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Synthesis and biological evaluation of 3,4-diphenyl-1,2,5-oxadiazole-2-oxides and 3,4-diphenyl-1,2,5-oxadiazoles as potential hybrid COX-2 inhibitor/nitric oxide donor agents

Carlos Velázquez,[a] P. N. Praveen Rao,[a] Robert McDonald[b] and Edward E. Knaus[a,*]

[a]*Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2N8*
[b]*Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2*

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Abstract—A group of 3,4-diphenyl-1,2,5-oxadiazole-2-oxides (3,4-diphenylfuroxans) and the corresponding *N*-desoxy 3,4-diphenyl-1,2,5-oxadiazoles (3,4-diphenylfurazans) analogs, were synthesized for in vitro evaluation as hybrid cyclooxygenase (COX) inhibitor/nitric oxide donor agents. Reaction of 1-[4-(methylsulfonyl)phenyl]-2-phenylethene with an aqueous sodium nitrite solution in acetic acid afforded a mixture (3:1 ratio) of the inseparable 4-[4-(methylsulfonyl)phenyl]-3-phenyl-1,2,5-oxadiazole-2-oxide (**13a**) and 3-[4-(methylsulfonyl)phenyl]-4-phenyl-1,2,5-oxadiazole-2-oxide (**13b**) regioisomers. A group of related regioisomers possessing either a *p*-aminosulfonylphenyl (**16**) or a *p*-azidosulfonylphenyl (**17**), moiety were obtained by chlorosulfonation of the unsubstituted 3,4-diphenylfuroxan (**10**) and subsequent reaction with either ammonium hydroxide or sodium azide, respectively. The methanesulfonyl regioisomers **13a**,**b** [COX-1 IC_{50} = 11.6 μM; COX-2 IC_{50} = 0.12 μM; COX-2 selectivity index (SI) = 97] and aminosulfonyl regioisomers **16** (COX-1 IC_{50} = 9.8 μM; COX-2 IC_{50} = 0.78 μM; COX-2 SI = 12), like the reference drug celecoxib (COX-1 IC_{50} = 33.1 μM; COX-2 IC_{50} = 0.07 μM; COX-2 SI = 472), were potent in vitro COX-2 inhibitors with a good COX-2 selectivity index. Release of nitric oxide (NO) from the 3,4-diphenylfuroxan compounds (**10**, **13a**,**b**, **16**, **17**) was thiol-dependent since the % NO released was higher upon incubation in the presence of L-cysteine (0.57–3.18%) compared to that in phosphate buffer solution at pH 7.4 (0.06–0.15%). Molecular modeling (docking) studies show that the methanesulfonyl ($MeSO_2$) COX-2 pharmacophore present in regioisomers **13a**,**b** is positioned in the vicinity of the COX-2 secondary pocket. The in vitro NO release data, COX-1/COX-2 inhibition and COX-2 SI structure–activity relationships acquired, and molecular modeling docking studies suggest that the 1,2,5-oxadiazole-2-oxide (furoxan) ring possesses beneficial features that should be present in a suitable central ring template (bioisostere) pertinent to the design novel hybrid COX-2 inhibitor/nitric oxide donor agents with a low ulcerogenicity profile that may be free from adverse cardiovascular effects.

1. Introduction

The nonselective inhibition of both cyclooxygenase isozymes (COX-1 and COX-2) by traditional ulcerogenic nonsteroidal antiinflammatory drugs (NSAIDs) clearly illustrated the clinical need for a new generation of non-ulcerogenic selective COX-2 inhibitors.[1–3] In this regard, the differential tissue distribution of COX-1 and COX-2 provided a rationale for the design of selective COX-2 inhibitors as antiinflammatory and analgesic agents with a lower incidence of associated gastrointestinal (GI) side effects than NSAIDs.[4] This hypothesis served as the basis for design of the highly selective tricyclic COX-2 inhibitors celecoxib (**1**) and rofecoxib (**2**), which have a central heterocyclic ring scaffold (see Fig. 1). A diverse group of structurally related tricyclic compounds that possess vicinal diaryl substituents on a central five- or six-membered ring template such as pyrazole (**1**),[5] 2-(5*H*)furanone (**2**),[6] cyclopentene (**3**),[7] isoxazole (**4** and **5**),[8,9] pyridine (**6**),[10] and pyranone (**7**)[11] have been investigated as selective COX-2 inhibitors.

Despite the relatively safe pharmacological profile of selective COX-2 inhibitors, there is now increasing concern regarding their use in patients at risk for an adverse cardiovascular event such as myocardial infarction. This increased risk is thought to be triggered by a reduction

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* Corresponding author. Tel.: +1 780 492 5993; fax: +1 780 492 1217; e-mail: eknaus@pharmacy.ualberta.ca

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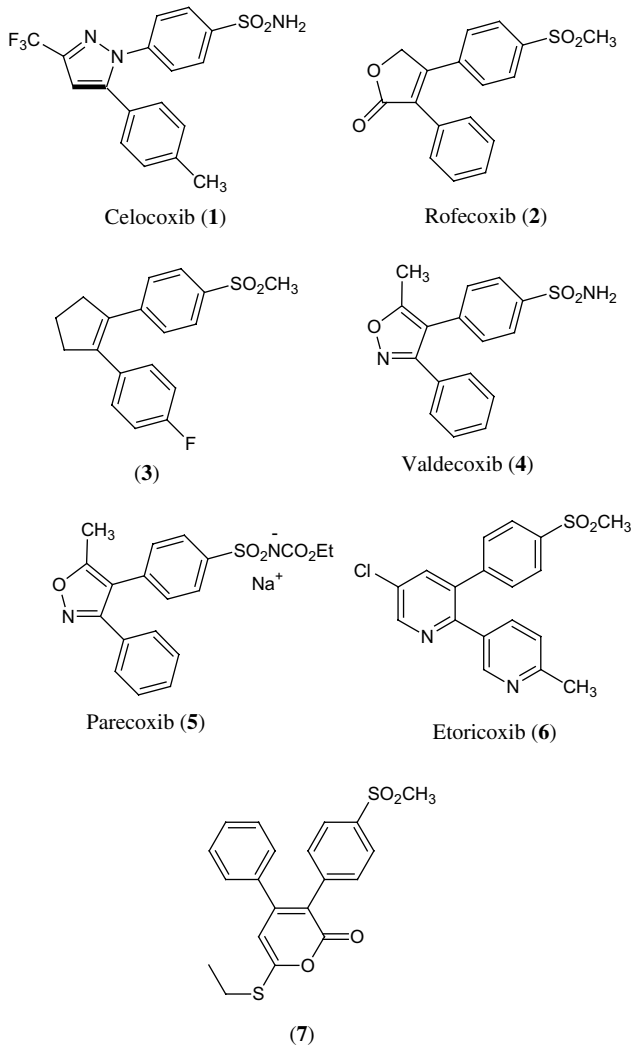


Figure 1. Some representative examples of selective tricyclic COX-2 inhibitors possessing a central five- or six-membered ring scaffold.

