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SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NEW FLOSULIDE ANALOGUES, SYNTHESIZED FROM NATURAL SAFROLE

Adriana S. Lages,[a,b] Kelli C. M. Silva,[a] Ana L. P. Miranda,[a] Carlos A. M. Fraga,[a] and Eliezer J. Barreiro, [a]

[a]*Laboratório de Avaliação e Síntese de Substâncias Bioativas (LASSBio), Faculdade de Farmácia, Universidade Federal do Rio de Janeiro, CP 68006, ZIP 21944-970, Rio de Janeiro - RJ, Brazil*

[a]*Departamento de Química Orgânica, Instituto de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro - RJ, Brazil*

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Abstract: Four new aryl-sulfonamide derivatives (3a, 4a, 5a~b), having methylenedioxy group attached to phenyl ring, were prepared from natural safrole and evaluated as anti-inflammatory agents. The N-methylsulfonamide 3a and corresponding retrosulfonamide derivative 5a were more active than standards indomethacin and nimesulide, at the same molar concentration, in carrageenan-induced pleurisy assay.

Nonsteroidal anti-inflammatory drugs (NSAIDs), the most commonly prescribed drugs in the world,[1] possess anti-inflammatory, analgesic, and antipyretic activities and have been widely used to treat acute and chronic inflammatory disorders.[2,3] The pharmacotherapeutic actions of NSAIDs are mediated through a reduction of prostanoids biosynthesis by direct inhibition of cyclooxygenase activity of prostaglandin-endoperoxide synthase (COX), which catalyzes the conversion of arachidonic acid to prostaglandin H_2 (PGH_2).[4,5] Unfortunately, inhibition of prostanoids production in organs such as the stomach and kidney by conventional NSAIDs results in a mechanism-based toxicity like irritation of stomach mucosa,[6] renal damage,[7] and increased bleending.[8] Thus, these unwanted side effects limit their therapeutic utility especially when long-term treatment is involved. Recently, a second isoform of COX (COX-2), which presents high homology at catalytic aminoacid residues and similar K_m values for arachidonic acid, was discovered.[9] The most dramatic difference between the two enzymes is in the patterns of expression: COX-1 is constitutively expressed in most tissues and it has been described as a "housekeeping enzyme" responsible for prostaglandin production, involved in the maintenance of physiological functions such as platelet aggregation, cytoprotection in the stomach, and maintenance of normal kidney function; COX-2 is undetectable in most tissues, but its expression can be dramatically increased by pro-inflammatory and mitogenic stimulus, appearing to be primarily responsible for prostaglandins produced in inflammation and mitogenesis.[9]

The classical NSAIDs are nonselective COX inhibitors, which exerts your anti-inflammatory activity via inhibition of the COX-2 and their deleterious side effects by inhibition of the COX-1.[10] In order to provide an effective treatment for inflammatory disorders, the designing of new selective COX-2 has been widely described[11] as a new therapeutical approach, which conduct to safe NSAIDs, devoid of the side effects commonly associated with conventional NSAIDs.[10]

As part of research program aiming at the synthesis and pharmacological evaluation of new lead-

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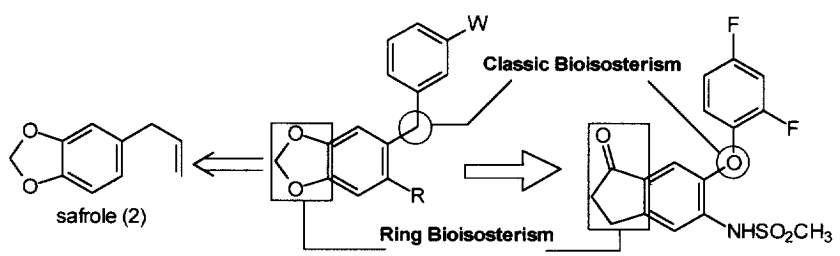
compounds, exploring rational principles of molecular designing , we described previously the development of new anti-inflammatory agents, analogues of classical NSAIDs.[12] Thus, we describe in this paper[13] the synthesis and the preliminary pharmacological evaluation of new analogues (3a~b, 4a~b and 5a~b) of flosulide (1), an important COX-2 inhibitor,[14,15] exploring the Brazilian natural safrole (2) as inexpensive starting material (Chart 1). These new analogues of 1 were structurally planned exploring the principles of bioisosterism,[16] where oxygen atom and indanone ring of flosulide (1) were substituted for methylene group and 1,2-benzodioxole ring in 3a~b, 4a~b and 5a~b, respectively (Chart 1).

