Antiedematogenic Activity of Two Thiazolidine Derivatives: *N*-Tryptophyl-5-(3,5-di-*tert*-butyl-4-hydroxybenzylidene) Rhodanine (GS26) and *N*-Tryptophyl-5-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-2,4-thiazolidinedione (GS28)

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The search for new anti-inflammatory drugs has been constant in several research centers. The use of the Bioisostery concept allows the elaboration of new bioactive compounds with different properties through the introduction of substitute groups in one or more positions of a main molecule with known biological activity. Preliminary works accomplished at our laboratory with 2,4-thiazolidinedione isosters demonstrated inhibitory activity on edema formation for *N*-tryptophyl-5-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-2,4-thiazolidinedione (GS28) and *N*-tryptophyl-5-(3,5-di-*tert*-butyl-4-hydroxybenzylidene) rhodanine (GS26). We verified the antiedematogenic and ulcerogenic activity of these two compounds in Wistar rats. The carrageenan induced paw edema suffered significant ($p<0.05$) inhibition (28.36% on average) for GS28 (100 mg/kg; v.o.) during the entire time of the experiment. GS26 (50 and 100 mg/kg; v.o.) significantly inhibited ($p<0.05$) the paw edema dextran induced (22.1 and 27.8%, for the respective doses) after 180 min. The compounds GS26 and GS28 did not show ulcerogenic activity on gastric mucous. The results suggest antiedematogenic action for both compounds without the appearance of gastric lesions.

Key words antiedematogenic activity; thiazolidine derivative; carrageenan paw edema; dextran paw edema

The main limitation in the use of anti-inflammatory drugs or drugs that inhibit some aspects of inflammation is their secondary effects, especially on the gastro-intestinal tract, during chronic use. Therefore, anti-inflammatory drugs without such effects have been one of the main objectives of researchers.[1)]

Modern organic chemistry development has provided an increased number of substances for various therapeutic aims. Among the methods for obtaining new drugs, molecular modification or variation using the Bioisostery concept is often used. Using this chemical concept, substitute groups can be introduced in one or more positions of a main molecule, creating similar compounds with varied biological activities that can show interesting medical properties. With this process, we can analyze the influence of the modification of an atom or a group of atoms on the main molecule's biological activity. The molecular alterations can result in molecules with similar, antagonistic or more larger potent activity than that of the original compound.[2)]

The thiazolidinic and imidazolidinic nuclei represent a category of compounds that present promising biological activities: insecticidal,[3)] antimicrobial, antifungal, narcotic, sedative, anesthetic, anticonvulsive and nematocidal.[4)] Góes *et al.* (1991)[5)] showed that thiazolidine and imidazolidine derivatives (or isosters) possess antifungal (against *Candida albicans* and *Neurospora crassa*) and moderate antibiotic activity (against *E. coli*, *S. aureus* and others).

Among the compounds that possess a thiazolidinic nucleus, 2,4-thiazolidinedione (Fig. 1) and rhodanine (Fig. 2) were studied by Lima (1999)[6)] with respect to the antiedematogenic properties of their isosters. In this study antiedematogenic activity was found for two bioisosters, *N*-tryptophyl-5-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-2,4-thiazolidinedione (GS28) (MW=476) (Fig. 3) and *N*-tryptophyl-5-(3,5-di-*tert*-butyl-4-hydroxybenzylidene) rhodanine (GS26) (MW=492) (Fig. 4). Studies related to acute toxicity (LD_{50}) of GS26 and GS28 in a range of oral doses of 25, 50, 100, 250, 500 and 1000 mg/kg did not indicate any toxicity. The aim of this study was to determine the existence of antiedematogenic properties on thiazolidinic isosters GS26 and GS28 in the response evoked by well-known edematogenic agents.