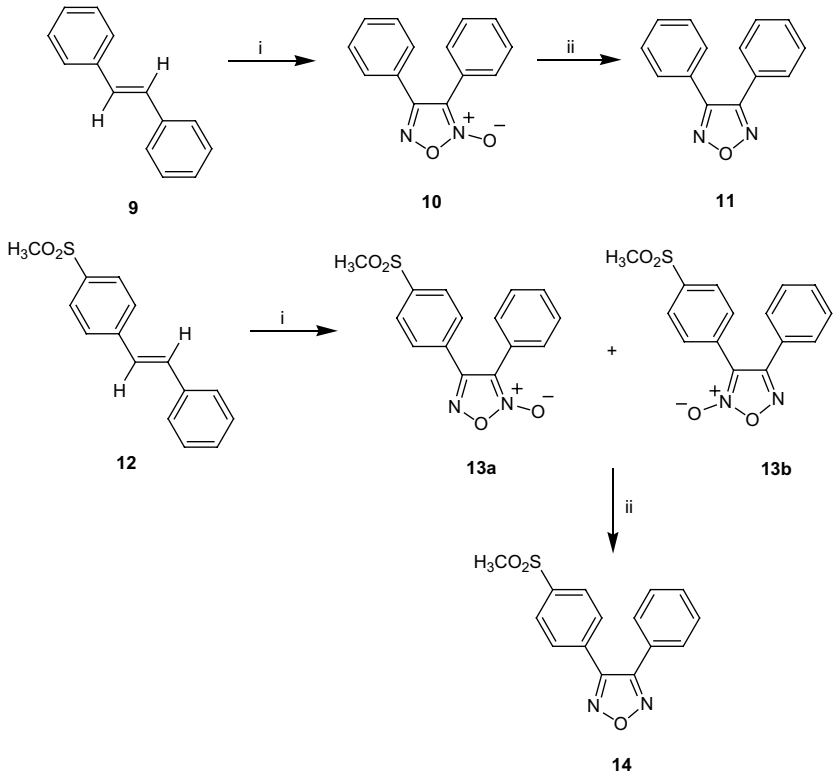
in the level of the desirable platelet aggregation inhibitor and vasodilatory prostacyclin (PGI_2) in conjunction with an increased level of the undesirable potent platelet activator and aggregator thromboxane A_2 (TxA_2).[12] This biochemical explanation constitutes the rationale for the recent voluntary worldwide withdrawal of Vioxx® (rofecoxib). This decision was based on data, from a three-year clinical trial evaluating the ability of rofecoxib to reduce colon polyp recurrence, that revealed a 'discernible and confirmed' higher risk of cardiovascular events such as stroke and heart attack in patients taking the drug for a period longer than 18 months.[13] This latter clinical evidence presents a new challenge in the design of selective COX-2 inhibitors since it is now necessary to also consider the physiological consequences associated with alterations in the arachidonic acid cascade due to unbalanced inhibition of the COX-1 and COX-2 isozymes.

Hybrid molecules, comprised of NSAID and nitric oxide (NO) donor moieties, constitute one of the more promising approaches for the design of drugs, which are devoid of the potential adverse cardiovascular effects associated with the use of selective COX-2 inhibitors, and which elicit a decreased ulcerogencity relative to that frequently observed on long-term use of traditional NSAIDs.[14] It has been reported that an increased generation of endothelial NO, or release of NO from a nitric oxide donor drug, is expected to produce beneficial effects such as a reduction in blood pressure and prevention of atherosclerosis.[15] At nanomolar concentrations, NO reversibly activates soluble guanylate cyclase by 400-fold, catalyzing the conversion of guanosine triphosphate to cyclic guanosine monophosphate (*c*GMP).[16] Elevation of *c*GMP relaxes smooth muscle in blood vessels, inhibits platelet aggregation and adhesion, and blocks the adhesion of white cells to blood vessel walls.[17] In addition to these cardiovascular effects, NO is now recognized as a critical mediator of gastrointestinal mucosal defense, exerting many of the same actions as prostaglandins in the gastrointestinal tract.[14,18,19] Drugs classified as NO–NSAIDs suppress COX-2 derived prostaglandin synthesis as effectively as the parent drugs, and they have been found to exert comparable antiinflammatory and antipyretic activity to the parent NSAID drug.[20,21]

1,2,5-Oxadiazole-2-oxides (furoxans) represent one class of heterocyclic compounds that are thiol-dependent NO donor agents. In this context, furoxans are considered to be NO releasing prodrugs whose biological activity is produced by action on the sGC–*c*GMP pathway. Compared to other NO donor agents, furoxans possess rather favorable pharmacological properties, since they frequently release NO slowly resulting in a longer duration of action. The absence of tolerance is an important distinctive feature of the furoxan moiety. A mechanism has been proposed by Granik and Grigor for the thiol-induced release of NO from furoxans that involves attack by a thiolate anion at C-3 and/or C-4 of the furoxan ring followed by ring opening and the subsequent release of NO.[22] As part of our ongoing research program, we now report the synthesis, in vitro COX-1/COX-2 inhibitory activities and nitric oxide release data, for a group of 3,4-diphenyl-1,2,5-oxadiazole-2-oxides (**10**, **13**, **16**, **17**, 3,4-diphenylfuroxans) and the corresponding desoxy 3,4-diphenyl-1,2,5-oxadiazole (**11**, **14**, **19**, **20**, 3,4-diphenylfurazans) analogs, possessing a C-4 H, SO_2CH_3, SO_2NH_2, or SO_2N_3 moiety on one of the phenyl groups, as potential hybrid COX-2 inhibitor/NO donor drugs.

