gently shaken for 10 minutes, and the colorimetric intensity was evaluated by spectrophotometry wavelength of 540 nm. All experiments were performed three times. The percentage of viability was calculated as AT/AC x 100; where AT and AC are the absorbance of treated and control cells, respectively.

4.6. Molecular docking

The molecular docking studies of thiazolidin-4-ones **4a-g** were performed in AutoDock Vina program [27]. Firstly, the compounds were sketched in GaussView 5.0.8 program, then compounds ideal conformations were calculated by semi-empirical method PM6 in Gaussian 09 program and the obtained structures were used to perform the docking. The crystal structures of the enzymes Cyclooxygenase-1 (COX-1) and Cyclooxygenase-2 (COX-2) were obtained from PDB (Protein Data Bank) with codes 1Q4G and 4PH9 respectively. The 1Q4G enzyme is complexed with 2-(1,1'-biphenyl-4-yl) propanoic acid. This enzyme is from Ovis aries organism and the resolution of the crystal Structure is 2.00 Å. The 4PH9 enzyme is complexed with ibuprofen, this enzyme is from Mus musculus organism and the resolution of the crystal Structure is 1.81 Å. As enzyme preparation, the water molecules were removed and added polar hydrogens only. In the AutoDock Vina, the grid size to perform docking in 1Q4G was x = 26; y = 26; z = 26 and the coordinates was x = 27.772; y = 34.177; z = 201.688. To perform docking in 4PH9 the grid size was x = 26; y = 26; z = 26 and the coordinates was x = 11.677; y = 23.272; z = 25.420. To validate the methodology, it was done redoking. Poses obtained by redocking of 2-(1,1'-biphenyl-4-yl)propanoic acid and ibuprofen are close to crystal binders

indicating that the docking conditions are correct. The results were analyzed using the PyMOL Molecular Graphics System, Version 1.7.2.1.

Supplementary information

The supplementary information contains the ^{1}H and ^{13}C NMR, Infrared and CG-MS spectrum for thiazolidin-4-ones **4a-g.**

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2-Aryl-3-(2-morpholinoethyl)thiazolidin-4-ones: Synthesis, anti-inflammatory *in vivo*, cytotoxicity *in vitro* and molecular docking studies.

Research Highlights:

- A serial of novel thiazolidinones were easily obtained by one-pot procedure
- Thiazolidinones showed anti-inflammatory activity in the ear edema model
- Four compounds had no toxic effect at 500 mg/ml in the Vero cell line
- One compound has six interaction with COX-2 enzyme according *in silico* docking study