Chemistry Two new compounds *N*-tryptophyl-5-(3,5-di-

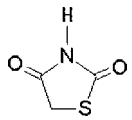

Fig. 1. 2,4-Thiazolidinedione

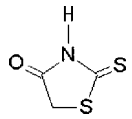

Fig. 2. Rhodanine

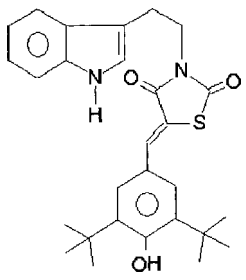

Fig. 3. *N*-Triptofil-5-substituted-2,4-thiazolidinedione (GS28)

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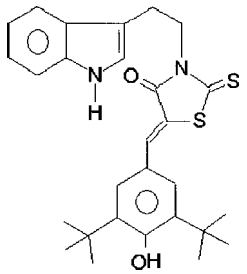

Fig. 4. *N*-Triptofil-5-substituted-rhodanine (GS26)

tert-butyl-4-hydroxybenzylidene) rhodanine (GS26) and *N*-tryptophyl-5-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-2,4-thiazolidinedione (GS28) were synthesized with satisfactory yield from Knoevenagel type condensation between 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde and *N*-tryptophyl-rhodanine and *N*-tryptophyl-2,4-thiazolidinedione, respectively. The intermediate *N*-tryptophyl-2,4-thiazolidinedione was obtained by the reaction of the 2,4-thiazolidinedione with the tryptophyl bromide in alkaline medium,[7] while *N*-tryptophyl-rhodanine was obtained by the reaction of carbon disulfide with tryptamine in amoniacal medium.[8] To establish the structure of the compounds (GS26) and (GS28) we used infrared, proton nuclear magnetic resonance and mass spectrometry. Data concerning the electron impact mass spectra of GS26 and GS28 have been published.[9]

Experimental

Melting points were determined on a Buchi apparatus and are uncorrected. TLC was carried out on precoated plates of silica gel 60 Merck with a fluorescent indicator. The ^{1}H-NMR spectra were obtained in $CDCl_3$ measured on a Gemini-200 Varian spectrometer at 300 MHz using tetramethylsilane as internal reference. Abbreviations for the signals are: s=singlet, d=doublet, dd=double doublet, t=triplet and m=multiplet. IR spectra were recorded on a Bruker IFS66 spectrometer. Electron impact mass spectra were recorded using an Ion-Trap Finnigan Mat GCQ instrument operating at 70 eV ionizing energy.

***N*-Tryptophyl-5-(3,5-di-*tert*-butyl-4-hydroxybenzylidene) Rhodanine (GS26)** To a mixture of 200 mg (0.725 mmol) of *N*-tryptophyl-rhodanine and 204 (0.87 mmol) of 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde in 3 ml of solution, NH_3/H_2O 1 : 2 and 2 ml of ethyl alcohol were added with a solution of 1.6 g of ammonium chloride in 3 ml of hot water. The mixture was stirred for 3 h at 60 °C. The resulting solid was purified by chromatographic column (CH_2Cl_2–*n*-hexane 7 : 3), yield 217 mg (61%) as yellow solid, mp 221—222 °C; IR (KBr): 3474 (NH), 1708 (C=O), 1585 (C=C); ^{1}H-NMR ($CDCl_3$): 8.02 (s, 1H), 7.86 (d, J=7.5 Hz, 1H), 7.71 (s, 1H) 7.37 (m, 1H), 7.19 (m, 2H), 7.13 (d, J=2.4 Hz, 1H), 4.42 (m, 2H), 3.18 (m, 2H), 1.48 (s, 18H); MS: *m/z* 492 (1.5, M^+), 247 (5.04), 143 (100), 130 (4.69), 116 (3.03).

***N*-Tryptophyl-5-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-2,4-thiazolidinedione (GS28)** To a mixture of 300 mg (1.154 mmol) of *N*-tryptophyl-2,4-thiazolidinedione and 324 mg (1.38 mmol) of 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde in 5 ml of solution, NH_3/H_2O 1 : 2 and 3 ml of ethyl alcohol was added with a solution of 2.5 g of ammonium chloride in 5 ml of hot water. The mixture was stirred for 11 h at 60 °C. The resulting solid was purified by chromatographic column (CH_2Cl_2–*n*-hexane 2 : 8), yield 223 mg (41%) as yellow solid, mp 207—208 °C; IR (KBr): 3362 (NH), 1724 (C=O), 1671 (C=O), 1587 (C=C); ^{1}H-NMR ($CDCl_3$): 8.04 (s, 1H), 7.86 (s, 1H), 7.76 (d, J=7.5 Hz, 1H), 7.36 (m, 1H) 7.18 (m, 2H), 7.09 (d, J=2.4 Hz 1H), 4.06 (m, 2H), 3.14 (m, 2H), 1.47 (s, 18H); MS: *m/z* 476 (8.8, M^+), 247 (2.91), 143 (100), 130 (14.39), 116 (2.24).

