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Studies on antiplatelet agents from natural safrole II. Synthesis and pharmacological properties of novel functionalized oxime *O*-benzylether derivatives [1]

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Abstract

In an ongoing research program aiming at the synthesis and pharmacological evaluation of new possible prototype candidates exploring the molecular hybridation and bioisosterism principles for molecular designing, we describe in this paper the design and synthesis of a series of new functionalized oxime *O*-benzylethers (**4a–b**) and (**14a–b**) as antiplatelet agents based on the inhibition of arachidonic acid (AA) cascade enzymes. For the synthesis of these new bioactive derivatives we used safrole (**5**), a Brazilian abundant natural product, as starting material. The platelet anti-aggregating evaluation of these oxime *O*-benzylether compounds (**4a–b**) and (**14a–b**) in model induced by ADP, collagen and AA, has permitted to evidence an antithrombotic profile to these new derivatives, being the most active the derivative methyl [[3,4-methylenedioxyphenyl]methylene]amino]oxy]-4-methylenephenylacetic acid (**14a**).

Keywords: Platelet aggregation; Oxime *O*-benzylethers derivatives; Safrole in synthesis

1. Introduction

During an ongoing research program to identify new lead compounds with antiplatelet activity, acting at AA cascade level, we described in previous papers, the anti-aggregating profile of new arylsulfonamides derivatives (**1a–b**) (Lima et al., 1999), synthesized from natural safrole as TPant and new *N*-phenylpyrazole oxime-*O*-benzylether derivatives (**2a–b**) (Muri et al., 1998), designed as dual TXSi/TPant (Fig. 1). In our continuing studies, we synthesized a new class of conformationally restricted PAF antagonists candidates (**3**) belonging to the 2-oxabicyclo[3.3.0]octane and tested them for their antiplatelet effects (Peçanha et al., 1998) (Fig. 1). These novel antiplatelet agents presented important biological profile, where the most active derivative was represented by compound (**1a**) (Lima et al., 1999).

Following these efforts, we wish to describe in this report, the synthesis and antiplatelet properties of novel oxime *O*-benzylether derivatives (**4a–b**), structurally planned as dual TXSi/TPant. candidates, synthesized from safrole (**5**), an abundant Brazilian natural product, occurring in the Sassafras oil, as starting material (Fig. 1).

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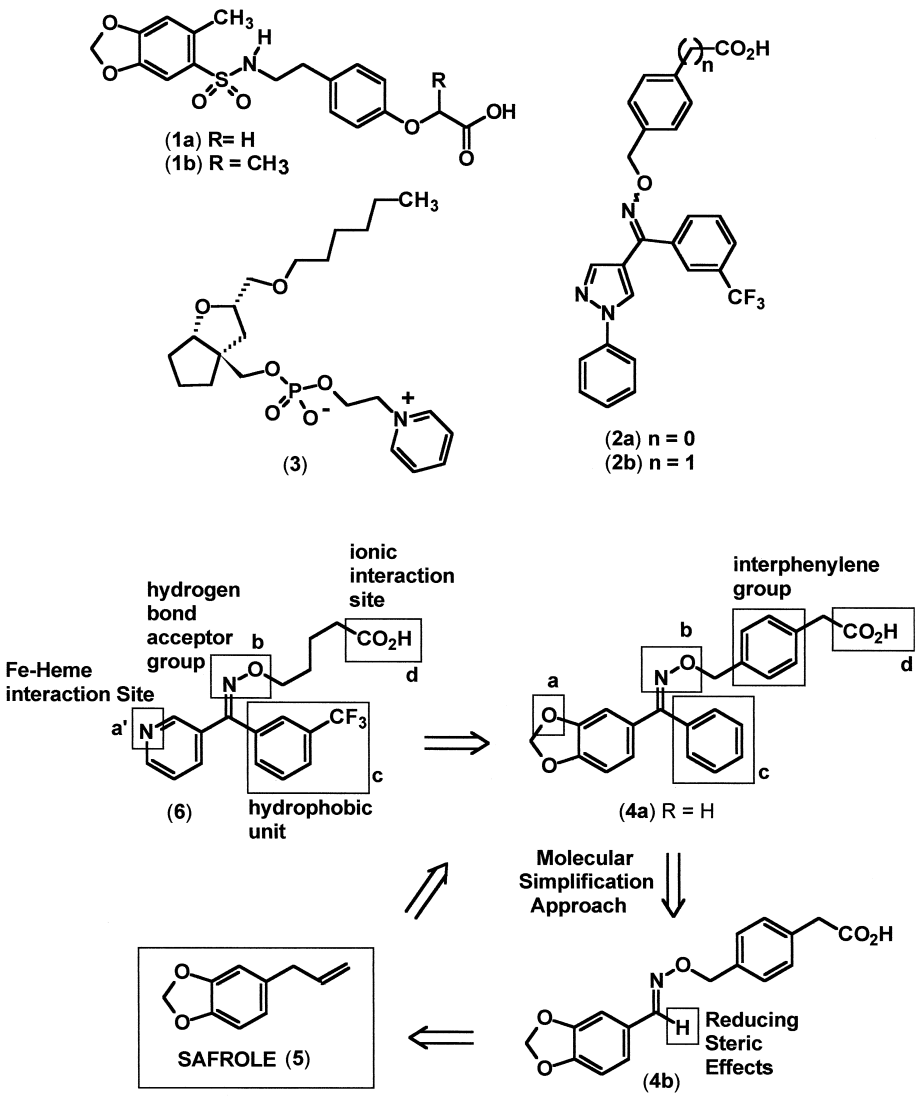


Fig. 1.