2. Chemistry

Reaction of (*E*)-1,2-diphenylethene (*trans*-stilbene, **9**) with a saturated aqueous solution of sodium nitrite in a mixture of acetic acid and 1,4-dioxane, a modification of the procedure reported by Gasco and co-workers,[23,24] afforded 3,4-diphenyl-1,2,5-oxadiazole-2-oxide (**10**, 26% yield) as illustrated in Scheme 1. A similar reaction using (*E*)-1-[4-(methylsulfonyl)phenyl]-2-phenylethene (**12**) yielded a mixture of the two furoxan regioisomers **13a** and **13b**. This product (**13**) was originally believed to be a single regioisomer since a single spot was observed on micro TLC plates irrespective of the polarity of the



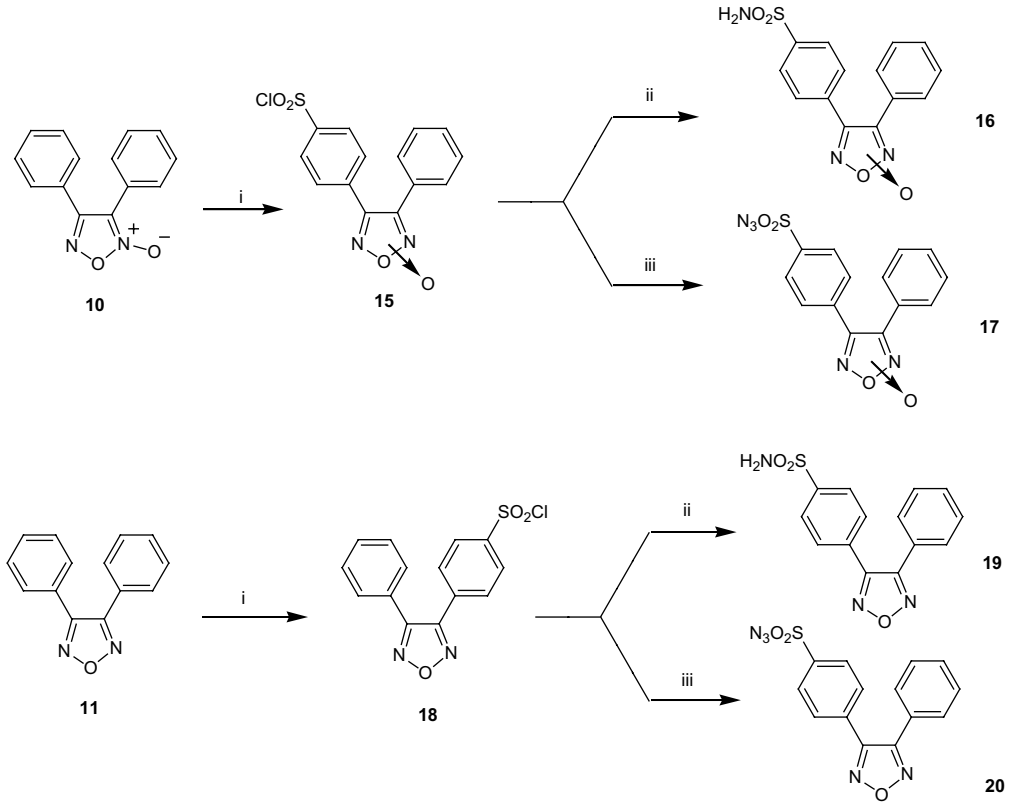
Scheme 1. Reagents and conditions: (i) $NaNO_2$, CH_3CO_2H, 1,4-dioxane, 50–60 °C, 6–24 h; (ii) $(EtO)_3P$, reflux, 19–24 h.

development solvent. Subsequent purification by silica gel column chromatography and then recrystallization from hexanes provided a product that showed a single set of resonances in both the ^1H NMR and ^{13}C NMR spectra. In order to determine which regioisomer of **13** was isolated, a X-ray crystallographic structure was determined, which indicated that this product was a mixture of the two regioisomers 4-[4-(methylsulfonyl)-phenyl]-3-phenyl-1,2,5-oxadiazole-2-oxide (**13a**) and 3-[4-(methylsulfonyl)phenyl]-4-phenyl-1,2,5-oxadiazole-2-oxide (**13b**) that were present in a 3:1 ratio (see Fig. 2). Reaction of furoxan **10**, or the regioisomers **13a**,**b**, with triethylphosphite at reflux for 19–24 h afforded the respective deoxygenated product 3,4-diphenyl-1,2,5-oxadiazole (**11**, 70%), or 3-[4-(methylsulfonyl)phenyl]-4-phenyl-1,2,5-oxadiazole (**14**, 84%).

Chlorosulfonation of the unsymmetrical furoxan **10** with chlorosulfonic acid at 25 °C for 17 h afforded the sulfonyl chloride **15** that was purified by silica gel column chromatography to provide a 30% isolated yield. Although the ^1H NMR spectrum for the sulfonyl chloride product **15** showed a single set of resonances similar to **13a**,**b**, it may also exist as a mixture of the regioisomers 4-[4-(chlorosulfonyl)phenyl]-3-phenyl-1,2,5-oxadiazole-2-oxide and 3-[4-(chlorosulfonyl)phenyl]-4-phenyl-1,2,5-oxadiazole-2-oxide as shown in Scheme 2. A similar chlorosulfonation of the symmetrical furazan **11** furnished 3-[4-(chlorosulfonyl)phenyl]-4-phenyl-1,2,5-oxadiazole (**18**), which unlike **15** cannot exist as a mixture of two regioisomers, in 16% isolated yield after purification by silica gel column chromatography. The subsequent reaction of the sulfonyl chloride **15** with NH_4OH afforded the sulfonamide product (**16**, presumed to be a mixture of two regioisomers, 69%). Alternatively, reaction of **15** with the nucleophile NaN_3 yielded the respective sulfonylazide product (**17**, 76%) that is assumed to exist as a mixture of two regioisomers. Similar reactions of the sulfonyl chloride **18** with NH_4OH furnished 3-[4-(aminosulfonyl)phenyl]-4-phenyl-1,2,5-oxadiazole (**19**, 82%), or with NaN_3 yielded 3-[4-(azidosulfonyl)phenyl]-4-phenyl-1,2,5-oxadiazole (**20**, 86%).