Animals Wistar rats (160—280 g) of either sex were maintained under standard environmental conditions (26±1 °C, 40% relative humidity and 12 h light/dark cycle), and deprived of standard food for 12 h before the assays.

Materials Saline solution (NaCl 0.9%); Carrageenan Type IV and Dextran (MW=77000) (SIGMA); Sulfuric ether (REAGEN); Indomethacin (CEME); GS26 and GS28 synthesized by Prof. Dr. Alexandre José da Silva Góes (Department of Antibiotics of UFPE) were used. For administration to the animals, the drugs to be tested were dissolved in vehicle (saline solution 0.9% and tween 80).

Biological Procedures. Hind Paw Edema in Rats[10] Four groups of 6 animals (Wistar rats, 200—280 g) were randomly selected and had their right hind paw injected with 0.1 ml of carrageenan type IV (1% w/v in saline) one hour after oral pre-treatment with GS26 (100 mg/kg), GS28 (100 mg/kg) or indomethacin (10 mg/kg) dissolved in vehicle. The control group received vehicle (5 ml/kg). Edema was measured plethysmographically (Ugo Basile plethysmometer, Italy) before and 60, 120, 180 and 240 min after the carrageenan challenge. The increase in paw volume was obtained by subtracting the paw volume measured before stimuli injection from the volume measured at different times later.

Paw Edema Induced by Dextran Eight groups of 6 animals (Wistar rats, 160—240 g) were randomly selected. The procedure was identical to that described previously, except that the edematogenic agent was dextran (PM=77000, 0.1% in saline). One hour before the hind paw injection of dextran (0.1 ml) the animals were orally treated with GS26 (25, 50, 100 mg/kg) or GS28 (25, 50, 100 mg/kg) dissolved in vehicle. The control group received vehicle (5 ml/kg).

Activity on Gastric Mucous Membrane[11] Animals of the two previous biological assays were sacrificed with an ether overdose and their stomachs were removed and split up along the larger curvature. After washing in running water the stomachs were examined in full detail. The ulcerogenic potential was determined by presence or absence of macroscopically detectable ulcers.

Statistical Tests The results obtained were expressed by average±S.D. (standard deviation). Results that presented the probability of occurrence of a null hypothesis less than 5% ($p<0.05$) were considered statistically different. The data groups were submitted to the Kolmogorov–Smirnov test of normalcy. The data groups that did not follow a normal distribution were submitted to the Kruskal–Wallis test for non parametric values, following by modified Tukey test for comparison among the groups.

Results

Effect of GS26 and GS28 on Carrageenan Induced Paw Edema The carrageenan induced paw edema was reduced ($p<0.05$) by oral GS28 (100 mg/kg) in the time intervals of 60 (28.5%), 120 (36.1%) and 180 (20.5%) minutes, when compared with the group control. The indomethacin (10 mg/kg) reduced the edema development during the entire experiment (38.6, 55.8, 50.6, 32.2%). GS26 did not exhibit significant antiedematogenic activity in any experimental time interval (Fig. 5).

Effect of GS26 and GS28 on the Dextran Induced Paw Edema GS26 (50, 100 mg/kg) reduced ($p<0.05$) the dextran induced paw edema in comparison to the control group 180 min (22.1, 27.8%) after administration of the agent (Fig. 6). GS28 did not alter significantly the edema evolution after the dextran injection.

Macroscopic observation of the gastric mucous membrane of all the animals submitted to the models of acute inflammation did not reveal any lesion.