The design of these new derivatives (**4a–b**) (Fig. 1), considered the 1,3-benzodioxole ring, present in the natural starting material (**5**), as a hydrogen-bonding acceptor site, as well explore the presence of the oxygen atom ***a*** (Fig. 1) as an equivalent site to coordinate with the Fe-heme presents in TXS, mimicking the nitrogen-containing ring of ridogrel (**6**) (De Clerck et al., 1989a,b; Bossche et al., 1992) (a′, Fig. 1). The compound (**4a**) was planned by introduction of a second aromatic ring, correspondent to a hydrophobic unit, which could be essential, due to its lipophilic properties, to present a dual profile (Fig. 1). In addition, to compare the contribution of this second aromatic ring in the antiplatelet activity, the corresponding nor-phenyl derivative (**4b**) was also prepared. In all these derivatives (**4a–b**), the carboxylic acid end-chain was maintained in order to assure the ionic site for binding with TP and TXS, as presented in numerous dual antiplatelet agents (Jin and Hopfinger, 1994; Albuquerque et al., 1995). The interphenylene nature of this acidic chain, corresponding to a phenyl acetic acid residue, was introduced as a conformational restriction tool in order also to prevents the metabolic β-oxidation process that reduce the length of fatty acid-like chains (Schulz, 1991).

2. Experimental

2.1. Chemistry

Melting points were determined with a Thomas–Hoover apparatus and are uncorrected. Proton magnetic resonance

(^{1}H NMR), unless otherwise stated, was determined in deuterochloroform containing ca. 1% tetramethylsilane as internal standard with a Brucker AC 200 at 200 MHz. Carbon magnetic resonance (^{13}C NMR) was determined in the same spectrometer described above at 50 MHz, using deuterochloroform as internal standard. Infrared (IR) spectra were obtained with Nicolet-205 spectrophotometers by using potassium bromide plates or neat films on sodium chloride plates. Ultraviolet (UV) spectra were determined in methanol solution on a Shimadzu UV-1601 spectrophotometer. The mass spectra (MS) were obtained by electron impact with a GC/VG Micromass 12 spectrometer.

The progress of all reactions was monitored by tlc which was performed on 2.0 cm × 6.0 cm aluminum sheets precoated with silica gel 60 (HF-254, Merck) to a thickness of 0.25 mm. The developed chromatograms were viewed under ultraviolet light. For column chromatography Merck silica gel (70–230 mesh) was used. Solvents used in the reactions were generally redistilled prior to use and stored over 3–4 Å molecular sieves. Reactions were generally stirred under a dry nitrogen atmosphere. The usual work-up means that the organic extracts prior to concentration, under reduced pressure, were treated with a saturated aqueous sodium chloride solution, referred to as brine, dried over anhydrous sodium sulfate and filtered.

*2.2. (3,4-Methylenedioxyphenyl) phenylmethanol (**9**) (Klix et al., 1995)*

To a suspension of 0.43 g (18.00 mmol) of pre-activated magnesium in 5 ml of dry THF containing a catalytic iodine crystal, was added 1.8 ml (2.82 g; 18.00 mmol) of dry bromobenzene. The reaction mixture was stirred until total consumption of the magnesium turnings and then a solution of 0.90 g (6.00 mmol) of aldehyde (**8**) in 4 ml of dry THF was added, dropwise, at 0°C. The reaction mixture was stirred at room temperature for 40 min, then poured in an ice-water mixture, neutralized with 4% aq. HCl and extracted with diethyl ether (3 × 30 ml). The organic extracts were submitted at usual work-up to give crude residue, which was purified by column chromatography on neutral alumina, using a hexanes:dichloromethane gradient from 100:0 to 0:100, to give 0.82 g (60%) of the diarylmethanol derivative (**9**), as a brownish oil; **^{1}H NMR** (200 MHz): δ 7.38–7.22 (5H, m, H-2′, H-3′ and H-4′), 6.87–6.82 (2H, m, H-2 and H-6), 6.76 (1H, d, $J_{6-5} = 8.0$ Hz, H-5), 5.92 (s, 2H, OCH_2O), 5.73 (s, 1H, C*H*OH), 2.42 (br, 1H, D_2O exangeable, OH) ppm; **^{13}C NMR** (50 MHz): δ 147.7 (C-3), 146.9 (C-4), 143.7 (C-1′), 137.9 (C-1), 128.4 (C-3′), 127.4 (C-4′), 126.2 (C-2′), 119.9 (C-6), 108.0 (C-5), 107.1 (C-2), 100.9 (O*C*H_2O), 75.9 (*C*HOH) ppm; **IR** (Film): 3405 (ν O0-H), 1245 and 1038 (ν C–O) cm^{-1}; **MS** (m/z): 228 (M^+, 100%), 211 (17%), 151 (50%), 123 (75%), 105 (65%).

*2.3. (3,4-Methylenedioxyphenyl) phenylmethanone (**10**) (Takeuchi et al., 1995)*

To a solution of 0.60 g (2.63 mmol) of alcohol derivative (**9**) in 10 ml of dry THF were added 3.60 g of previously thermo-activated MnO_2. Next, the reaction mixture was refluxed for 30 min, then filtered in a Celite packed column. The filtrate was concentrated at reduced pressure to furnish 0.41 g (70%) of the benzophenone derivative (**10**), as a white solid, mp 49–53°C; **^{1}H NMR** (200 MHz): δ 7.73 (2H, dt, H-2′), 7.60–7.40 (3H, m, H-3′ and H-4′), 7.37 (1H, dd, $J_{6-5} = 8$ Hz and $J_{6-2} = 2$ Hz, H-6), 7.35 (1H, d, $J_{6-2} = 2$ Hz, H-2), 6.85 (1H, d, $J_{6-5} = 8$ Hz, H-5) ppm; **^{13}C NMR** (50 MHz): δ 195.0 (C=O), 151.4 (C-4), 147.8 (C-3), 138.0 (C-1′), 131.8 (C-4′), 131.7 (C-1), 129.5 (C-3′), 128.1 (C-2′), 126.7 (C-6), 109.8 (C-5), 107.5 (C-2), 101.7 (O*C*H_2O) ppm; **IR** (KBr): 1642 (ν C=O), 1236 and 1039 (ν C–O) cm^{-1}; **MS** (m/z): 226 (M^+, 46%), 149 (100%), 121 (15%), 105 (33%), 77 (50%).