3. Results and discussion

In vitro COX-1 and COX-2 enzyme inhibition data (Table 1) showed that replacement of the 2-(5*H*)furanone central ring present in rofecoxib (**1**) by a 1,2,5-oxadiazole-2-oxide ring maintains COX-2 inhibitory activity. Compounds **10** (COX-1 IC_{50} = 12.0 μM; COX-2 IC_{50} > 100 μM) and **11** (COX-1 = 0.15 μM; COX-2 IC_{50} > 100 μM), having unsubstituted C-3 and C-4 phenyl substituents, are selective COX-1 inhibitors. However, incorporation of a *para*-SO_2Me phenyl substituent (COX-2 pharmacophore) provided a mixture of the two regioisomers **13a** and **13b** [COX-1 IC_{50} = 11.6 μM; COX-2 IC_{50} = 0.12 μM; COX-2 selectivity index (SI) = 97], and **14** (COX-1 IC_{50} = 1.6 μM; COX-2 IC_{50} = 0.74 μM; COX-2 SI = 2), that possess a selectivity for the COX-2 isozyme. Compounds **16** and **19**, possessing a *para*-SO_2NH_2 phenyl substituent (COX-2 pharmacophore), like **13** and **14**, were also selective COX-2 inhibitors where the respective COX-2 selectivity indexes were 12 and 5, respectively. In an earlier investigation,[25] we



Scheme 2. Reagents and conditions: (i) $ClSO_3H$, 25 °C, 17 h; (ii) NH_4OH, THF, 25 °C, 1.5 h; (iii) NaN_3, H_2O, THF, 25 °C, 20 h.

Table 1. In vitro COX inhibition data for 3,4-diphenylfuroxans (**10**, **13a**,**b**, **16**, and **17**) and 3,4-diphenylfurazans (**11**, **14**, **19**, and **20**)

Compound	COX-1 IC_{50}[a] (μM)	COX-2 IC_{50}[a] (μM)	COX-2 SI[b]
10	12.0	>100	—
11	0.15	>100	—
13a,**b**	11.6	0.12	97
14	1.6	0.74	2
16	9.8	0.78	12
17	>100	>100	—
19	4.6	0.91	5
20	>100	>100	—
Rofecoxib	>100	0.50	>200
Celecoxib	33.1	0.07	472

[a] Values are means of two determinations acquired using an ovine COX-1/COX-2 assay kit (catalog no. 560101, Cayman Chemicals Inc., Ann Arbor, MI, USA) and the deviation from the mean is <10% of the mean value.

[b] In vitro COX-2 selectivity index (COX-1/COX-2 IC_{50}).

showed that 2-(5*H*)furanone derivatives possessing a *para*-SO_2N_3 phenyl substituent exhibited significant COX-2 inhibitory activity. In contrast, in this study replacement of the SO_2Me substituent present in compounds **13** and **14**, or the SO_2NH_2 substituent present in compounds **16** and **19**, by a SO_2N_3 phenyl substituent provided compounds **17** and **20**, which were completely devoid of both COX-1 and COX-2 inhibitory activity (COX-1 and COX-2 $IC_{50} > 100$ μM). A comparison of the furoxan regioisomers (**13a**,**b**) having a *N*-oxido substituent, with the corresponding furazan (**14**) that does not possess a *N*-oxido substituent, shows that *N*-deoxygenation provided a small increase in COX-1 potency, and a modest decrease in COX-2 potency, which resulted in a lower COX-2 inhibitory selectivity index.

The percent nitric oxide (NO) released from the 3,4-diphenyl-1,2,5-oxadiazole-2-oxides (furoxans **10**, **13a**–**b**, **16**, and **17**) upon in vitro incubation with L-cysteine, for 1.5 h at 37 °C, was determined and the results are summarized in Table 2. It has been reported that a reduced thiol such as L-cysteine, L-cysteamine, or glutathione is required for the release of NO from certain NO donor agents such as those containing a furoxan (1,2,5-oxadiazole-2-oxide) moiety.[26] The % NO release data acquired in this study (see Table 2) is consistent with this literature precedent since the % NO released from the 3,4-diphenylfuroxan compounds (**10**, **13a**,**b**, **16**, and **17**) was higher upon incubation in the presence of L-cysteine (0.57–3.18%) compared to that determined in phosphate buffer solution at pH 7.4 (0.06–0.15%). In comparison, the reference drug glycerine trinitrate released 5.86% NO upon incubation in the presence of L-cysteine, and 0.72% NO in phosphate buffer at pH 7.4, per nitrooxy group. Antiinflammatory agents releasing NO that reversibly activates soluble guanylate cyclase which in turn catalyzes the conversion of guanosine triphosphate to cyclic guanosine monophosphate (*c*GMP)[16] would be expected to relax smooth muscle in blood vessels, inhibit platelet aggregation and adhesion, and block the adhesion of white cells to blood vessel walls.[17] Accordingly, hybrid COX-2 inhibitor/NO donor agents should not cause adverse cardiovascular

Table 2. Nitric oxide release from 3,4-diphenylfuroxans possessing a C-4 H (**10**), SO_2Me (**13a**,**b**), SO_2NH_2 (**16**), or SO_2N_3 (**17**) substituent

Compound	% NO release[a]	
	PBS[b]	L-Cysteine[c]
10	0.15	0.57
13a,**b**	0.06	1.48
16	0.10	0.84
17	0.08	3.18
Glycerine trinitrate[d]	0.72	5.86

[a] Percent of nitric oxide released (mean value, $n = 3$) relative to a theoretical maximum release of 1 mol of NO/mol of test compound was determined using the Griess reaction. Variation from the mean % value was $\leqslant 0.02\%$.

[b] Incubated in phosphate buffer solution (PBS, pH 7.4) at 37 °C for 1.5 h.

[c] Incubated in the presence of 5 mM L-cysteine in phosphate buffer solution (pH 7.4) at 37 °C for 1.5 h.

[d] The percent nitric oxide released was estimated as the % NO produced/nitrooxy (ONO_2) group present in the reference drug glycerine trinitrate [$O_2NCH_2CH(ONO_2)CH_2ONO_2$].

events such as an increased incidence of heart attacks and strokes.

