Nitrooxymethyl-Substituted Analogues of Rofecoxib: Synthesis and Pharmacological Characterization

Nitrooxymethyl-Substituted Analogues of Rofecoxib: Synthesis and Pharmacological Characterization

by **Donatella Boschi**[a]), **Clara Cena**[a]), **Antonella Di Stilo**[a]), **Barbara Rolando**[a]), **Paola Manzini**[b]), **Roberta Fruttero**[a]), and **Alberto Gasco***[a])

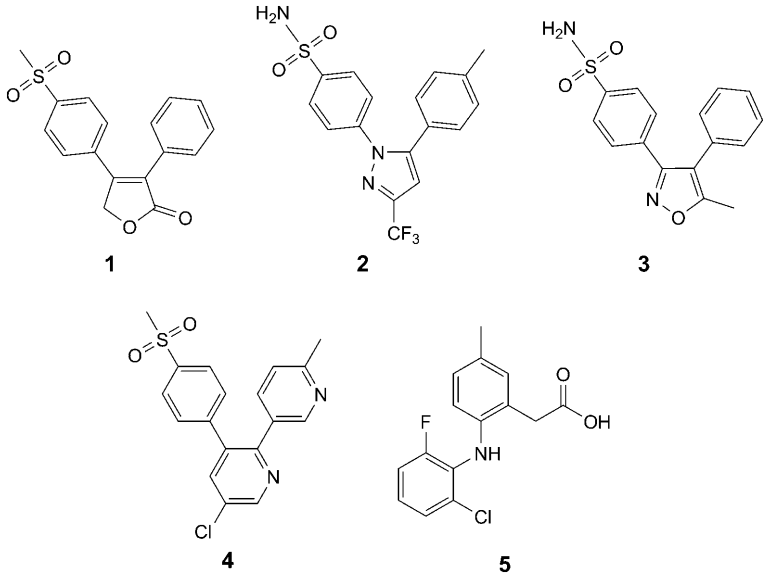
[a]) Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Via Pietro Giuria 9, I-10125 Torino (phone: +39011 6707670; fax: +39011 6707286; e-mail: alberto.gasco@unito.it)

[b]) SIMT Banca del Sangue, AOU S. Giovanni Battista, C.so Bramante 88, I-10126 Torino

Nitrooxymethyl-substituted derivatives of Rofecoxib were synthesized and tested for their cyclooxygenase (COX)-inhibiting activity in whole human blood, vasodilator potency on rat aorta strips, and for their capacity of inhibiting platelet aggregation of human platelet-rich plasma. The results show that their potency and selectivity in inhibiting COX isoforms, as well as their anti-aggregatory properties, are closely dependent on the position at which the NO-donor nitrooxymethyl function is introduced into the Rofecoxib scaffold. All the products were capable of dilating rat aorta strips precontracted with phenylephrine in a dose-dependent manner, through a cGMP-dependent mechanism. Compound **10** emerged as a quite potent COX-2-selective inhibitor endowed with good vasodilator activity. Interestingly, compound **19** behaved as a potent selective COX-1 inhibitor, and displayed good vasodilator and anti-aggregatory properties. The hydroxymethyl derivatives, potential metabolites of the nitrooxymethyl analogues, were similarly studied for a comparison.

Introduction. – The principal pharmacological effects of the traditional non-steroidal anti-inflammatory drugs (tNSAIDs) are related to their ability to inhibit the cyclooxygenase isoforms COX-1 and COX-2, two enzymes involved in the production of prostanoids from arachidonic acid [1]. A wide amount of experimental evidence shows that the inhibition of the COX-2 isoform is principally associated with the anti-inflammatory and analgesic properties of the NSAIDs, while the inhibition of the COX-1 isoform is responsible for their anti-aggregatory and gastrotoxic effects [2][3]. On these bases, a new class of inhibitors endowed with a high selectivity for the COX-2 isoform was developed as anti-inflammatory drugs [4]. These products, called Coxibs, are characterized by reducing prostaglandin (PG)-dependent inflammation, while maintaining protective gastric PG synthesis [2][5][6]. A number of them, Rofecoxib (**1**) [7], Celecoxib (**2**), Valdecoxib (**3**), Etoricoxib (**4**), and Lumiracoxib (**5**), were introduced into the market [8].