*2.4. (3,4-Methylenedioxyphenyl) phenylmethanone oxime (**7a**) (Furniss et al., 1989)*

To a mixture containing 0.70 g (3.10 mmol) of the ketone derivative (**10**), 0.64 g (9.30 mmol) of hydroxylamine hydrochloride, 10 ml of ethanol and 2 ml of water, were added slowly 0.62 g (15.5 mmol) of NaOH, under vigorous stirring. The reaction mixture was refluxed for 30 min, then neutralized with a 1 M aqueous HCl solution and extracted with dichloromethane (3 × 30 ml). The organic extracts were joined and evaporated under reduced pressure to give 0.47 g (64%) of an 1.1:1 (*E*)–(*Z*) diastereomeric mixture of oxime derivative (**7a**), as a white solid, mp 143-4°C; **^{1}H NMR** (200 MHz): δ 7.50–7.26 (6H, m, H-2′, H-3′, H-4′ and OH), 7.06 (1H, dd, $J_{6-5} = 2$ Hz and $J_{5-2} < 1$ Hz, H-5 (*Z*)), 7.01 (1H, t, $J_{6-2} = 1$ Hz and $J_{5-2} < 1$ Hz, H-2 (*Z*)), 6.89 (2H, m, H-6 (*Z*) and H-2 (*E*)), 6.84 (1H, dd, $J_{6-5} = 8$ Hz and $J_{6-2} = 2$ Hz, H-6 (*E*)), 6.72 (1H, dd, $J_{6-5} = 8$ Hz and $J_{5-2} = 1$ Hz, H-5 (*E*)), 6.02 (2H, s, OCH_2O (*Z*)), 5.97 (2H, s, OCH_2O (*E*)) ppm; **^{13}C NMR** (50 MHz): δ 157.5 (C=N (*Z*)), 157.2 (C=N (*E*)), 148.7 (C-4 (*Z*)), 148.1 (C-4 (*E*)), 147.7 (C-3 (*Z*)), 147.3 (C-3 (*E*)), 136.3 (C-1′ (*Z*)), 132.7 (C-1′ (*E*)), 130.2 (C-1 (*E*)), 129.3 (C-4′ (*E*)), 129.0 (C-4′ (*Z*)), 128.9 (C-3′(*E*)), 128.2 (C-3′ (*Z*)), 128.1 (C-2′ (*E*)), 127.9 (C-2′ (*Z*)), 125.9 (C-1 (*Z*)), 123.6 (C-6 (*Z*)), 122.7 (C-6 (*E*)); 110.0 (C-2 (*Z*)), 108.1 (C-2 (*E*)), 107.8 (C-5 (*E*)), 107.4

(C-5 (*Z*)), 101.2 (OCH_2O) ppm; **IR** (KBr): 3294 (ν O–H), 1500 and 1487 (ν C=N), 1232 and 1040 (ν C–O) cm^{-1}; **MS** (*m/z*): 241 (M^+, 100%), 224 (63%), 122 (44%), 77 (30%); **UV** (MeOH): λ 295.0 (ε, 5520), 224.0 (ε, 15 900) nm.

*2.5. 3,4-Methylenedioxybenzaldehyde oxime (**7b**) (Furniss et al., 1989)*

To a mixture containing 0.45 g (3.00 mmol) of aldehyde (**8**), 0.31 g (4.50 mmol) of hydroxylamine hydrochloride, 7 ml of ethanol and 1.5 ml of water, were added slowly 0.60 g (15.0 mmol) of NaOH, under vigorous stirring. The reaction mixture was refluxed for 5 min, then poured in 10 ml of a 1 M aqueous HCl solution. The resulting precipitate was filtered out, washed with 10 ml of water and air dried, furnishing 0.25 g (51%) of the desired oxime derivative (**7b**), as a white solid, mp 108°C; **^{1}H NMR** (200 MHz): δ 8.87 (1H, br, O*H*), 8.06 (1H, s, *H* C=N), 7.17 (1H, d, $J_{6-2} = 2$ Hz, H-2), 6.96 (1H, dd, $J_{6-5} = 8$ Hz and $J_{6-2} = 2$ Hz, H-6), 6.79 (1H, d, $J_{6-2} = 2$ Hz, H-5), 5.98 (2H, s, OCH_2O) ppm; **^{13}C NMR** (50 MHz): δ 149.9 (C=N), 149.2 (C-4), 148.1 (C-3), 126.0 (C-1), 123.0 (C-6), 108.2 (C-5), 105.6 (C-2), 101.3 (OCH_2O) ppm; **IR** (KBr): 3225 (ν O–H), 1499 (ν C=N), 1256 and 1035 (ν C–O) cm^{-1}; **MS** (*m/z*): 165 (M^+, 100%), 149 (4%), 122 (40%); **UV** (MeOH): λ 306.5 (ε, 8390), 268.0 (ε, 12 290), 223.0 (ε, 14 110) nm.

*2.6. (3,4-Methylenedioxyphenyl) phenylmethanone oxime, O-methyl ether (**11**) (adapted from Seebach et al., 1994)*

An 80% suspension of sodium hydride in mineral oil (0.050 g, 1.66 mmol) was washed with dry *n*-hexane until a white pale solid was obtained which was suspended in 1 ml of dry DME. Then, a solution of 0.044 g (0.18 mmol) of the oxime derivative (**7a**) in 2 ml of dry DME was added dropwise and the reaction mixture was refluxed under stirring for 30 min. After this time, 0.1 ml (1.6 mmol) of methyl iodide was added at room temperature. After 5 min, the reaction mixture was poured into an ice–water mixture containing 2 ml of an 10% aqueous HCl solution and extracted with ethyl ether (3×15 ml). The organic extracts were submitted at usual work-up, furnishing a crude oily residue which was purified by silica gel column chromatography, furnishing 0.012 g (33%) of the benzophenone derivative (**10**) and 0.026 g (57%) of the an 1.1:1 (*E*)–(*Z*) diastereomeric mixture of the desired oxyme methylether (**11**), as a dark yellow oil; **^{1}H NMR** (200 MHz): δ 7.50–7.26 (6H, m, H-2′, H-3′, H-4′ and OH), 7.10 (1H, d, $J_{6-5} = 2$ Hz, H-5 (*Z*)), 6.90 (1H, br, H-2 (*Z*)), 6.82 (2H, m, H-6 (*Z*) and H-2 (*E*)), 6.81 (1H, dd, $J_{6-5} = 8$ Hz and $J_{6-2} = 2$ Hz, H-6 (*E*)), 6.70 (1H, d, $J_{6-5} = 8$ Hz, H-5 (*E*)), 5.96 (2H, s, OCH_2O (*Z*)), 5.92 (2H, s, OCH_2O (*E*)), 3.97 (3H, s, OCH_3 (*E*)), 3.92 (3H, s, OCH_3 (*Z*)) ppm; ^{13}C NMR (50 MHz): δ 156.3 (C=N (*Z*)), 156.1 (C=N (*E*)), 148.7 (C-4 (*Z*)), 148.0 (C-4 (*E*)), 147.8 (C-3 (*Z*)), 147.4 (C-3 (*E*)), 136.8 (C-1′ (*Z*)), 133.6 (C-1′ (*E*)), 130.8 (C-1 (*E*)), 129.1 (C-4′), 128.6 (C-3′ (*E*)), 128.1 (C-3′ (*Z*)), 128.0 (C-2′), 126.0 (C-1 (*Z*)), 123.6 (C-6 (*Z*)), 122.7 (C-6 (*E*)), 110.1 (C-2 (*Z*)), 108.0 (C-2 (*E*)), 107.8 (C-5 (*E*)), 107.6 (C-5 (*Z*)), 101.2 (OCH_2O), 62.3 (OCH_3 (*Z*)), 62.2 (OCH_3 (*E*)) ppm; **MS** (*m/z*): 255 (M^+, 76%), 224 (100%), 121 (20%), 77 (35%).