The binding interactions of the 3,4-diphenyl-1,2,5-oxadiazole-2-oxide (3,4-diphenylfuroxan) regioisomers (**13a**,**b**, COX-2 $IC_{50} = 0.12$ μM; COX-2 SI = 96) within the COX-2 binding site were examined by molecular modeling (docking) experiments (Figs. 3 and 4). The most stable conformer of the regioisomer **13a** orients within the COX-2 primary binding site such that the C-4 *para*-methanesulfonylphenyl substituent is positioned in the vicinity of the COX-2 secondary pocket where it is surrounded by amino acid residues Phe[518], Arg[513], Gln[192], Val[523], and Leu[352] (Fig. 3). One of the *O*-atoms of the SO_2Me substituent forms a favorable hydrogen bond with the backbone N*H* of Ile[517] (distance = 2.22 Å) and a weak hydrogen bond with the NH_2 of Gln[192] (distance = 3.62 Å). The distance between the second *O*-atom of SO_2Me and the NH_2 of Arg[513] was about 7.58 Å. The unsubstituted C-3 phenyl ring was oriented

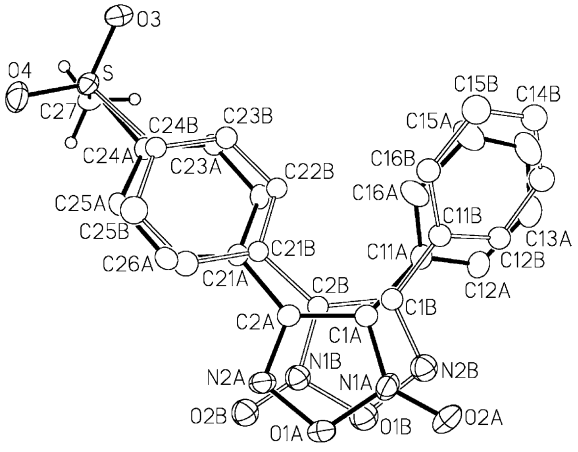


Figure 2. X-ray crystal structure of a mixture (3:1 ratio) of the 4-[4-(methylsulfonyl)phenyl]-3-phenyl-1,2,5-oxadiazole-2-oxide (**13a**), and 3-[4-(methylsulfonyl)phenyl]-3-phenyl-1,2,5-oxadiazole-2-oxide (**13b**), regioisomers. Atom labels ending in 'A' (e.g., O1A, N1A, C1A) belong to **13a**, while those ending in 'B' (O1B, N1B, C1B) belong to **13b** (the $MeSO_2$ group is common to both).

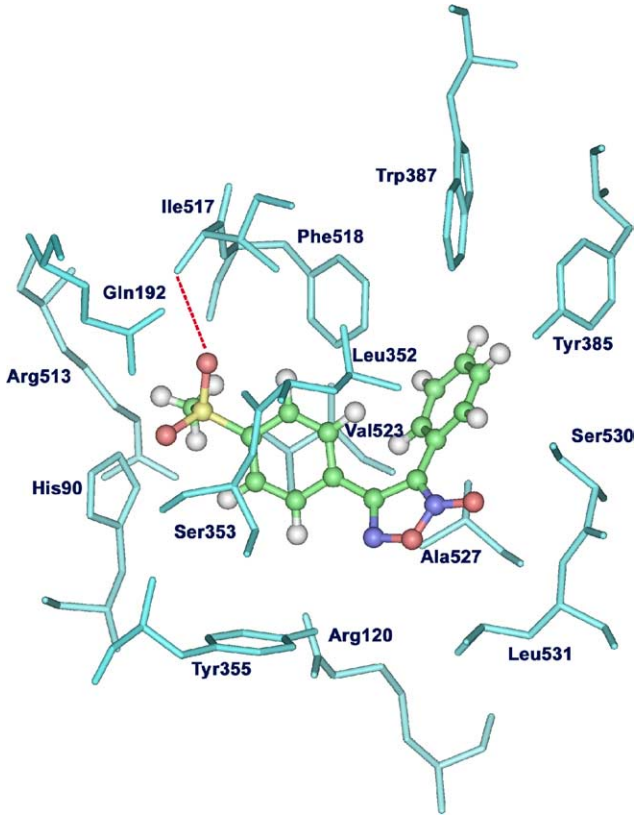


Figure 3. Docking of 4-[4-(methylsulfonyl)phenyl]-3-phenyl-1,2,5-oxadiazole-2-oxide (**13a**) (ball-and-stick) in the active site of murine COX-2. Hydrogen atoms of the amino acid residues have been removed to improve clarity.

toward the top of the COX-2 binding site closer to Trp[387] and Tyr[385]. The N^5-atom of the central furoxan ring participates in a hydrogen bonding interaction with the O*H* of Tyr[355] (distance = 3.16 Å) and it is positioned about 4.25 Å away from the NH_2 of Arg[120] that is located near the mouth of the COX-2 binding site. Accordingly, the *O*-atom of the central furoxan ring undergoes a weak hydrogen bonding interaction with the O*H* of Tyr[355] (distance = 3.63 Å) and it is located about 5.4 Å from the NH_2 of Arg[120]. It is interesting to note that the *N*-oxide moiety of the central furoxan ring is oriented close to Ser[530], the acetylation site of aspirin. The distance between negatively charged N^2-oxido *O*-atom and the O*H* of Ser[530] was about 2.5 Å. This observation is consistent with previous studies where the C=*O* of the central furanone ring of rofecoxib undergoes a favorable hydrogen bonding interaction with the O*H* of Ser[530].[27]

A similar molecular modeling experiment where the regioisomer **13b** was docked in the COX-2 binding site shows, like that observed for **13a**, that the C-3 phenyl ring possessing the *p*-SO_2Me COX-2 pharmacophore is also oriented in the vicinity of the COX-2 secondary pocket (Phe[518], Arg[513], Gln[192], Val[523], Ser[353], and Leu[352]) as shown in Figure 4. One of the *O*-atoms of the –SO_2Me substituent is hydrogen bonding with the backbone N*H* of Ile[517] (distance = 2.28 Å) and the NH_2 of Gln[192] (distance = 2.08 Å). The C-4 unsubstituted phenyl ring present in **13b**, similar to that observed