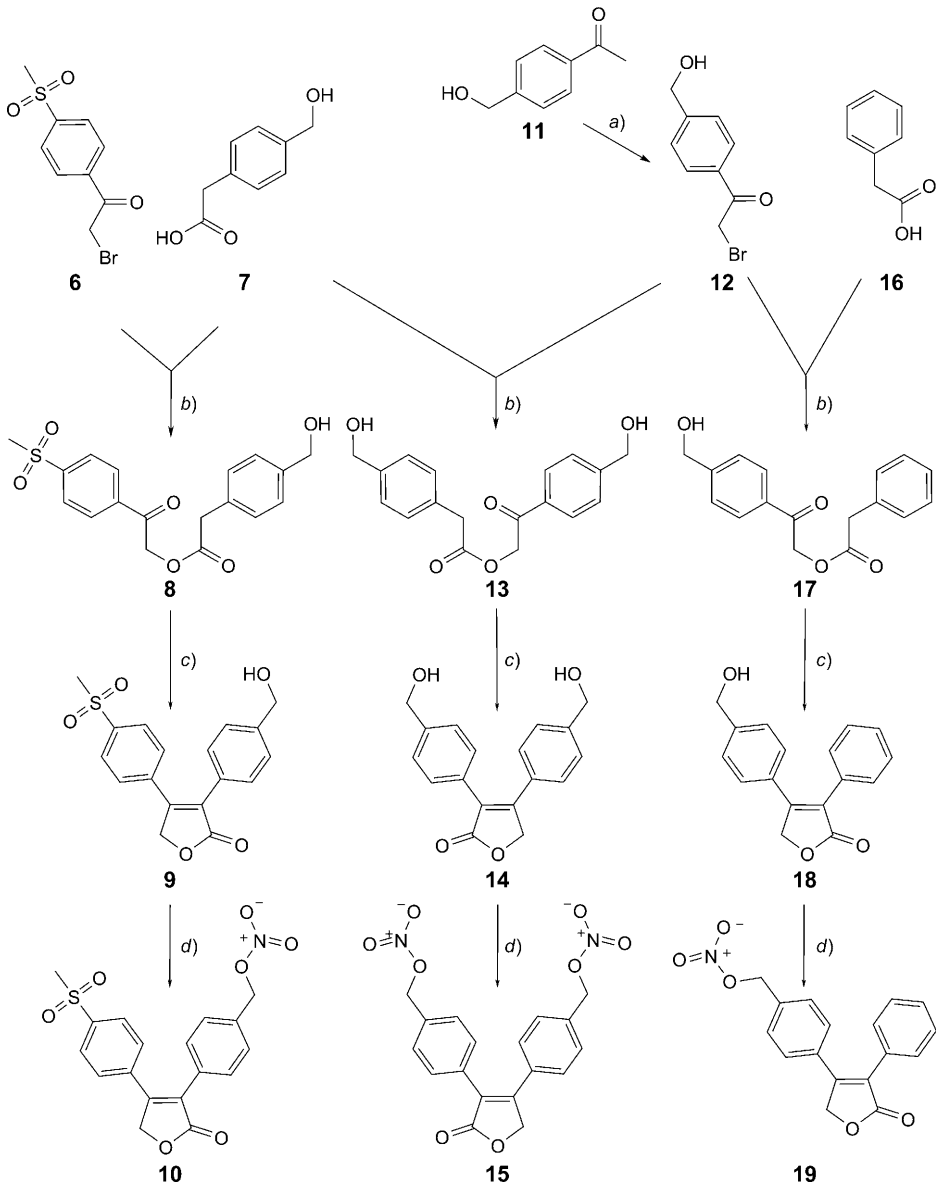
During the clinical use of these Coxibs, it was found that they can increase the risk of heart attack and stroke. This cardiotoxicity is due to their capacity of reducing the biosynthesis of prostacycline (PGI_2) *in vivo*, with consequent tipping of prostanoid balance in favor of thrombogenic tromboxane [9–11]. Because of their adverse cardiovascular effects, Rofecoxib and Valdecoxib were withdrawn from the market [8]. This removal decreased the interest in the selective COX-2 inhibitors and discouraged the pharmaceutical companies to develop further this kind of drugs. Today, there is a



renewed attention to Coxibs based on the finding that a meta-analysis of 140 randomized trials of five different Coxibs showed that their use was associated with a relatively low incidence of major vascular events [12][13]. Therefore, these drugs could be used with appropriate population of patients at low cardiovascular risk. In addition, recent discoveries revealed new functions for COX-2 enzyme, including an important role in tumorigenesis [14] with consequent possible applications of Coxibs in the treatment and in the detection of cancer [15]. The use of these drugs has been proposed also for the management of *Alzheimer*'s disease [16]. A possible way of attempting to reduce cardiotoxicity of Coxibs, and consequently to improve their benefit–risk profile, is to design nitric oxide (NO)-releasing coxibs (NO-Coxibs). Indeed, NO is an endogenous messenger which displays very important roles in maintaining micro- and macrovascular homeostasis, among them vasodilation, inhibition of platelet aggregation, modulation of platelet and leukocyte adherence to vessels, and inhibition of smooth muscle-cell proliferation. In addition, it is essential for normal physiological function in central nervous system (CNS), and it is one of the final effectors in the immune system [17]. Consequently, NO donors are potential useful tools in cancer therapy and in the management of *Alzheimer*'s disease [18][19]. There are a number of NO-Coxibs reported in literature. They were obtained by using NO donor 3,4-diphenylfuroxan system bearing appropriate substituents [20][21], or by linking selective COX-2 inhibitors with either diazen-1-ium 1,2-diolate [22], or nitrooxy NO-donor moieties [23–26]. As development of our work in this field, we now describe the synthesis, COX-inhibition profile studied in human whole blood, and anti-aggregatory and *in vitro* vasodilator activities of the compounds **10**, **15**, and **19**, containing nitrooxy function(s) inserted into the Rofecoxib scaffold. The inhibitory activity of the related OH analogs **9**, **14**, and **18**, used as intermediates for the preparation of the target compounds, is also reported in view of their possible role of metabolites [27].

Results and Discussion. – 1. *Chemistry.* The synthetic pathway used to prepare the nitrooxy-substituted Rofecoxib derivatives is depicted in the *Scheme*. The method used to prepare furan-2(5*H*)-one derivatives is the same reported by *Thèrien et al.* for the

Scheme



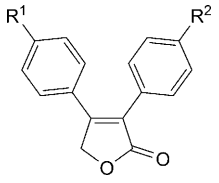
a) HBr/Br_2, CH_2Cl_2, 0°. *b*) Et_3N, MeCN. *c*) 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU), MeCN, 60° (r.t. for **9**). *d*) Ph_3P, $AgNO_3$, *N*-Bromosuccinimide (NBS), −15°→r.t. (60° for **19**).