The sulfonamide moiety described as an essential structural requirement for COX-2 activity due to the acidic hydrogen,[14] was maintained as a retrobioisostere in 3a~b and 4a~b, in order to elucidate the role of the position of the acidic hydrogen in the biological activity. The 3-trifluoromethyl substituent of 3b, 4b and 5b was chosen as an electron-withdrawing mimetic group of 2,4-difluoro substituents present in flosulide (2) and the nonsubstituted analogues (3a, 4a and 5a) were elected in order to compare the eventual contribution of electron-withdrawing groups in the COX-2 activity. Finally, the phenylsulfonamide group present in 4a~b may reveal the importance of additional π electrons to this system and consequently in the bioactivity (Chart 1).



3a W = H; R = SO_2NHCH_3
3b W = CF_3; R = SO_2NHCH_3
4a W = H; R = SO_2NHPh
4b W = CF_3; R = SO_2NHPh
5a W = H; R = $NHSO_2CH_3$
5b W = CF_3; R = $NHSO_2CH_3$

Chart 1

The initial planned synthetic route to these new derivatives (3a~b, 4a~b and 5a~b) (Schemes 1 and 2) suggests the diphenylmethane derivatives (6a~b), as a common key intermediate. Thus, 6a~b were initially prepared by coupling the appropriate aryl Grignard reagent with piperonal (7)[17] (Scheme 1), which can be easily obtained from safrole (2) by isomerization followed oxidative cleavage of double bond, a technique widely employed in our laboratory.[13] Grignard reaction of 7, in THF, furnished, after chromatographic purification, the corresponding diarylmethanol derivatives 8a~b, in 80% yield (Scheme 1).

Subsequently, 8a~b were deoxygenated by using sodium borohydride in trifluoroacetic acid media[18] to produce the corresponding diphenylmethane derivatives (6a~b) in excellent yield (90%), after chromatographic purification (Scheme 1).

The next step to prepare the target-analogues (3a~b and 4a~b) was the functionalization of 6a~b at