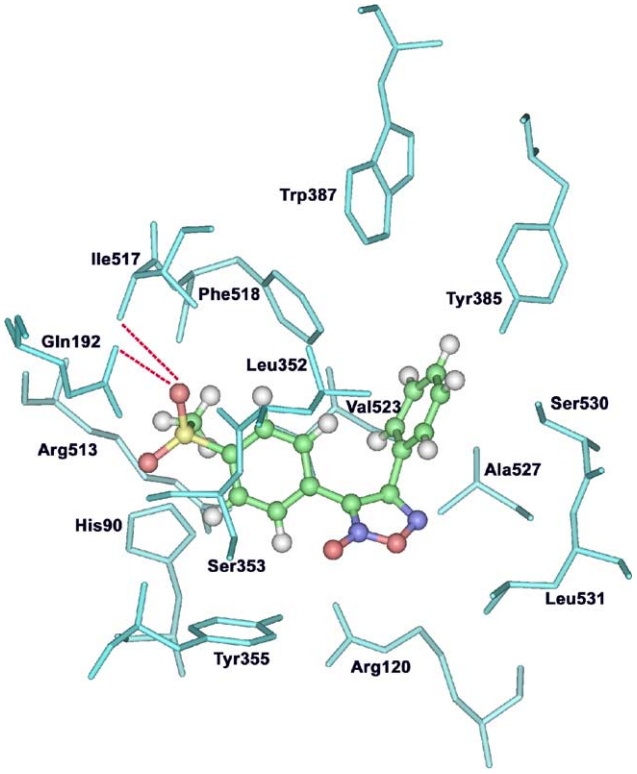


Figure 4. Docking of 3-[4-(methylsulfonyl)phenyl]-4-phenyl-1,2,5-oxadiazole-2-oxide (**13b**) (ball-and-stick) in the active site of murine COX-2. Hydrogen atoms of the amino acid residues have been removed to improve clarity.

for regioisomer **13a**, is also oriented toward a hydrophobic area comprised of Trp^{387} and Tyr^{385} at the top of the COX-2 binding site. The major difference between the binding modes observed for the two regioisomers **13a** and **13b** within the COX-2 binding site is the orientation of the *N*-oxido moiety of the central furoxan ring. In the case of regioisomer **13b**, the *N*-oxido moiety is oriented toward the mouth of the COX-2 binding site close to Tyr^{355} and Arg^{120}. In contrast, the *N*-oxido moiety present in **13a** was oriented in a direction close to Ser^{530}. The distance between the negatively charged *O*-atom of the *N*-oxido moiety in **13b** and the O*H* of Tyr^{355} is about 3.3 Å, whereas the distance between the charged guanidino side chain of Arg^{120} and the *O*-atom of the *N*-oxido moiety is about 6.0 Å. Molecular dynamics (MD) simulations on the stabilities of the enzyme–ligand complexes revealed that **13a** ($E_{\text{intermolecular}} = -52.30$ kcal/mol) has a slightly higher binding affinity for the COX-2 isozyme as compared to **13b** ($E_{\text{intermolecular}} = -52.10$ kcal/mol).

4. Conclusions

Replacement of the 2-(5*H*)furanone central ring present in rofecoxib by an isosteric 1,2,5-oxadiazole-2-oxide ring maintains COX-2 inhibitory activity, although there is a decrease in COX-2 selectivity, relative to the reference compounds celecoxib (**1**) and rofecoxib (**2**). Structure–activity data acquired for the 3,4-diphenyl-1,2,5-oxadiazole-2-oxides (3,4-diphenylfuroxans) and 3,4-diphenyl-1,2,5-oxadiazoles (3,4-diphenylfurazans) investigated indicate that a methylsulfonyl (SO_2Me), or aminosulfonyl (SO_2NH_2), COX-2 pharmacophore located at the *para*-position of either a C-3 or C-4 phenyl ring is essential for COX-2 inhibitory activity. In contrast, compounds having an unsubstituted C-3 or C-4 phenyl substituent (**10** and **11**) were selective COX-1 inhibitors. The sulfonylazido (SO_2N_3) substituent is not a suitable COX pharmacophore since compounds possessing this substituent were devoid of both COX-1 and COX-2 inhibitory activity. A central ring *N*-oxido substituent is not a major determinant of COX inhibitory potency and/or selectivity. In this regard, the presence of a *N*-oxido moiety provided small increases in COX-2 potency and selectivity. Alternatively, removal of the *N*-oxido oxygen atom, which gives rise to the *N*-deoxy derivatives, results in a small increase in COX-1 potency and a small decrease in COX-2 potency. The thiol-dependent release of NO, in conjunction with relatively potent and selective COX-2 inhibitory activity, suggest that the 1,2,5-oxadiazole-2-oxide (furoxan) ring system possesses beneficial features that would be desirable for the design of hybrid COX-2 inhibitor/NO donor antiarthritic agents with a low ulcerogencity profile and minimal potential to induce adverse cardiovascular events such as heart attacks and strokes.[13]

5. Experimental

Melting points were determined using a Thomas–Hoover capillary apparatus and are uncorrected. Infrared (IR) spectra were recorded using a Nicolet 550 Series II Magna FT-IR spectrometer. Nuclear magnetic resonance spectra (^1H) were recorded on a Bruker AM-300 spectrophotometer. The assignment of exchangeable protons (N*H*) was confirmed by the addition of D_2O. Ultraviolet (UV) spectra and quantitative analyses were measured using a Philips PU 8740 UV/vis scanning spectrophotometer. Silica gel column chromatography was performed using Silicycle® (silica gel 70–230 mesh). X-ray crystallographic (crystallography laboratory) and elemental analyses (microanalytical service laboratory), were performed in the Department of Chemistry, University of Alberta. Molecular modeling experiments were performed on a Sillicon Graphics Workstation (Octane 2) equipped with a R14000A microprocessor using the Insight II software version 2000.1 (Accelerys Inc.). 1-[4-(Methylsulfonyl)phenyl]-2-phenylethene (**12**) was prepared according to a reported procedure.[28] All other reagents were purchased from Aldrich Chemical (Milwaukee, WI) and used without further purification.