Discussion

Inflammation is an organism defense reaction that involves a series of events which can be initiated by numerous stimuli (infectious agents, ischemia, antigen-antibody interactions and thermal or other kinds of lesions).[12] The process is usually characterized by clinical signs of erythema, edema, heat, hyperalgesia and pain. Inflammatory response occur in three different stages, each one apparently mediated by a different mechanism: (1) a transitory acute phase, characterized by local vasodilatation and increased capillary permeability; (2) a retarded sub chronic phase, characterized by leukocyte infiltration and phagocytic cells; and (3) a proliferative chronic

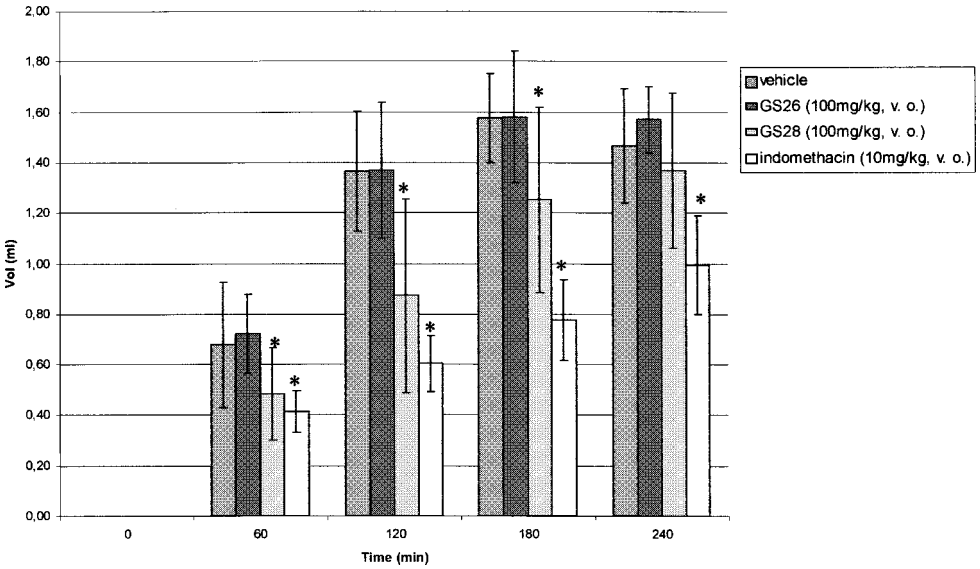

Fig. 5. GS26, GS28 and Indomethacin Effect on Paw Edema 60, 120, 180 and 240 min after 0.1 ml of Carrageenan Injection ($n=6$ Animals/Group)
$*p<0.05$. Kruskal–Wallis followed by modified Tukey test.