synthesis of Rofecoxib [28]. The Br-substituted ketone **6** treated with the [4-(hydroxymethyl)phenyl]acetic acid (**7**) afforded, in the presence of Et_3N, esther **8**. This product underwent cyclization, under the action of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), to give the 4-hydroxymethyl-substituted Rofecoxib **9**. Treatment of **9**, dissolved in MeCN, with *N*-bromosuccinimide (NBS) in the presence of Ph_3P, and then with $AgNO_3$, afforded the expected mononitrooxy substituted final compound **10**. This is a recent method for the preparation of nitrates under mild conditions [29]. The sequence of reactions aforementioned was used to prepare **13**, **14**, and the dinitrooxy-substituted final compound **15**, starting from **7** and **12**. This latter intermediate was obtained by bromination of the known 1-[4-(hydroxymethyl)phenyl]ethanone (**11**). Similarly, the intermediates **17**, **18**, and the mononitrooxy-substituted Rofecoxib analog **19** were sequentially synthesized starting from **12** and 2-phenylacetic acid (**16**).

2. *COX Inhibition and Platelet Anti-aggregatory Activity.* The ability of the products and of their alcoholic analogues to inhibit the COX enzymes was evaluated in human whole blood according to a well-established procedure [30]. To assess the extent of COX-2 isoform inhibition, human heparinized whole blood was incubated with lipopolysaccharide (LPS) overnight in the presence of the inhibitors, and plasma was assayed for PGE_2 production as a function of COX-2 inhibition. For COX-1 inhibition activity, whole blood samples without any anticoagulant were incubated with the inhibitors for 1 h. Then, plasma was collected and analyzed for TXB_2 production. The potencies of the products expressed as IC_{50} values are collected in *Table 1*. When these values could not be derived because inhibition did not reach 50%, the inhibition at maximal concentration tested (100 μM) was calculated. Analysis of the data shows that the introduction of one nitrooxymethyl group in *para*-position of the 3-phenyl ring of Rofecoxib gives rise to **10**, which is a COX-2 inhibitor *ca.* 15 times less potent than the lead, but it retains a quite good degree of COX-2/COX-1 selectivity. Its OH analogue **9** behaves similarly. By contrast, when in **1** the $MeSO_2$ group, which is an important determinant for the COX-2 selectivity [4], is replaced with the nitrooxymethyl moiety, **19** is obtained that is a COX-1 inhibitor *ca.* 100 times more potent and a COX-2 inhibitor about sixfold less potent than the lead. Inhibitory potencies and selectivity are even more evident in its OH analog **18**. Finally, the simultaneous presence of two nitrooxymethyl groups in *para*-positions of the two phenyl rings of **1** provides **15**, which displays very feeble COX inhibition, when tested on both the two isoforms. By contrast, its dihydroxy analog **14** is a quite potent and selective COX-1 inhibitor. The COX inhibitory profiles of the nitrooxy-substituted Rofecoxib compounds and of their OH analogs here described parallel very well those of the related Celecoxib derivatives previously described [26]. The only difference is the definitively lower inhibitor activities of the bis(hydroxymethyl)- and bis(nitrooxymethyl)-substituted compounds **14** and **15**, respectively. All products were also tested for their ability to inhibit collagen-induced platelet aggregation of human platelet-rich plasma (PRP). Only the nitrooxymethyl-substituted final compound **19** displayed potent anti-aggregatory activity in the micromolar range, in keeping with its high ability to inhibit COX-1 isoform (*Table 2*). A similar behavior was shown by the $HOCH_2$-substituted intermediates **14** and **18**.

3. *Vasodilation.* The *in vitro* vasodilator activity of the NO-donor analogs of Rofecoxib **10**, **15**, and **19** was assessed on rat aorta strips precontracted with

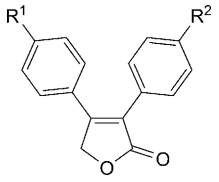
Table 1. *COX-1 and COX-2 inhibition data for compounds and Rofecoxib* **1**, *taken as reference*



Compound	R^1	R^2	COX-1		COX-2	
			$IC_{50} \pm$ SE [μM][a])	% Inhibition ± SE[b]) (100 μM)[c])	$IC_{50} \pm$ SE [μM][a])	% Inhibition ± SE[b]) (100 μM)[c])
1	SO_2Me	H	64 ± 13	–	1.5 ± 0.8	
9	SO_2Me	CH_2OH	[d])	7.6 ± 6.5	22 ± 5	
10	SO_2Me	CH_2ONO_2	[d])	11 ± 4	22 ± 6	
14	CH_2OH	CH_2OH	5.7 ± 1.7		[d])	21 ± 13
15	CH_2ONO_2	CH_2ONO_2	[d])	36 ± 2	[d])	39 ± 11
18	CH_2OH	H	0.24 ± 0.03		8.4 ± 2.8	
19	CH_2ONO_2	H	0.61 ± 0.17		9.6 ± 4.2	

[a]) Values are expressed as $IC_{50} \pm$ standard errors. [b]) Values are expressed as mean percentage of inhibition ± standard errors. [c]) Maximum concentration tested. [d]) Inhibition of control prostanoid production did not reach 50%.