5.1. 3,4-Diphenyl-1,2,5-oxadiazole-2-oxide (10)

trans-Stilbene (**9**, 2 g, 11.1 mmol) was dissolved in a warm mixture (50–55 °C) of glacial acetic acid (6 mL) and 1,4-dioxane (10 mL). Aqueous sodium nitrite (5.34 g, 77.6 mmol in 10 mL of water) was added dropwise during a period of 1 h, the reaction was allowed to proceed with stirring at 50–60 °C for 6.5 h, and the reaction was quenched by addition of ice-water (100 mL of a

50:50 v/v mixture) at 25 °C. This mixture was extracted with EtOAc (3 × 30 mL), the organic phase was dried (Na_2SO_4), and the solvent was removed in vacuo. The residue obtained was purified by silica gel column chromatography (hexanes–ether, 3:1 v/v) prior to recrystallization from hexanes to yield **10** (0.63 g, 26%) as pale yellow crystals; mp 104–105 °C; IR (KBr): 3059 (CH), 1588 (NO), 1420 (NO) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.43–7.55 (m, 10H, phenyl hydrogens). Anal. Calcd for $C_{14}H_{10}N_2O_2$: C, 70.58; H, 4.23; N, 11.76. Found: C, 70.33; H, 4.19; N, 11.61.

5.2. 4-[4-(Methylsulfonyl)phenyl]-3-phenyl-1,2,5-oxadiazole-2-oxide (13a) and 3-[4-(methylsulfonyl)phenyl]-4-phenyl-1,2,5-oxadiazole-2-oxide (13b) regioisomers (ratio 3:1)

1-[4-(Methylsulfonyl)phenyl]-2-phenylethene (**12**, 1.12 g, 4.3 mmol) was dissolved in a warm mixture (55–60 °C) of glacial acetic acid (5 mL) and 1,4-dioxane (50 mL). Aqueous sodium nitrite (2.2 g, 31.9 mmol in 4 mL of water) was added dropwise during a period of 1 h, the reaction was allowed to proceed with stirring at 60 °C for 17 h, and the reaction was quenched by addition of ice-water (100 mL of a 50:50 v/v mixture) at 25 °C. This mixture was extracted with CH_2Cl_2 (3 × 40 mL), the organic extract was dried (Na_2SO_4), and the solvent was removed in vacuo. The residue obtained was purified by silica gel column chromatography (hexanes–$CHCl_3$–EtOAc, 50:35:15 v/v/v) to yield a mixture of the two regioisomers **13a** and **13b** that were subsequently shown by X-ray crystallography to be present in a ratio of 3:1 (0.65 g, 47%) as a white powder; mp 121–123 °C; IR (KBr): 3059 (CH_{arom}), 2918 (CH_{aliph}), 1595 (NO), 1313 (SO) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.96 (d, J_{ortho} = 8.4 Hz, 2H, 4-methylsulfonylphenyl *H*-3, *H*-5), 7.68 (d, J_{ortho} = 8.4 Hz, 2H, 4-methylsulfonylphenyl *H*-2, *H*-6), 7.43–7.40 (m, 5H, phenyl hydrogens), 3.12 (s, 3H, CH_3). Anal. Calcd for $C_{15}H_{12}N_2O_4S$: C, 56.95; H, 3.82; N, 8.86. Found: C, 56.96; H, 3.76; N, 8.89.

5.3. 3,4-Diphenyl-1,2,5-oxadiazole (11)

A solution of **10** (0.3 g, 1.26 mmol) in triethylphosphite (10 mL) was heated at reflux for 24 h with stirring, the solution was cooled to 25 °C, and the reaction mixture was stirred for 25 °C for 2 h. The reaction was quenched by addition of H_2SO_4 (100 mL of 2 N), the mixture was stirred for 20 min, and the product was extracted with CH_2Cl_2 (2 × 50 mL). The combined CH_2Cl_2 extracts were washed with water (6 × 50 mL), the organic phase was dried (Na_2SO_4), and the solvent was removed in vacuo. The residue obtained was purified by silica gel column chromatography (hexanes–ether, 4:1 v/v) to yield **11** (0.19 g, 70%) as a white powder; mp 67–70 °C; ^1H NMR ($CDCl_3$): δ 7.41–7.56 (m, 10H, phenyl hydrogens). Anal. Calcd for $C_{14}H_{10}N_2O$: C, 75.66; H, 4.54; N, 12.60. Found: C, 75.39; H, 4.80; N, 12.78.

5.4. 3-[4-(Methylsulfonyl)phenyl]-4-phenyl-1,2,5-oxadiazole (14)

A mixture (ratio of 3:1) of the two regioisomers **13a** and **13b** (0.65 g, 2.05 mmol) dissolved in triethylphosphite (10 mL) was heated at reflux for 24 h with stirring. The reaction mixture was cooled to 25 °C, the reaction was allowed to proceed at 25 °C for an additional 1 h with stirring, and the reaction was quenched by addition of H_2SO_4 (10 mL of 2 N) with subsequent stirring for 20 min. This mixture was extracted with CH_2Cl_2 (3 × 30 mL), the combined CH_2Cl_2 extracts were washed with water (6 × 30 mL), the organic fraction was dried (Na_2SO_4), and the solvent was removed in vacuo. The residue obtained was purified by silica gel column chromatography (hexanes–ether, 3:1 v/v) to yield **14** (0.51 g, 84%) as a white powder; mp 135–137 °C; IR (KBr): 3012 (CH_{arom}), 2925 (CH_{aliph}), 1595 (NO), 1313 (SO) cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.03 (d, J_{ortho} = 8.4 Hz, 2H, 4-methylsulfonylphenyl *H*-3, *H*-5), 7.78 (d, J_{ortho} = 8.4 Hz, 2H, 4-methylsulfonylphenyl *H*-2, *H*-6), 7.56–7.48 (m, 5H, phenyl hydrogens), 3.12 (s, 3H, CH_3). Anal. Calcd for $C_{15}H_{12}N_2O_3S$: C, 59.99; H, 4.03; N, 9.33. Found: C, 59.71; H, 4.01; N, 9.09.