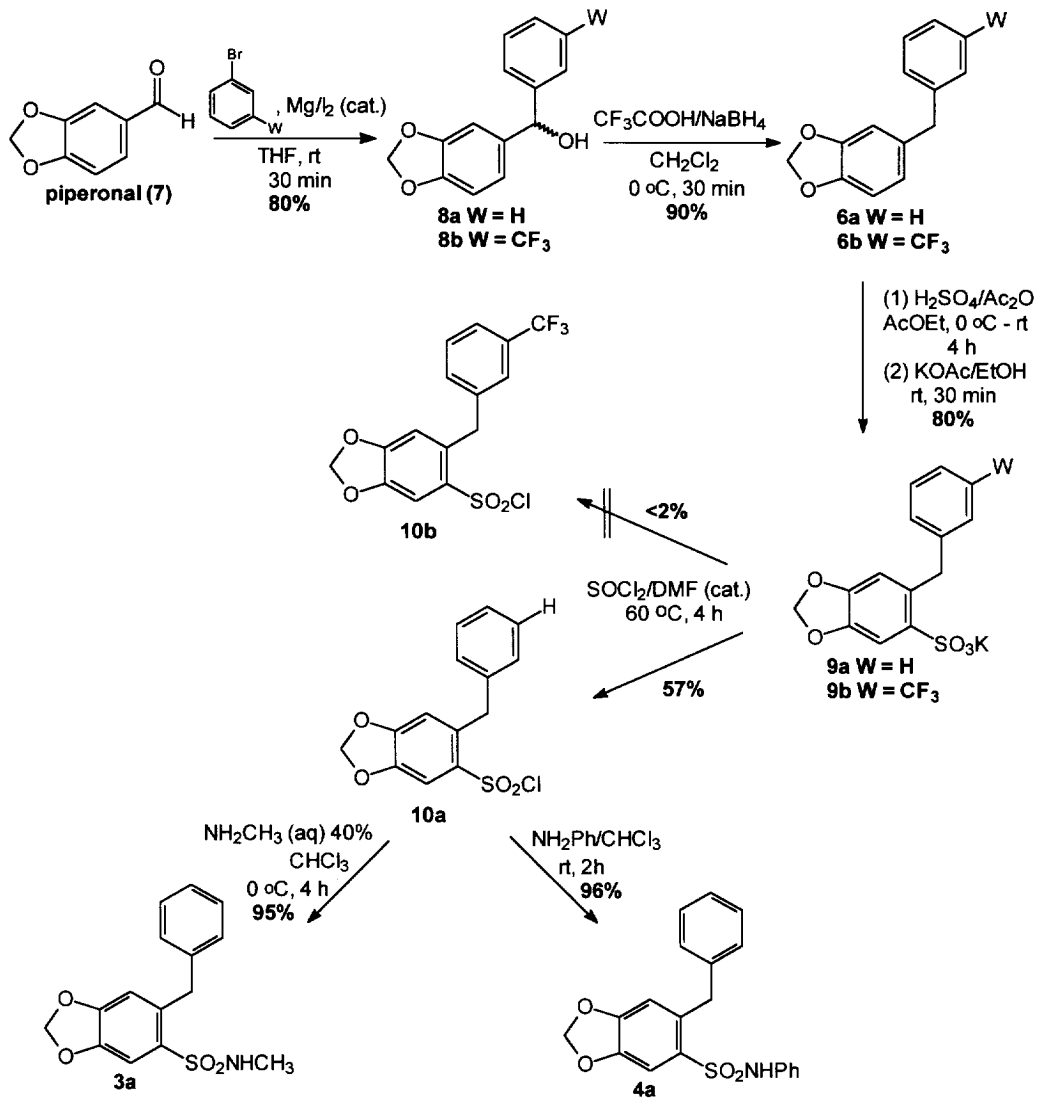
the 5-position of the 1,2-benzodioxole ring. Anticipating that the electron-donating character of the methylenedioxy group could orientate regioselectively towards a aromatic eletrophilic substitution, we used this synthetic strategy to attempt the desired functionalization of 6a~b. In fact, following the procedure previously described by Fraga[12b] for mild sulfonation of safrole derivatives, the treatment of 6a~b with sulfuric acid and acetic anhydride in ethyl acetate followed by addition of ethanolic potassium acetate solution afforded, as the only product, the potassium salts (9a~b) in 80% yield (Scheme 1). The ^{1}H NMR analysis of these compounds confirmed the regioselectivity of this process, as evidenced by presence of two double signals at δ 7.38 and δ 6.64 ppm in compound 9a and 7.37 and δ 6.64 ppm in compound 9b, an AB pattern typical of the tetrasubstituted phenyl system.

The salt derivatives (9a~b) were next converted to respective sulfonyl chloride derivatives (10a~b) by treatment with thionyl chloride containing a catalytic amount of DMF.[12b] The intermediates (10a~b) were obtained in low yields, especially the CF_3 analogue (10b), which was obtained in only 2% yield (Scheme 2). A possible explanation to the very low reactivity of 9b compared with 9a could be an unconventional anion-π interaction between the negatively charged oxygen atom of sulfonate group and the electron-poor 3-trifluoromethylphenyl ring indisposing the sulfonate moiety to nucleophilic reaction.

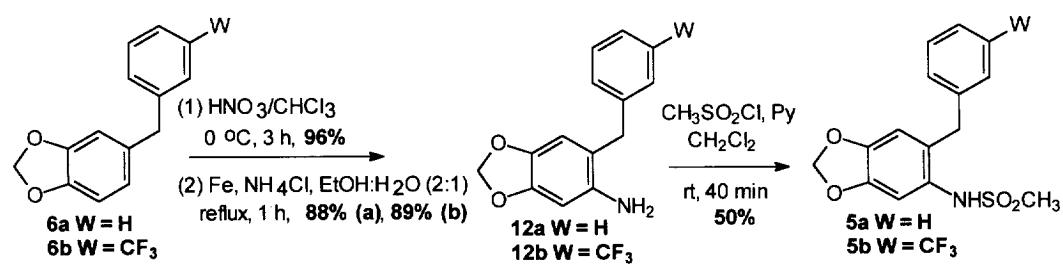
Finally, the synthesis of the designed products 3a and 4a was accomplished, in high yield, by nucleophilic substitution of the adequate amines with the crude adduct of chlorosulfonylation reaction.[12b] In fact, we are able to detect that compound 10a is highly air-sensitive, being hydrolyzed during workup even by atmospheric water, to the corresponding sulfonic acid.