Table 2. *Anti-Aggregatory and Vasodilating Properties of Derivatives*



Compound	R^1	R^2	Anti-aggregatory activity		Vasodilating activity
			IC_{50} (95% CL) [μM][a])	% Inhibition ± SE[b]) (100 μM)[c])	$EC_{50} \pm$ SE [μM][d])
1	SO_2Me	H	48 (44–51)		inactive
9	SO_2Me	CH_2OH	[e])	1.7 ± 1.7	[f])
10	SO_2Me	CH_2ONO_2	[e])	29 ± 15	0.21 ± 0.06 11 ± 1.3[g])
14	CH_2OH	CH_2OH	23 (19–28)		[f])
15	CH_2ONO_2	CH_2ONO_2	[e])	5.3 ± 4.4	0.28 ± 0.05 15 ± 4[g])
18	CH_2OH	H	39 (35–42)		[f])
19	CH_2ONO_2	H	46 (42–50)		0.22 ± 0.08 35 ± 3[g])
ISDN[h])			[f])	[f])	4.7 ± 0.6 > 100[g])

[a]) Values are expressed as IC_{50} with 95% confidential limits in brackets. [b]) Values are expressed as mean percentage of inhibition ± standard errors. [c]) Maximum concentration tested. [d]) Values are expressed as $EC_{50} \pm$ standard errors. [e]) Inhibition of control aggregation effect did not reach 50%. [f]) Not tested. [g]) In the presence of 1 μM 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ). [h]) Isosorbide dinitrate (ISDN) was used as a reference compound.

phenylephrine. All the products were capable of relaxing the contracted tissues in a concentration-dependent manner. Their potencies, expressed as EC_{50} values, are collected in *Table 2*. All of them behave as potent vasodilators, more active than isosorbide dinitrate (ISDN) taken as a reference. The vasodilator potencies of the products were reduced, when the experiments were repeated in the presence of 1 μM ODQ (1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one), a well-known heme site inhibitor of sGC. This is in keeping with the involvement of NO in the vasodilator action.

Conclusions. – Introduction of nitrooxymethyl and of hydroxymethyl groups on the scaffold of Rofecoxib (**1**) gives rise to compounds with different COX-inhibitor profiles. The two most interesting products obtained are the compounds **19** and **10**. The former is derived from replacement of methylsulfonyl group with nitrooxymethyl moiety in **1**. It is a rather potent and selective COX-1 inhibitor, endowed with vasodilator and anti-aggregatory properties. This finding confirms the importance of $MeSO_2$ group for COX-2 selectivity of the lead. To our knowledge, it is the first example of NO-donor selective COX-1 inhibitor which might be further studied as cardioprotective, chemopreventive, and analgesic agent [31]. The latter arises from introduction of nitrooxymethyl group in the *para*-position of the 3-phenyl ring of **1**. It is a selective and quite potent COX-2 inhibitor, which displays good vasodilator activity. This product is certainly an interesting NO-Coxib, worthy of additional study owing to its potential low cardiotoxicity.

This work was supported by a grant from *Regione Piemonte Progetto Ricerca Sanitaria Finalizzata 2007.*

Experimental Part

General. Anh. $MgSO_4$ was used as drying agent for the org. phases. Flash column chromatography (FC): silica gel (*Merck Kieselgel 60*, 230–400 mesh ASTM) using the reported eluents. TLC: 5×20 cm plates with 0.25-mm layer thickness. M.p.: in capillaries (*Büchi 540* instrument); m.p. with decomposition were determined after introduction of the sample in the bath at 10° below the m.p.; a heating rate of 1° min^{-1} was used. All compounds were routinely checked by FT-IR (*Perkin-Elmer SPECTRUM BXII*), ^{1}H- and ^{13}C-NMR (*Bruker Avance 300*), and MS (*Finnigan-Mat TSQ-700*). Elemental analysis (C, H, N): *REDOX* (*Monza*); the results were within ±0.4% of the theoretical values. Compounds **6** [28] and **11** [32] were synthesized according to the methods described in the literature, **7** and **16** were supplied by *Sigma-Aldrich*.

2-Bromo-1-[4-(hydroxymethyl)phenyl]ethanone (**12**). A soln. of Br_2 (4.38 g, 27.3 mmol) in CH_2Cl_2 (50 ml) was added at 0°, during 2 h, to a stirred soln. of **11** (4.11 g, 27.0 mmol) in CH_2Cl_2 (200 ml) containing conc. HBr (4 drops). The mixture was washed with H_2O and with a sat. soln. of $NaHCO_3$, then dried, and evaporated to give a crude compound that was purified by FC (CH_2Cl_2/AcOEt 98:2) to give **12** (2.23 g, 36%). White solid. M.p. 68–69° (i-Pr_2O). ^{1}H-NMR ($CDCl_3$): 7.98 (*AA′BB′*, *J* = 8.4, 2 arom. H); 7.49 (*AA′BB′*, *J* = 8.4, 2 arom. H); 4.80 (*s*, CH_2OH); 4.45 (*s*, CH_2Br); 1.94 (*s*, OH). ^{13}C-NMR ($CDCl_3$): 191.1; 147.3; 133.1; 129.2; 126.8; 64.5; 30.9. EI-MS: 230, 228 (M^+), 135 (100). Anal. calc. for $C_9H_9BrO_2$ (229.07): C 47.19, H 3.96; found: C 47.14, H 3.98.

2-[4-(Methylsulfonyl)phenyl]-2-oxoethyl [4-(Hydroxymethyl)phenyl]acetate (**8**). A soln. of **6** (1.66 g, 6.0 mmol) in MeCN (25 ml) was added dropwise to a soln. of **7** (1.00 g, 6.0 mmol) and Et_3N (0.83 ml, 6.0 mmol) in MeCN (30 ml) at r.t. After 7 h, the mixture was concentrated *in vacuo*, and the residue was dissolved in AcOEt. The combined org. phases were washed with H_2O, 1N HCl, and brine, dried and evaporated. FC (petroleum ether (PE)/i-PrOH 7:3) of the crude product gave **8** (1.85 g, 85%). White

solid. M.p. 107–108° (MeOH). ^{1}H-NMR ($CDCl_3$): 7.98 (*s*, 4 arom. H); 7.34–7.26 (*m*, 4 arom. H); 5.28 (*s*, CH_2O); 4.69 (*s*, CH_2OH); 3.81 (*s*, CH_2CO); 3.07 (*s*, Me); 2.03 (*s*, OH). ^{13}C-NMR ($CDCl_3$): 191.9; 170.8; 144.6; 140.3; 138.1; 132.4; 129.5; 128.8; 127.9; 127.3; 66.7; 64.9; 44.3; 40.6. EI-MS: 362 (M^+), 165 (100). Anal. calc. for $C_{18}H_{18}O_6S$ (362.40): C 59.66, H 5.01; found: C 59.85, H 5.05.