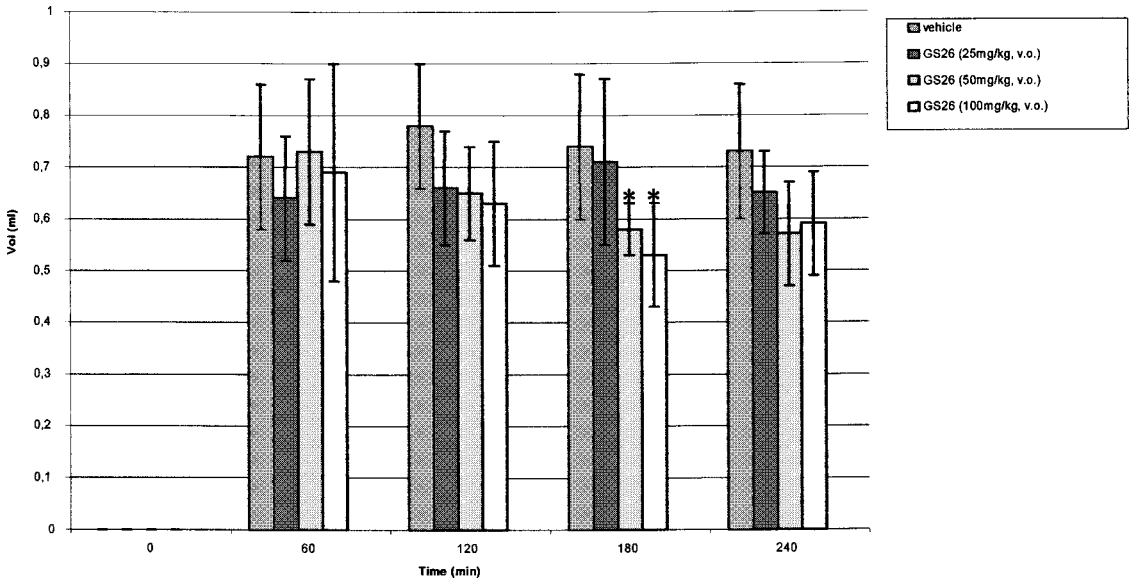

Fig. 6. GS26 Effect on Paw Edema 60, 120, 180 and 240 min after 0.1 ml of Dextran Injection ($n=6$ Animals/Group)
$*p<0.05$. Kruskal–Wallis followed by modified Tukey test.

phase in which occurs tissue regeneration and fibrosis.[13] During the tissual aggression, the flogistic agent determines local cell destruction, whose intracellular enzymatic content, once extravasated for the interstitial environment, induces tissular protein alterations. These altered proteins produce vasodilatation and act on complement system activation and Hageman's factor. Among the components of the complement system, the anaphilatoxins precursors (C3a and C5a) have the capacity to determine mastocyte degranulation, with histamine liberation following. Histamine is the first liberated chemical mediator and is related with the acute phase of the inflammation. The later phases do not depend on histamine action, but on other mediators (kinins, prostaglandins, *etc.*) that will appear as the process develops. When inflammation begins, a neutrophil and macrophage recruitment occurs to liberate the tissues of all xenobiotic agents.[14]

All of these inflammatory reactions result in the liberation of tissue mediators, among them radicals of oxygen (free radicals), simple amines such as histamine and serotonin, vasoactive peptides like bradykinin and correlates, as well as proteins of high molecular weight such as the chemotaxis factor of eosinophils and complement system components. Of considerable importance in the process, arachidonic acid (AA) derivatives, prostaglandins, tromboxanes and leukotrienes act as mediators of several processes, as vasodilatation, leukocyte attraction and in pain sensibility modulation.[12]

The antiedematogenic action of the compounds GS26 and GS28 was evaluated initially in two experimental models of acute inflammation, paw edema induced by carrageenan and paw edema induced by dextran.

Carrageenan, a polysaccharide obtained from sea algae

(the Irish moss *Chondrus*), is considered a nonspecific edematogenic agent that promotes liberation of several inflammation mediators when injected to the mouse paw.[10] The acute inflammatory reaction induced by carrageenan occurs as a complex organic response in which the chemical mediators histamine, serotonin, bradykinin and prostaglandins are liberated locally. Di Rosa *et al.* (1971)[15] showed that this experimental model is characterized by a sequential liberation of different inflammatory mediators as time progresses: (1) in the first hour histamine and serotonin liberation occurs predominantly; (2) kinins are the main mediators during the second hour; (3) the third hour is characterized by solid liberation of prostaglandins, a period in which the maximum peak of the edema happens. After the third hour the edema volume is stabilized. It should be evident that components of the complement system are also activated during this whole process. Compounds that act in the first hour demonstrate potential interference with the activity of the mediators that are liberated in the first hour (histamine and serotonin).

Drugs that act to inhibit prostaglandin formation (Aspirin and other NSAIDs) reduce the edema peak.[16] Selective COX-2 inhibitors act in the prostaglandin formation involved in the process, without, however, interfering in the action of the prostaglandins involved in the homeostasis processes.[17]

Pinto *et al.* (1995)[18] demonstrated that the kinins, mainly bradykinin, are the main mediator involved in above model. According to these data, the kinin could act not only for its own activity, but also for the interacting with other factors, as prostaglandins.

Our results on the carrageenan-induced edema model demonstrated that orally administered GS28 (100 mg/kg) possesses antiedematogenic activity similar to that found by Lima (1999).[6] In accord with these results, GS28 possibly interferes with different inflammatory mediators, since a significant reduction percentage of the edema over several time intervals is verified: 60 min (29.4%), 120 min (36.4%) and 180 min (20.8%), or specifically reduces kinin activity because of its interaction with other inflammatory mediators.