*2.7. Methyl 4-[[[(3,4-methylenedioxyphenyl)phenylmethylene]amino]oxy]methyl] phenyl acetate (**14a**) (adapted from Seebach et al., 1994)*

An 80% suspension of sodium hydride in mineral oil (0.06 g, 2 mmol) was washed with dry *n*-hexane until a white pale solid was obtained which was suspended in 1 ml of dry DME. Then, a solution of 0.066 g (0.27 mmol) of the oxime derivative (**7a**) in 2 ml of dry DME was added dropwise and the reaction mixture was refluxed under stirring for 30 min. After this time, a solution of 0.10 g (0.43 mmol) of 4-bromomethylphenylacetic acid (**12**) in 2 ml of dry DME was added and the reaction was additionally refluxed for 18 h, then poured into an ice–water mixture containing 2 ml of an 10% aqueous HCl solution and extracted with ethyl ether (5×15 ml). The organic extracts were submitted at usual work-up, furnishing a crude residue which was next treated with an ethereal diazomethane solution (Monson, 1971). The obtained mixture was purified by silica gel column chromatography, using hexanes:EtOAc as eluent (gradient, 100:0 to 94:6), to give 0.035 g (58%) of the ketone derivative (**10**), 0.005 g (8%) of the precursor oxime derivative (**7a**) and 0.031 g (28%) of an 1:1 (*E*)–(*Z*) diastereomeric mixture of methyl ester derivative (**14a**), as brownish solid, mp 80-84°C; **^{1}H NMR** (200 MHz): δ 7.50–7.20 (9H, m, H-2′, H-3′, H-4′, H-2′, H-3′), 7.10 (1H, dd, $J_{6-5} = 2$ Hz and $J_{5-2} < 1$ Hz, H-5 (*Z*)), 6.91 (1H, dd, $J_{6-2} = \sim 1$ Hz and $J_{5-2} < 1$ Hz, H-2 (*Z*)), 6.82 (2H, m, H-6 (*Z*) and H-2 (*E*)), 6.79 (1H, dd, $J_{6-5} = 8$ Hz and $J_{6-2} = 2$ Hz, H-6 (*E*)), 6.69 (1H, dd, $J_{6-5} = 8$ Hz and $J_{5-2} = 1$ Hz, H-5(*E*)), 5.99 (2H, s, OCH_2O (*Z*)), 5.94 (2H, s, OCH_2O (*E*)), 5.21 (2H, s, OCH_2Ar (*E*)), 5.16 (2H, s, OCH_2Ar (*Z*)), 3.67 (6H, s, $COOCH_3$ (*E*) and (*Z*)), 3.61 (4H, s, $ArCH_2C=O$ (*E*) and (*Z*)) ppm; **^{13}C NMR** (50 MHz): δ 171.9 (C=O), 156.6 (C=N (*Z*)), 156.4 (C=N (*E*)), 148.6 (C-4 (*Z*)), 147.8 (C-4 (*E*)), 147.6 (C-3 (*Z*)), 147.1 (C-3 (*E*)), 137.1 (C-1′ (*Z*)), 136.9 (C-1′ (*E*)), 136.6 (C-1′ (*Z*)), 133.3 (C-1′ (*E*)),

133.2 (C-4′ (*Z*)), 133.1 (C-4′ (*E*)), 130.5 (C-1 (*E*)), 129.17 (C-3′), 129.12 (C-4′), 129.0 (C-2′), 128.6 (C-3′ (*E*)), 128.2 (C-3′ (*Z*)), 128.1 (C-2′ (*E*)), 128.0 (C-2′ (*Z*)), 126.6 (C-1 (*Z*)), 123.6 (C-6 (*Z*)), 122.7 (C-6 (*E*)), 110.0 (C-2 (*Z*)), 107.9 (C-2 (*E*)), 107.7 (C-5 (*E*)), 107.5 (C-5 (*Z*)), 101.1 (OCH_2O), 76.1 (OCH_2Ar (*Z*)), 75.9 (OCH_2Ar (*E*)), 52.0 ($COOCH_3$), 40.8 ($ArCH_2C=O$) ppm; **MS** (*m/z*): 403 (M^+, 49%), 372 (100%), 121 (18%), 77 (38%).

Anal. Calcd. for $C_{24}H_{21}NO_5$: C, 71.44; H, 5.25; N, 3.47. Found: C, 71.53; H, 5,17; N, 3.55.