2-[4-(Hydroxymethyl)phenyl]-2-oxoethyl [4-(Hydroxymethyl)phenyl]acetate (**13**). Compound **13** was obtained by the above described procedure for **8**, starting from **7** and **12**. After 24 h, the mixture was poured into ice/H_2O, and the pure white precipitate was filtered and dried (yield 82%). M.p. 92–94° ((i-Pr)$_2$O/AcOEt 3:1). ^{1}H-NMR ((D$_6$)DMSO): 7.92 (*AA′BB′*, *J* = 8.4, 2 arom. H); 7.48 (*AA′BB′*, *J* = 8.4, 2 arom. H); 7.28 (*s*, 4 arom. H); 5.49 (*s*, CH_2O); 5.40 (*t*, *J* = 5.7, OH); 5.16 (*t*, *J* = 5.7, OH); 4.59 (*d*, *J* = 5.7, CH_2OH); 4.48 (*d*, *J* = 5.7, CH_2OH); 3.81 (*s*, CH_2CO). ^{13}C-NMR ((D$_6$)DMSO): 193.3; 171.9; 149.5; 141.5; 133.1; 133.0; 129.9; 128.5; 127.4; 127.3; 67.4; 63.3; 63.0; 40.4. EI-MS: 296 ([$M - H_2O$]$^+$), 135 (100). Anal. calc. for $C_{18}H_{18}O_5$ (314.33): C 68.78, H 5.77; found: C 68.65, H 5.78.

2-[4-(Hydroxymethyl)phenyl]-2-oxoethyl 2-Phenylacetate (**17**). Compound **17** was obtained by the above described procedure for **8**, starting from **12** and **16**. The mixture was kept at r.t. for 24 h. The mixture was poured into ice/H_2O, and the pure white precipitate was filtered and dried (yield 52%). M.p. 60–62° ((i-Pr)$_2$O). ^{1}H-NMR ($CDCl_3$): 7.86 (*AA′BB′*, *J* = 8.4, 2 arom. H); 7.45 (*AA′BB′*, *J* = 8.4, 2 arom. H); 7.26–7.36 (*m*, 5 arom. H); 5.33 (*s*, CH_2O); 4.76 (*s*, CH_2OH); 3.82 (*s*, CH_2CO); 1.98 (br. *s*, OH). ^{13}C-NMR ($CDCl_3$): 192.2; 171.6; 147.7; 134.0; 133.7; 129.3; 128.5; 127.7; 127.3; 126.4; 66.9; 64.9; 41.3. EI-MS: 284 (M^+), 135 (100). Anal. calc. for $C_{17}H_{16}O_4$ (284.31): C 71.82, H 5.67; found: C 71.57, H 5.71.

*3-[4-(Hydroxymethyl)phenyl]-4-[4-(methylsulfonyl)phenyl]furan-2(5*H*)-one* (**9**). A soln. of DBU (0.36 g, 2.4 mmol) in dry MeCN (15 ml) was added dropwise during 1 h to a soln. of **8** (1.70 g, 4.7 mmol) in dry MeCN (50 ml) at r.t. under N_2. Then, the mixture was poured into H_2O, and the product was extracted with CH_2Cl_2. The combined org. phase was treated with brine, dried, and evaporated. FC (CH_2Cl_2/MeOH 98:2) of the crude product gave **9** (1.15 g, 71%). White solid. M.p. 177–180° (MeOH/Et$_2$O). ^{1}H-NMR ((D$_6$)DMSO): 8.00 (*AA′BB′*, *J* = 8.4, 2 arom. H); 7.63 (*AA′BB′*, *J* = 8.4, 2 arom. H); 7.38–7.29 (*m*, 4 arom. H); 5.40 (*s*, 1 CH_2 of furan); 5.27 (*t*, *J* = 5.4, OH); 4.53 (*d*, *J* = 5.4, CH_2OH); 3.26 (*s*, Me). ^{13}C-NMR ((D$_6$)DMSO): 172.7; 155.7; 143.5; 142.0; 135.9; 128.9; 128.7; 128.0; 127.5; 126.9; 126.7; 70.9; 62.6; 43.2. EI-MS: 344 (M^+, 100%). Anal. calc. for $C_{18}H_{16}O_5S$ (344.39): C 62.78, H 4.68; found: C 62.45, H 4.68.

*3,4-Bis[4-(hydroxymethyl)phenyl]furan-2(5*H*)-one* (**14**). A soln. of DBU (0.27 g, 1.7 mmol) in dry MeCN (20 ml) was added dropwise during 10 min to a soln. of **13** (1.08 g, 3.4 mmol) in dry MeCN (180 ml) kept at 60° under N_2. After 45 min, the soln. was concentrated to 20 ml under vacuum, the residue was poured into H_2O, and the product was extracted with AcOEt. The combined org. phases were treated with H_2O and brine, and dried and evaporated. The crude product was purified by crystallization from 1,2-dichloroethane to give the title product as a yellow solid (0.70 g, 69% yield). M.p. 161–162°. ^{1}H-NMR ((D$_6$)DMSO): 7.37–7.28 (*m*, 8 arom. H); 5.36 (*s*, CH_2 of furan); 5.24–5.30 (*m*, 2 OH); 4.54–4.49 (*m*, 2 CH_2OH). ^{13}C-NMR ((D$_6$)DMSO): 173.1; 157.2; 145.2; 142.3; 128.9; 128.7; 127.3; 126.6; 126.5; 126.4; 124.1; 70.5; 62.5; 62.4. EI-MS: 296 (M^+, 100%). Anal. calc. for $C_{18}H_{16}O_4 \cdot 0.05\ C_2H_4Cl_2$ (301.27): C 72.16, H 5.42; found: C 71.78, H 5.37.