The greatest inhibition (36.4%) exhibited by orally administered GS28 (100 mg/kg) happened 120 min after injection of the edematogenic agent. Such results suggest a larger involvement of this compound on the bradykinin action since it is the main inflammatory mediator in this interval of time. However, our results do not allow us to identify the mechanism through which the compound exercises its antiedematogenic effect.

Differently from that found by Lima (1999),[6] orally administered GS26 (100 mg/kg) did not exhibit activity against the carrageenan edema in the doses used. Some explanations can be made for this result. Although our objective was not to investigate the GS26 and GS28 pharmacokinetcs, a reasonable possibility is the temporary variations that happen in the animal physiology, and that interfere with the pharmacokinetics of the product. In accord with Labrecque *et al.* (1995),[19] circannual variations happen in the pharmacokinetics and therapeutic effectiveness of drugs, as for instance, NSAIDs, therefore, the same drug may not exhibit the same effects in different seasons of the year. The higher antiinflammatory activity of GS28 may also be attributable to its prolonged lifetime in circulation, whereas GS26 could not exhibit effective suppression presumably because it was rapidly cleared from the circulation. Further insight to these pharmacokinetic considerations awaits further investigation. Problems regarding the stability of the compound can be ruled out, since the same lot of the product was once tested for several months without loss of activity (data not shown).

Dextran, a polysaccharide produced by the bacterium *Leuconostoc mesenteróides*, possesses the property of causing mastocyte degranulation with consequent histamine liberation in the tissues. The second model of acute inflammation, dextran induced paw edema, allows evaluation of the activity of compounds on processes mediated predominantly by histamine. Dextran induces fluid accumulation due to mast cell granulation with little protein and few neutrophils.[20,21] The histamine liberated from mastocyte granules is the main factor responsible for inflammation, whose effects involve two of the three histamine receptors, H1 and H2.[22]

The paw edema inhibition promoted by oral administration of GS26 (50, 100 mg/kg) in the dextran-induced model suggests the utility of this compound in histamine induced processes. The moment at which the effect of GS26 is seen causes doubt about the real activity of the product in this model, since the compound action occurs only after the edema peak, when the stabilization and regression of the edematous process begins.

The macroscopic observation of the gastric mucous membrane is a good indication of the ulcerogenic capacity of a drug.[11] The new NSAIDs meloxicam,[17] CGP 28238,[23] NS-398 and nimesulide[24] selectively inhibit COX-2 and do not have side effects on the gastric mucous membrane. According to Walsh *et al.* (1990),[25] evidence has accumulated which implicates uninhibited or increased leukotriene production in addition to prostaglandin inhibition as a more complete explanation for the NSAID-induced side effects. Thus, as more lipoxygenase (5-LO) inhibitory activity is introduced into NSAID pharmacophore, a commensurate decrease in ulceration is realized. The stomachs analyzed during the two treatments did not demonstrate any indication of lesions provoked by the compounds, however these results, did not permit us to make a conclusion about the action mechanism. Further experiments remain to be conducted to clarify the advantage of the products concerning their activity against 5-LO and COX, as well other inflammatory mediators such as cytokine.

Several drugs introduced in this century for treatment of specific diseases with average use and observation have also been found to have other indications. For instance, estrogens used initially as replacers of hormone deficiencies, are used today as anti-neoplastics, prophylactics of osteoporosis and contraceptives.[26—28] A more detailed screening on *N*-tryptophyl-5-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-2,4-thiazolidinedione (GS28) and *N*-tryptophyl-5-(3,5-di-*tert*-butyl-4-hydroxybenzylidene) rhodanine (GS26) may reveal a promising therapeutic indications for these and other thiazolidine derivatives.

Conclusion

The compounds GS26 and GS28 exhibited significant antiedematogenic activity in different experimental models of acute inflammation.

The studied compounds did not present ulcerogenic activity in the used doses.

New studies, with different formulations and posologic outlines of GS26 and of GS28, can reveal better antiedematogenic capacity.

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