*2.8. 4-[[[(3,4-Methylenedioxyphenyl)methylene]amino] oxy]methyl] phenylacetic acid (**4b**) (adapted from Seebach et al., 1994)*

An 80% suspension of sodium hydride in mineral oil (0.12 g, 5 mmol) was washed with dry *n*-hexane until a white pale solid was obtained which was suspended in 1 ml of dry THF. Then, a solution of 0.166 g (1 mmol) of the oxime derivative (**7b**) in 2 ml of dry THF was added dropwise and the reaction mixture was refluxed under stirring for 30 min. After this time, a solution of 0.298 g (1.30 mmol) of 4-bromomethylphenylacetic acid (**12**) in 4 ml of dry THF was added and the reaction was additionally refluxed for 1.5 h, then poured into an ice–water mixture containing 5 ml of an 10% aqueous HCl solution and extracted with dichloromethane (3 × 20 ml). The organic extracts were submitted at usual work-up, furnishing 0.190 g (61%) of the acid derivative (**4b**), as a white solid, mp 154–156°C; **^{1}H NMR** (200 MHz): δ 8.05 (1H, s, *H*C=N), 7.37 (2H, d, $J = 8$ Hz, H-3′), 7.28 (2H, d, $J = 8$ Hz, H-2′), 7.26 (1H, s, CO_2H), 7.19 (1H, d, $J_{6-2} = 2$ Hz, H-2), 6.93 (1H, dd, $J_{6-5} = 8$ Hz and $J_{6-2} = 2$ Hz, H-6), 6.78 (1H, d, $J_{6-5} = 8$ Hz, H-5), 6.00 (2H, s, OCH_2O), 5.15 (2H, s, OCH_2Ar), 3.65 (2H, s, $ArCH_2C=O$) ppm; **^{13}C NMR** (50 MHz): δ 172.7 (C=O), 148.9 (C-4), 148.8 (C=N), 147.8 (C-3), 136.0 (C-1′), 134.7 (C-4′); 129.4 (C-3′), 128.3 (C-2′), 126.1 (C-1), 122.9 (C-6), 108.5 (C-5), 105.3 (C-2), 101.5 (OCH_2O), 75.1 (OCH_2Ar), 40.4 ($ArCH_2C=O$) ppm; **IR** (KBr): 3444 (ν O–H), 1703 (ν C=O), 1603 (ν C=N), 1256 and 1033 (ν C–O) cm^{-1}; **MS** (*m/z*): 313 (M^+, 14%), 149 (100%), 121 (6%), 77 (14%).

Anal. Calcd. for $C_{17}H_{15}NO_5$: C, 65.16; H, 4.83; N, 4.47. Found: C, 65.23; H, 4,87; N, 4.55.

*2.9. 4-[[[(3,4-Methylenedioxyphenyl)phenylmethylene] amino]oxy]methyl] phenylacetic acid (**4a**) (Barrow et al., 1995)*

To a solution of the 0.023 g (0.057 mmol) of the methyl ester derivative (**14a**) in 7 ml of acetone were added 4 ml of 1 N aqueous LiOH solution. The resulting mixture was stirred at room temperature for 7 h then neutralized with 1 N aqueous HCl (ca. 3 ml) until pH 4 and extracted with diethyl ether (3 × 10 ml). The organic extracts were joined and submitted at usual work-up to give 0.020 g (90%) of the respective acid derivative (**4a**), as a viscous brownish oil; **^{1}H NMR** (200 MHz): δ 7.50–7.18 (10H, m, H-2′, H-3′, H-4′, H-2′, H-3′, CO_2H), 7.08 (1H, d, $J_{6-5} = \sim 1$ Hz, H-5 (*Z*)), 6.88 (1H, t, $J_{6-2} = \sim 1$ Hz and $J_{5-2} < 1$ Hz, H-2 (*Z*)), 6.81 (2H, m, H-6 (*Z*) and H-2 (*E*)), 6.80 (1H, dd, $J_{6-5} = 8$ Hz and $J_{6-2} = 2$ Hz, H-6 (*E*)), 6.68 (1H, dd, $J_{6-5} = 8$ Hz and $J_{5-2} = 1$ Hz, H-5 (*E*)), 5.90 (2H, s, OCH_2O (*Z*)), 5.86 (2H, s, OCH_2O (*E*)), 5.13 (2H, s, OCH_2Ar (*E*)), 5.08 (2H, s, OCH_2Ar (*Z*)), 3.55 (4H, s, $ArCH_2C=O$ (*E*) and (*Z*)) ppm; **^{13}C NMR** (50 MHz): δ 176.1 (C=O), 156.8 (C=N (*Z*)), 156.6 (C=N (*E*)), 148.7 (C-4 (*Z*)), 147.9 (C-4 (*E*)), 147.8 (C-3 (*Z*)), 147.3 (C-3 (*E*)), 137.5 (C-1′ (*Z*)), 137.3 (C-1′ (*E*)), 136.8 (C-1′ (*Z*)), 133.6 (C-1′ (*E*)), 132.7 (C-4′ (*Z*)), 132.6 (C-4′ (*E*)), 130.8 (C-1 (*E*)), 129.2 (C-4′ and C-3′), 129.1 (C-2′), 128.6 (C-3′ (*E*)), 128.3 (C-3′ (*Z*)), 128.1 (C-2′ (*E*)), 128.0 (C-2′ (*Z*)), 126.9 (C-1 (*Z*)), 123.7 (C-6 (*Z*)), 122.8 (C-6 (*E*)), 110.1 (C-2 (*Z*)), 108.0 (C-2 (*E*)), 107.8 (C-5 (*E*)), 107.7 (C-5 (*Z*)), 101.2 (OCH_2O), 76.2 (OCH_2Ar (*Z*)), 76.0 (OCH_2Ar (*E*)), 40.6 ($ArCH_2C=O$) ppm; **IR** (Film): 3447 (ν O–H), 1709 (ν C=O), 1487 (ν C=N), 1237 and 1038 (ν C–O) cm^{-1}.

Anal. Calcd. for $C_{23}H_{19}NO_5$: C, 70.93; H, 4.92; N, 3.60. Found: C, 71.03; H, 4.98; N, 3.55.