*4-[4-(Hydroxymethyl)phenyl]-3-phenylfuran-2(5*H*)-one* (**18**). A soln. of DBU (0.41 g, 2.6 mmol) in dry MeCN (10 ml) was added dropwise during 10 min to a soln. of **17** (1.50 g, 3.4 mmol) in dry MeCN (20 ml) kept at 60° under N_2. After 60 min, the mixture was concentrated to 20 ml under vacuum, and the residue was poured into H_2O. The product was extracted with AcOEt, and the combined org. phases were treated with H_2O and brine, and dried ($MgSO_4$) and evaporated. The crude product obtained was crystallized by H_2O/EtOH to give **18** (1.05 g, 75%). White solid. M.p. 141–142°. ^{1}H-NMR ($CDCl_3$): 7.43–7.26 (*m*, 9 arom. H); 5.16 (*s*, CH_2 of furan); 4.69 (*s*, CH_2OH); 2.21 (br. *s*, OH). ^{13}C-NMR ($CDCl_3$): 173.7; 156.0; 143.8; 130.2; 129.8; 129.3; 128.8; 128.7; 127.6; 127.2; 126.0; 70.6; 64.4. EI-MS: 266 (100, M^+). Anal. calc. for $C_{17}H_{14}O_3$ (266.30): C 76.68, H 5.30; found: C 76.47, H 5.26.

4-[2,5-Dihydro-4-[4-(methylsulfonyl)phenyl]-2-oxofuran-3-yl]benzyl Nitrate (**10**). $AgNO_3$ (0.39 g, 2.3 mmol) and Ph_3P (0.51 g, 1.9 mmol) were added to a soln. of **9** (0.53 g, 1.5 mmol) in dry MeCN (15 ml) under N_2. The mixture was cooled to −15°, and NBS (1.69 g, 1.7 mmol) was added portionwise. Stirring was continued at −15° for 1 h and then at r.t. for 24 h. CH_2Cl_2 was added to the mixture, and the precipitate was removed by filtration. The filtrate was washed with H_2O and brine, and dried and

concentrated *in vacuo*. The residue was purified by FCC (CH_2Cl_2/AcOEt 95 :5) to give **10** (0.40 g, 67%). White solid. M.p. 170–172° (MeOH). ^{1}H-NMR ($CDCl_3$): 7.95 (*AA′BB′*, *J* = 8.4, 2 arom. H); 7.51 (*AA′BB′*, *J* = 8.4, 2 arom. H); 7.47–7.40 (*m*, 4 arom. H); 5.45 (*s*, CH_2ONO_2); 5.21 (*s*, CH_2 of furan); 3.09 (*s*, Me). ^{13}C-NMR ($CDCl_3$): 172.2; 154.4; 142.2; 136.0; 133.6; 130.3; 129.7; 129.5; 128.5; 128.3; 128.2; 74.0; 70.5; 44.3. EI-MS: 389 (M^+), 343 (100). Anal. calc. for $C_{18}H_{15}NO_7S$ (389.38): C 55.52, H 3.88; found: C 55.44, H 3.94.

(2,5-Dihydro-2-oxofuran-3,4-diyl)bis(benzene-4,1-diylmethanediyl) Dinitrate (**15**). $AgNO_3$ (1.71 g, 10.0 mmol) and Ph_3P (2.19 g, 8.0 mmol) were added to a soln. of **14** (0.99 g, 3.0 mmol) in dry MeCN (15 ml) under N_2. The mixture was cooled to −15°, and NBS (1.42 g, 8.0 mmol) was added portionwise. Stirring was continued at −15° for 1 h and then at r.t. for 4 h. AcOEt was added to the mixture, and the precipitate was removed by filtration. The filtrate was washed with H_2O and brine, and dried and concentrated *in vacuo*. The residue was purified by FCC (CH_2Cl_2) to give **15** (0.50 g, 39%). White solid. M.p. 100–101° (MeOH). ^{1}H-NMR ($CDCl_3$): 7.50–7.34 (*m*, 8 arom. H); 5.46 (*s*, CH_2ONO_2); 5.44 (*s*, CH_2ONO_2); 5.20 (*s*, CH_2 of furan). ^{13}C-NMR ($CDCl_3$): 172.8; 155.8; 135.1; 133.1; 131.7; 131.1; 129.5; 129.4; 129.0; 128.8; 126.3; 74.2; 73.6; 70.6. EI-MS: 386 (M^+), 264 (100). Anal. calc. for $C_{18}H_{14}N_2O_8$ (386.31): C 55.96, H 3.65; found: C 55.89, H 3.67.