Again the aromatic electrophilic substitution of 6a~b was used as the initial approach to retrosulfonamide series (5a~b). Thus, nitration of 6a~b with nitric acid in cooled chloroform[12a] furnished the nitro derivatives (11a~b), as the only product in 96% yield (Scheme 5). These compounds showed in ^{1}H NMR spectra the same AB pattern of tetrasubstituted phenyl ring at δ 7.51 and δ 6.62 ppm for 11a and 7.55 and δ 6.63 ppm for 11b, confirming the reaction regioselectivity.

The nitro derivatives (11a~b) were next reduced in 96% yield, to corresponding aniline derivatives (12a~b), employing iron powder in aqueous ethanol under reflux.[11b] Finally, the synthesis of the analogues (5a~b) was completed by selective mono-mesylation of compounds 12a~b, by treatment with mesyl chloride and pyridine,[11b] at room temperature (Scheme 2).



Scheme 1.



Scheme 2

The anti-inflammatory activity of the new compounds[19] (3a, 4a, 5a~b) was determined in vivo using the carrageenan-induced rat paw edema[20] and pleurisy,[21] which are considered as biological models that involves the COX-2 expression.[22] Fasted albino rats of both sexes (150~200 g) were used. Compounds were administered orally (100 μmol/kg; 0.1 mL/20g) as a suspension in 5% arabic gum in saline (vehicle), one hour before the injection of carrageenan. Indomethacin (100 μmol/kg) and a selective COX-2 inhibitor nimesulide[11e] (100 μmol/kg) were used as standard drugs in the same conditions. Anti-inflammatory activity was expressed as % of inhibition when compared with the vehicle control group.

Table 1: Effect of sulfonamide derivatives (3a, 4a and 5a~b), indomethacin and nimesulide in the inhibition of carrageenan-induced rat pleurisy and paw edema.

Compounds	Dose[a] (μmols/kg)	N[b]	Cell Number (1 x 10^6 cells/cavity)	% Inhibition[c]	Edema		
					N[b]	Volume Variation (μl)	% Inhibition[d]
Vehicle Control (arabic gum 5%)	-	16	34.1 ± 1.6	-	17	546.3 ± 26.7	-
Saline	-	5	10.6 ± 1.4	-	-	-	-
Indomethacin	100	10	23.3 ± 2.2	31.7 *	5	79.5 ± 33.6	85.4 *
Nimesulide	100	8	23.9 ± 1.5	29.9 *	5	208.9 ± 30.0	61.7 *
3a	100	5	18.4 ± 3.2	46.0 *	14	531.4 ± 40.6	2.7 n.s.
4a	100	5	25.7 ± 2.9	24.6 *	5	397.6 ± 82.9	27.2 *
5b	100	5	25.7 ± 1.2	24.6 *	5	438.6 ± 19.9	19.7 *
5a	100	5	19.4 ± 2.4	43.1 *	10	431.4 ± 23.6	21.0 *

[a]All compouds were administered po. [b]N = number of animals. [c]% of inhibition obtained by comparision with vehicle control group. [d]% of inhibition obtained by comparision with vehicle control group and expressed by the mean volume variation ± SEM at the 4th hour after carrageenan injection. *p< 0.05 (Student's t-test). Results are expressed as mean ± SEM. n.s. = no significant.

Just the compounds 4a, 5a~b were able to inhibit moderately the rat paw edema at the 4th hour after carrageenan edema-induction. Indomethacin and nimesulide inhibited the edema in 67.7 and 64.7% respectively (Table 1). Otherwise, compounds 3a and 5a were more active then indomethacin and nimesulide, in pleurisy assay at a dose of 100 μmol/kg, po, promoting a significative reduction (46 and 43.1%, respectively) of the cells number per cavity.

These results permitted us to evidence that the more active compounds 3a and 5a presenting a pharmacophoric N-methylsulfonamide moiety and the corresponding retrosulfonamide group, respectively, confirms the bioisosteric relationships between these functional groups. The observed anti-inflammatory activity is strongly influenced by replacement sulfonamide attached group, that is, SO_2NH-Me X SO_2NHPh [46% (3a) vs. 24.6 % (4a) inhibition] and by introduction of a electron-withdrawing group in the phenyl ring, i.e. (5a) vs. (5b) (43.1% vs. 24.6 % inhibition). Compounds 3a and 5a were selected for further studies using isolated COX-1 and COX-2 enzymes, in order to evaluate their selective inhibition profile.

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