*2.10. Methyl 4-[[[(3,4-methylenedioxyphenyl)methylene]amino]oxy]methyl] phenylacetate (**14b**) (Teixeira et al., 1998)*

A solution of 0.070 g (0.22 mmol) of the acid (**4b**) in 5 ml of methanol, containing two drops concentrated sulfuric acid, was refluxed for 2 h, then poured into an ice–water mixture. The precipitate formed was filtered out, washed with 5 ml of a 5% aqueous sodium bicarbonate solution and air-dried, to give 0.062 g (86%) of the corresponding methyl ester derivative (**14b**), as a white solid, mp 70-71° C; **^{1}H NMR** (200 MHz): δ 8.05 (1H, s, *H*C=N), 7.38 (2H, d, $J = 8$ Hz, H-3′), 7.28 (2H, d, $J = 8$ Hz, H-2′), 7.19 (1H, d, $J_{6-2} = 2$ Hz, H-2), 6.93 (1H, dd, $J_{6-5} = 8$ Hz and $J_{6-2} = 2$ Hz, H-6), 6.78 (1H, d, $J_{6-5} = 8$ Hz, H-5), 6.00 (2H, s, OCH_2O), 5.15 (2H, s, OCH_2Ar), 3.70 (3H, s, $COOCH_3$), 3.65 (2H, s, $ArCH_2C=O$) ppm; **^{13}C NMR** (50 MHz): δ 171.8 (C=O), 149.0 (C-4), 148.5 (C=N), 148.0 (C-3), 136.3 (C-1′), 133.5 (C-4′), 129.2 (C-3′), 128.5 (C-2′), 126.4 (C-1), 122.8 (C-6), 108.1 (C-5), 105.6 (C-2), 101.2 (OCH_2O), 75.8 (OCH_2Ar), 51.9

($COOCH_3$), 40.8 ($ArCH_2C=O$) ppm; **IR** (KBr): 1731 (ν C=O), 1500 (ν C=N), 1260 and 1043 (ν C–O) cm^{-1}; **MS** (m/z): 327 (M^+, 33%), 296 (100%), 149 (63%).

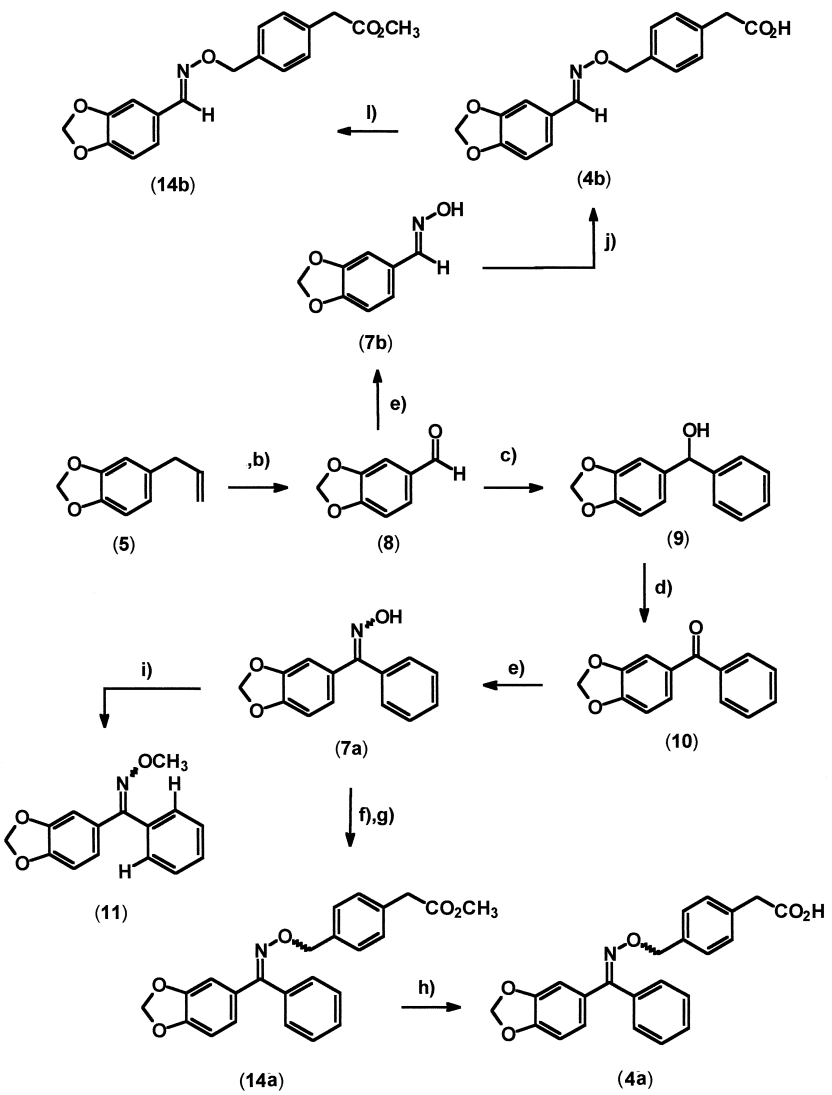
Anal. Calcd. for $C_{18}H_{17}NO_5$: C, 66.03; H, 5.24; N, 4.28. Found: C, 66.11; H, 5,27; N, 4.35.

2.11. Pharmacology

Blood was collected from rabbits by puncture of the central ear artery into 3.8% sodium citrate (9:1 v/v). Platelet-rich plasma (PRP) was prepared by centrifugation, $500 \times g$ for 10 min, at room temperature, and platelet count was adjusted to 5×10^8 platelets/ml.

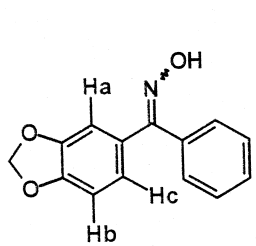
Platelet aggregation was monitored by the turbidimetric method (Born and Cross, 1963) in a Chrono-Log aggregometer. PRP (400 μl) was incubated at 37°C for 1 min with continuous stirring at 900 rpm and then stimulated with ADP (5 μM in distilled water), collagen (5 μg/ml in saline), arachidonic acid (AA — 200 μM in ethanol) or U-46619 (3 μM in ethanol).