4-(2,5-Dihydro-5-oxo-4-phenylfuran-3-yl)benzyl Nitrate (**19**). $AgNO_3$ (1.28 g, 7.5 mmol) and Ph_3P (1.23 g, 4.7 mmol) were added to a soln. of **18** (1.00 g, 3.8 mmol) in dry MeCN (50 ml) under N_2. The mixture was cooled to 5°, and NBS (0.84 g, 4.7 mmol) was added portionwise. Stirring was continued for 1 h at r.t. and then for 2.5 h at 60°. AcOEt was added to the mixture, and the precipitate was removed by filtration. The filtrate was washed with H_2O and brine, and dried and concentrated *in vacuo* to give a solid that was purified by crystallization from MeOH to give **19** (0.64 g, 55%). White solid. M.p. 78–79° (MeOH). ^{1}H-NMR ($CDCl_3$): 7.40–7.26 (*m*, 9 arom. H); 5.41 (*s*, CH_2ONO_2); 5.17 (*s*, CH_2 of furan). ^{13}C-NMR ($CDCl_3$): 173.1; 154.9; 134.8; 132.0; 129.8; 129.4; 129.2; 129.1; 128.8; 128.0; 127.2; 73.7; 70.5. EI-MS: 311 (M^+), 178 (100). Anal. calc. for $C_{17}H_{13}NO_5$ (311.29): C 65.59, H 4.21; found: C 65.68, H 4.28.

Cyclooxygenase Inhibition Studies. A whole blood assay [30] was performed to evaluate the ability of synthesized compounds to inhibit COX-1 and COX-2.

Blood samples were divided in two aliquots to test COX-1 and COX-2 inhibition. The COX-2 aliquots were treated with 10 IU/ml of sodium heparine, 10 μg/ml acetylsalicylic acid, and 10 μg/ml lipopolysaccaride from *E.coli* (LPS). MeOH solns. of the tested compounds at different concentrations were prepared, 10-μl aliquots were distributed in incubation tubes, and the solvent was evaporated. The residues were dissolved by vortexing either in 1 ml of heparinized blood to test COX-2 inhibition, or in 1 ml of untreated blood to test COX-1 inhibition. The final concentrations of the tested compounds were, therefore, diluted 100 times in the incubation tubes. The COX-1 aliquots were incubated in glass tubes for 1 h at 37°, which is sufficient to complete coagulation, then centrifuged at 2000*g* for 10 min, after which the serum was ready to be tested for platelet TXB_2 production. % Inhibition in samples treated with the test compounds was evaluated in comparison with control samples with basal TXB_2 production.

The COX-2 aliquots were incubated in polyethylene tubes for 24 h at 37° to allow COX-2 expression in monocytes and maximal PGE_2 production. They were then centrifuged at 2000*g* for 10 min, after which the plasma was ready to be tested for PGE_2 production. Basal PGE_2 production in blood untreated with LPS was subtracted from values for each sample, and % inhibition in samples incubated with tested compounds was calculated *vs.* control samples with maximal PGE_2 production.

Prostanoid production was evaluated by enzyme immunoassay, following the specific instructions provided by *Cayman Chemical*, based on a competitive reaction, for COX-1, between TXB_2 and a TXB_2–acetylcholinesterase conjugate (TXB_2 tracer) for a specific TXB_2 antiserum, and, for COX-2, between PGE_2 and PGE_2–acetylcholinesterase (PGE_2 tracer) for a specific PGE_2 antiserum. Standard curves with known concentrations of TXB_2 and of PGE_2 were used to determine prostanoid concentrations in the sample wells. Percent inhibition in compound-treated samples was calculated by comparison with untreated controls. The concentration of the tested compounds causing 50% inhibition (IC_{50}) was calculated from the concentration–inhibition response curve (5–6 experiments).

Inhibition of Platelet Aggregation in vitro. Platelet-rich plasma (PRP) was prepared by centrifugation of citrated blood at 200*g* for 20 min. Aliquots (500 μl) of PRP were added into aggregometer (*Chrono-log 4902D*) cuvettes, and aggregation was recorded as increased light transmission under

continuous stirring (1000 rpm) at 37° for 10 min after addition of the stimulus. Collagen (1.0 μg/ml) was used as platelet activator in PRP. The inhibitory activity of the compounds was tested by addition of drug to PRP 10 min before addition of the stimulus (collagen). Drug vehicle ($\leq$0.5% DMSO) added to PRP did not affect platelet function in control samples. At least five experiments were performed for each compound. The anti-aggregatory activity of the tested compounds was evaluated as % inhibition of platelet aggregation compared to controls (5–6 experiments), and IC_{50} values were calculated by nonlinear regression analysis.

Vasodilating-Activity Assay. Thoracic aortas were isolated from male *Wistar* rats weighing 180–200 g. The endothelium was removed, and the vessels were helically cut: three strips were obtained from each aorta. The tissue was placed in organ baths containing 30 ml of *Krebs*-bicarbonate buffer of the following composition [mM]: NaCl 111.2, KCl 5.0, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.0, $NaHCO_3$ 12, glucose 11.1, maintained at 37° and continuously gassed with 95% O_2 and 5% CO_2 (pH 7.4). The aortic strips were allowed to equilibrate for 120 min and then contracted with 1 μM (−)-phenylephrine. When the response to the agonist reached a plateau, cumulative concentration–response curves to compounds **10**, **15**, and **19** were determined. The effect of 1 μM ODQ was evaluated in a separate series of experiments in which the ODQ was added 5 min before contraction. EC_{50} Values are means of 4–10 determinations. Responses were recorded by an isometric transducer connected to the *MacLab System PowerLab®*.

REFERENCES

[1] A. L. Blobaum, L. J. Marnett, *J. Med. Chem.* **2007**, *50*, 1425.
[2] D. E. Griswold, J. L. Adams, *Med. Res. Rev.* **1996**, *16*, 181.
[3] J. A. Mitchell, T. D. Warner, *Br. J. Pharmacol.* **1999**, *128*, 1121.
[4] J. J. Talley, *Prog. Med. Chem.* **1999**, *36*, 201.
[5] X. de Leval, F. Julemont, V. Benoit, M. Frederich, B. Pirotte, J.-M. Dogne, *Mini-Rev. Med. Chem.* **2004**, *4*, 597.
[6] P. Singh, A. Mittal, *Mini-Rev. Med. Chem.* **2008**, *8*, 73.
[7] P. Prasit, Z. Wang, C. Brideau, C.-C. Chan, S. Charleson, W. Cromlish, D. Ethier, J. F. Evans, A. W. Ford-Hutchinson, J. Y. Gauthier, R. Gordon, J. Guay, M. Gresser, S. Kargman, B. Kennedy, Y. Leblanc, S. Léger, J. Mancini, G. P. O'Neill, M. Ouellet, M. D. Percival, H. Perrier, D. Riendeau, I. Rodger, P. Tagari, M. Thérien, P. Vickers, E. Wong, L.-J. Xu, R. N. Young, R. Zamboni, S. Boyce, N. Rupniak, N. Forrest, D. Visco, D. Patrick, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1773.
[8] S. Shi, U. Klotz, *Eur. J. Clin. Pharmacol.* **2008**, *64*, 233.
[9] J.-M. Dogné, C. T. Supuran, D. Pratico, *J. Med. Chem.* **2005**, *48*, 2251.
[10] E. M. Antman, D. DeMets, J. Loscalzo, *Circulation* **2005**, *112*, 759.
[11] F. Andersohn, S. Suissa, E. Garbe, *Circulation* **2006**, *113*, 1950.
[12] P. M. Kearney, C. Baigent, J. Godwin, H. Halls, J. R. Emberson, C. Patrono, *Br. Med. J.* **2006**, *332*, 1302.
[13] C. Patrono, B. Rocca, *Pharmacol. Res.* **2009**, *59*, 285.
[14] O. C. Trifan, T. Hla, *J. Cell. Mol. Med.* **2003**, *7*, 207.
[15] C. A. Rouzer, L. J. Marnett, *J. Lipid Res.* **2009**, *50*, S29.
[16] M. Nivsarkar, A. Banerjee, H. Padh, *Pharmacol. Rep.* **2008**, *60*, 692.
[17] J. F. Kerwin Jr., M. Heller, *Med. Res. Rev.* **1994**, *14*, 23.
[18] S. Huerta, S. Chilka, B. Bonavida, *Int. J. Oncol.* **2008**, *33*, 909.
[19] G. R. J. Thatcher, B. M. Bennett, J. N. Reynolds, *Curr. Alzheimer Res.* **2006**, *3*, 237.
[20] E. Del Grosso, D. Boschi, L. Lazzarato, C. Cena, A. Di Stilo, R. Fruttero, S. Moro, A. Gasco, *Chem. Biodiversity* **2005**, *2*, 886.
[21] C. Velázquez, P. N. P. Rao, R. McDonald, E. E. Knaus, *Bioorg. Med. Chem.* **2005**, *13*, 2749.
[22] K. R. A. Abdellatif, M. A. Chowdhury, Y. Dong, D. Das, G. Yu, C. Velázquez, M. R. Suresh, E. E. Knaus, *Bioorg. Med. Chem.* **2009**, *17*, 5182.
[23] F. C. Engelhardt, Y.-J. Shi, C. J. Cowden, D. A. Conlon, B. Pipik, G. Zhou, J. M. McNamara, U.-H. Dolling, *J. Org. Chem.* **2006**, *71*, 480.

[24] R. R. Ranatunge, M. Augustyniak, U. K. Bandarage, R. A. Earl, J. L. Ellis, D. S. Garvey, D. R. Janero, L. G. Letts, A. M. Martino, M. G. Murty, S. K. Richardson, J. D. Schroeder, M. J. Shumway, S. W. Tam, A. M. Trocha, D. V. Young, *J. Med. Chem.* **2004**, *47*, 2180.
[25] K. Chegaev, L. Lazzarato, P. Tosco, C. Cena, E. Marini, B. Rolando, P.-A. Carrupt, R. Fruttero, A. Gasco, *J. Med. Chem.* **2007**, *50*, 1449.
[26] D. Boschi, L. Lazzarato, B. Rolando, A. Filieri, C. Cena, A. Di Stilo, R. Fruttero, A. Gasco, *Chem. Biodiversity* **2009**, *6*, 369.
[27] B. Testa, J. M. Mayer, in 'Hydrolysis in Drug and Prodrug Metabolism – Chemistry, Biochemistry and Enzymology', Eds. B. Testa, J. M. Mayer, VHCA/Wiley-VCH, Zurich, 2003, p. 535.
[28] M. Thérien, J. Y. Gauthier, Y. Leblanc, S. Léger, H. Perrier, P. Prasit, Z. Wang, *Synthesis* **2001**, 1778.
[29] P. Frøsyen, *Phosphorus, Sulfur Silicon Relat. Elem.* **1997**, *129*, 89.
[30] P. Patrignani, M. R. Panara, A. Greco, O. Fusco, C. Natoli, S. Iacobelli, F. Cipollone, A. Ganci, C. Créminon, J. Maclouf, *J. Pharmacol. Exp. Ther.* **1994**, *271*, 1705.
[31] N. Handler, G. Brunhofer, C. Studenik, K. Leisser, W. Jaeger, S. Parth, T. Erker, *Bioorg. Med. Chem.* **2007**, *15*, 6109.
[32] S. Ganapathy, B. B. V. Sekhar, S. M. Cairns, K. Akutagawa, W. G. Bentrude, *J. Am. Chem. Soc.* **1999**, *121*, 2085.

Received December 23, 2009