The new oxime derivatives (**4a–b**), (**14a–b**) and the vehicle (0.5% DMSO, 2 μl) were added to the PRP

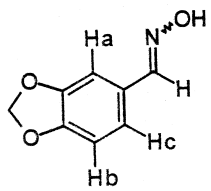


Scheme 1. (a) aq. 3*N* KOH, *n*-BuOH, reflux, 3 h, 98%; (b) i: O_3–O_2, AcOH, −10°C, 4 h; ii: Zn, AcOH, 0°C, 2 h; 78%; (c) PhMgBr, THF, r.t., 40 min., 60%; (d) MnO_2, THF, reflux, 30 min, 70%; (e) $NH_2OH \cdot HCl$, NaOH, EtOH, H_2O, reflux; 30 min, 64% (**7a**); 5 min, 94% (**7b**); (f) i: NaH, DME, 30 min; ii: *para*-$BrCH_2C_6H_4CH_2COOH$ (**12**), DME, r.t. ⇒ reflux, 18 h; (g) i: CH_2N_2, Et_2O, r.t.; ii: chromatographic purification (SiO_2), 28% (two steps); (h) LiOH, acetone, r.t., 4 h, 90%; (i) i: NaH, DME, reflux, 30 min; ii: CH_3I, r.t., 5 min, 57%; (j) i: NaH, THF, 30 min; ii: *para*-$BrCH_2C_6H_4CH_2COOH$ (**12**), THF, r.t. 10 h, 85%; (l) CH_3OH, H_2SO_4, reflux, 2 h, 86%.

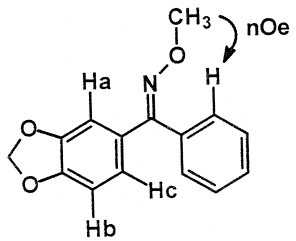
Table 1
Diastereomer ratio and chemical shift of the 1,3-benzodioxole ring hydrogens from ^{1}H NMR spectra of the (*E*)- and (*Z*)-ketoxime derivatives (**7a–b**) and (**11**)



(**7a**)



(**7b**)



(**11**)

Compound	*E*/*Z* ratio[b]	δ (ppm)[b]							
		–OCH$_2$O–		Ha		Hb		Hc	
		(*E*)	(*Z*)	(*E*)	(*Z*)	(*E*)	(*Z*)	(*E*)	(*Z*)
7a	1.1:1	5.97	6.02	6.89	7.01	6.72	7.06	6.84	6.89
11	1.1:1	5.92	5.96	6.82[c]	6.90	6.70	7.10	6.81	6.82[c]
7b	100:0	5.98	–	7.17	–	6.79	–	6.96	–

[a] Determined by relative integration of methylenedioxy singlet signals.
[b] Performed in $CDCl_3$ at 200 MHz.
[c] The signals corresponding to the hydrogens (*E*)–Ha and (*Z*)–Hb appears together in a multiplete at δ 6.82 ppm.

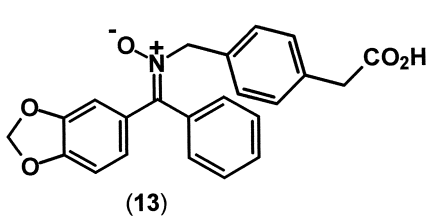
samples 5 min before addition of the aggregating agent. The DMSO used as vehicle did not have either pro- or antiplatelet aggregation activity. Indomethacin (10 μM), a classical cyclooxygenase inhibitor, was used as standard.

The platelet aggregation was expressed as percentage of aggregation for ADP, AA, U-46619 and as the maximum rate of aggregation (slope) for collagen. Data were analyzed statistically by Student's ''t'' test for a p value of < 0.05 and are expressed as mean ± S.D. for n experiments in triplicate.

3. Results and discussion

3.1. Chemistry

The target new oximes *O*-benzylethers derivatives (**4a–b**) were synthesized by using the methodology illustrated in Scheme 1. Our synthetic approach to these new compounds identify the functionalized arylketone-oxime (**7a**) and the 3,4-methylenedioxybenzaldehyde-oxime (**7b**) as the key intermediates. These compounds can be easily obtained from the natural product used as starting-material (**5**) by exploring a well-known synthetic sequence from this laboratory (Barreiro and Lima, 1992; Barreiro et al., 1985) (Scheme 1), including the basic isomerization of safrole (**5**) double bond, followed by ozonolysis cleavage with reductive work-up furnishing the corresponding 3,4-methylenedioxybenzaldehyde (**8**). The aldehyde (**8**), precursor of the desired oxime (**7a**), was next treated with the phenyl Grignard reagent (Klix et al., 1995) furnishing the aryl-benzylic alcohol (**9**), in high yield (Scheme 1). The next step of the planned synthetic route to access the new oxime (**7a**), was the MnO_2 oxidation (Takeuchi et al., 1995) of the benzylic alcohol (**9**), to produce the corresponding aryl-ketone (**10**). Treatment of the aryl–ketone derivative (**10**), with hydroxylamine gave the desired oxime (**7a**) (Furniss et al., 1989). The oxime (**7a**) was obtained as 1.1:1 mixture of diastereomers (*E*) and (*Z*), as indicated by the relative integration of singlets refereed to methylenedioxy hydrogens in ^{1}H NMR spectra (Table 1). The diastereomer attribution was made in agreement with previous paper of Karabatsos and His (1967) and by relative comparison of the its ^{1}H NMR signals with those of the diastereomerically pure nor-phenyl derivative (**7b**) (Table 1). Additionally, the stereochemical assignment of (**7a**) was aided by nOe experiment on the correspondent methyl ether derivative (**11**), obtained by treatment of the sodium salt of the diastereomeric mixture of oximes (**7a**) with



(**13**)

Fig. 2.

Table 2
Effect of oxime *O*-benzylether derivatives (**4a–b**) and (**14a–b**) on in vitro platelet aggregation of citrated rabbit platelet-rich plasma induced by arachidonic acid, ADP, collagen and U-46619

Compounds	C^a (μM)	n^b	Arachidonic Acid (200 μM)		n^b	ADP (5 μM)		n^b	Collagen (5 μg/ml)		n^b	U-46619 (3 μM)	
			Aggregation (%)	Inhibition (%)		Aggregation (%)	Inhibition (%)		Aggregation (slope)	Inhibition (%)		Aggregation (%)	Inhibition (%)
Control	–	6	68.9 ± 5.1	–	5	32.5 ± 2.5	–	5	8.7 ± 0.5	–	5	52.7 ± 4.3	–
Indomethacin	10	4	–	100.0*	3	–	1.2	4	–	94.8*	5	–	1.9
4a	100	4	53.9 ± 5.5	21.8	4	25.3 ± 0.5	22.1	4	4.7 ± 1.6	46.0*	5	48.6 ± 4.2	7.8
14a	100	4	9.5 ± 4.1	86.2*	4	23.2 ± 1.2	28.6*	4	0.7 ± 0.7	91.9*	5	48.0 ± 4.6	8.9
	75	4	50.5 ± 6.5	26.7	–	–	–	–	–	–	–	–	–
	50	4	67.9 ± 3.5	1.4	–	–	–	–	–	–	–	–	–
4b	100	4	64.9 ± 6.7	5.8	4	26.1 ± 0.6	19.7	4	6.7 ± 1.3	23.0	–	–	–
14b	100	4	56.0 ± 4.6	18.7	4	24.7 ± 1.2	24.0	4	4.6 ± 1.6	47.1*	–	–	–

[a] C = concentration.
[b] n = number of independent experiments carried out in triplicate.
* $p < 0.05$ compared to appropriate control (Student's ''t'' test).

methyl iodide (Scheme 1). For instance, irradiation of the OCH_3 protons of the major isomer of the compound (**11**) at δ 3.97 ppm in the ^1H NMR spectrum, showed a positive nOe effect into *ortho*-hydrogens present in phenyl group (Table 1), indicating the relative configuration (*E*).

The key-step for access to new oxime *O*-benzylether (**4a**) was the *O*-benzylation of the oxime (**7a**). This step was accomplished by treatment sodium salt of oxime derivative (**7a**), prepared from treatment with NaH in THF at room temperature, with *para*-bromophenylacetic acid (**12**) at reflux, to produce the corresponding acid (**4a**), in appropriated yield (Seebach et al., 1994). In fact, the O-alkylation of benzophenone-like oximes can produce a mixture of *N*,*O*-alkylation products (Grigg et al., 1990). For instance, in the alkylation of benzophenone-oxime (**7a**), we are able to detect by tlc the formation of secondary nitrone derivative (**13**) (Smith and Robertson, 1962) (Fig. 2) and also the recovery of 58% yield of the corresponding aryl–ketone derivative (**10**).

In order to obtain the desired *O*-benzylated product (**4a**), in good purity degree, we are obligated to esterify them with diazomethane followed by carefully chromatographic purification. This procedure gave the pure corresponding methyl esters (**14a**), as a mixture of (*E*)- and (*Z*)-diastereomers. The basic hydrolysis of the methyl ester (**14a**) with an aqueous LiOH solution in acetone (Barrow et al., 1995) at room temperature regenerated the desired acid compound (**4a**), in 90% yield. The nor-aryl oxime *O*-benzylether derivative (**4b**) was prepared from the aldehyde (**8**) (Scheme 1) by oximation with hydroxylamine, to produce pure (*E*)-diastereomer of (**7b**), which after O-benzylation with (**12**), using the same experimental conditions, gives the acid (**4b**) in good yield.

Finally, in order to compare the antiplatelet profile of planned acid derivatives (**4a–b**) with the corresponding methyl esters (**14a–b**), we submitted the acid (**4b**) to classical Fisher's esterification conditions (Teixeira et al., 1998), to obtain the ester (**14b**), in 86% yield.

3.2. Pharmacology

The antithrombotic activity of these novel oxime *O*-benzylethers **(4a–b)** and its corresponding methyl ester derivatives (**14a–b**) were evaluated by their ability to inhibit platelet aggregation of rabbit platelet-rich plasma (PRP) induced by either adenosine diphosphate (ADP, 5 μM), collagen (100 μM), arachidonic acid (AA, 200 μM) and U-46619 (3 μM), a stable TP agonist. The results of the antiplatelet profile of **(4a–b)** and **(14a–b)** are displayed in Table 2.

In this series, methyl esters (**14a**) and (**14b**) were more active than the corresponding acid derivatives (**4a**) and (**4b**), in models induced by AA and collagen (Table 2). Compound **(4b)** did not shown any significant activity for all agonists studied.

Compound (**14a**) was the most active one. It inhibited strongly the platelet aggregation induced by AA and collagen, by 86.2% and 91.9% respectively, and while also inhibited the ADP induced aggregation, by 28.6%. The IC_{50} obtained from AA induced aggregation of the compound (**14a**) was 81.5 μM (Table 2).

In addition, compound (**14a**) inhibited the second wave of aggregation induced by ADP on human PRP (data not shown).

Compounds (**4a**) and (**14b**) presented a similar activity. Both compounds inhibited moderately the platelet aggregation induced by collagen [46% (**4a**) vs. 47.1% (**14b**)] and AA [21.8% (**4a**) vs. 18.7% (**14b**)] (Table 2).

Compounds (**4a**) and (**14a**) did not inhibited the platelet aggregation induced by U-46619 (Table 2), suggesting that the anti-aggregating activity presented by these compounds is not probably through antagonism of TP receptors.

These results stand out the relevance, to the antiplatelet profile, of the presence of a second phenyl unit at the oxime moiety, characterized by pattern benzophenone-oxime of compound (**14a**), just as described in the lead compound ridogrel (**6**). Curiously, the results obtained from this study seems to indicate that the interphenylene acidic chain present in compound (**14a**) prevents the TPant profile as shown the data using U-46619 (Table 2), contrasting with the lead compound (**6**). In addition, the more pronounced activity of the methyl ester (**14a**) can be probably due to its less effective plasmatic protein binding in comparison with the corresponding acid derivative (**4a**) (Kragh-Hansen, 1981).

Finally, the results described herein demonstrated an important antithrombotic profile for compound (**14a**), suggesting an activity at the TXS level.

4. Conclusions

The synthetic route described herein represents an useful method to access the new antiplatelet oxime *O*-benzylether derivatives (**4a**) and (**14a**), exploring the 3,4-methylenedioxybenzophenone oxime (**7a**) as a key intermediate and safrole (**5**) as starting material. The antiplatelet profile of these new compounds identify the new compound (**14a**) as inhibitor of the platelet aggregation induced by arachidonic acid presenting an $IC_{50} = 81.5$ μM. These results indicated that the compounds (**4a–b**) and (**14a–b**) represents a new class of antiplatelet agents, possessing a new molecular pattern, synthesized from Brazilian abundant natural product safrole (**5**).

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