5.5. 4-[4-(Chlorosulfonyl)phenyl]-3-phenyl-1,2,5-oxadiazole-2-oxide and 3-[4-(chlorosulfonyl)phenyl]-4-phenyl-1,2,5-oxadiazole-2-oxide (15) regioisomers

3,4-Diphenyl-1,2,5-oxadiazole-2-oxide (**10**, 0.5 g, 2.1 mmol) was added slowly to a solution of chlorosulfonic acid (5 mL) at ice-bath temperature, the reaction mixture was stirred at this temperature for 10 min, the ice bath was removed, and the reaction was allowed to proceed at 25 °C for 17 h. The brown reaction mixture was poured dropwise with caution onto crushed ice (100 g). Extraction with EtOAc (3 × 30 mL), repeated washing of the EtOAc extract with water until the water wash achieved a neutral pH of 7, drying the EtOAc fraction (Na_2SO_4), and the solvent was removed in vacuo. The residue obtained was purified by silica gel column chromatography (hexanes–ether, 1:1 v/v) to afford **15** (0.21 g, 30%) as a pale brown liquid; ^1H NMR ($CDCl_3$): δ 8.10 (d, J_{ortho} = 8.4 Hz, 2H, 4-chlorosulfonylphenyl *H*-3, *H*-5), 7.83 (d, J_{ortho} = 8.4 Hz, 2H, 4-chlorosulfonylphenyl *H*-2, *H*-6), 7.50–7.53 (m, 5H, phenyl hydrogens). The sulfonyl chloride product **15** was used immediately after purification for the synthesis of compounds **16** and **17**.

5.6. 3-[4-(Chlorosulfonyl)phenyl]-4-phenyl-1,2,5-oxadiazole (18)

3,4-Diphenyl-1,2,5-oxadiazole (**11**, 0.86 g, 3.87 mmol) was added to chlorosulfonic acid (5 mL) at ice-bath temperature with stirring, after 10 min the ice bath was removed, the reaction mixture was allowed to warm to 25 °C, and the reaction was allowed to proceed at 25 °C for 17 h. The brown reaction mixture was poured dropwise with caution onto crushed ice (100 g). The product was extracted with EtOAc (3 × 30 mL), and the organic phase was washed repeatedly with water until the pH was neutral. The EtOAc fraction was dried (Na_2SO_4), the solvent was removed in vacuo, and the residue obtained was purified by silica gel column chromatography (hexanes–EtOAc, 96:4 v/v) to furnish the sulfonyl chloride product **18** (0.20 g, 16%) as a pale brown liquid; ^1H NMR ($CDCl_3$): δ 8.11 (d, J_{ortho} = 8.1 Hz, 2H, 4-chlorosulfonylphenyl *H*-3, *H*-5),

7.84 (d, J_{ortho} = 8.1 Hz, 2H, 4-chlorosulfonylphenyl *H*-2, *H*-6), 7.50–7.52 (m, 5H, phenyl hydrogens). The sulfonyl chloride **18** was used immediately after purification for the synthesis of compounds **19** and **20**.

5.7. 4-[4-(Aminosulfonyl)phenyl]-3-phenyl-1,2,5-oxadiazole-2-oxide and 3-[4-(aminosulfonyl)phenyl]-4-phenyl-1,2,5-oxadiazole-2-oxide regioisomers (16)

An excess of ammonium hydroxide (2 mL of 30% w/v) was added dropwise to a mixture of the sulfonyl chloride regioisomers **15** (0.24 g, 0.71 mmol) in THF (10 mL) at 25 °C with stirring. The reaction was allowed to proceed for 1.5 h at 25 °C, water (100 mL) was added to quench the reaction, and this mixture was extracted with EtOAc (3 × 20 mL). The combined EtOAc extracts were washed with water (3 × 25 mL) and the EtOAc fraction was dried (Na_2SO_4). Removal of the solvent in vacuo afforded **16** (0.15 g, 69%) as a white powder; mp 245–247 °C; IR (KBr): 3321 (NH), 3261 (NH), 1595 (NO), 1326 (SO) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.82 (d, J_{ortho} = 8.4 Hz, 2H, 4-aminosulfonylphenyl *H*-3, *H*-5), 7.47 (d, J_{ortho} = 8.4 Hz, 2H, 4-aminosulfonylphenyl *H*-2, *H*-6), 7.31–7.37 (m, 5H, phenyl hydrogens), 6.70 (s, 2H, NH_2). Anal. Calcd for $C_{14}H_{11}N_3O_4S$: C, 52.99; H, 3.49; N, 13.24. Found: C, 53.31; H, 3.34; N, 13.15.

5.8. 4-[4-(Azidosulfonyl)phenyl]-3-phenyl-1,2,5-oxadiazole-2-oxide and 3-[4-(azidosulfonyl)phenyl]-4-phenyl-1,2,5-oxadiazole-2-oxide regioisomers (17)

Sodium azide (87 mg, 1.26 mmol) was added to a mixture of regioisomers **15** (0.21 g, 0.63 mmol) in THF (10 mL) and water (2 mL) at 25 °C. The reaction was allowed to proceed for 12 h with stirring at 25 °C, EtOAc (40 mL) was added to quench the reaction, the insoluble solid was removed by filtration and discarded, the organic phase was washed with water (2 × 15 mL), dried (Na_2SO_4), and the solvent was removed in vacuo. The residue obtained was purified by silica gel column chromatography (hexanes–ether, 3:1 v/v) to furnish **17** (0.16 g, 76%) as white crystals; mp 85–90 °C; IR (KBr): 3059 (CH), 2146 (N_3), 1595 (NO), 1380 (SO) cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.04 (d, J_{ortho} = 8.4 Hz, 2H, 4-azidosulfonylphenyl *H*-3, *H*-5), 7.82 (d, J_{ortho} = 8.4 Hz, 2H, 4-azidosulfonylphenyl *H*-2, *H*-6), 7.56–7.59 (m, 5H, phenyl hydrogens). Anal. Calcd for $C_{14}H_9N_5O_4S$: C, 48.98; H, 2.64; N, 20.40. Found: C, 49.16; H, 2.40; N, 20.43.

5.9. 3-[4-(Aminosulfonyl)phenyl]-4-phenyl-1,2,5-oxadiazole (19)

Excess ammonium hydroxide (1 mL of 30% w/v) was added dropwise to a solution of the sulfonyl chloride **18** (81 mg, 0.25 mmol) in THF (10 mL) at 25 °C. The reaction was allowed to proceed for 1.5 h at 25 °C with stirring and water (100 mL) was added to quench the reaction. Extraction with EtOAc (3 × 20 mL), washing the combined EtOAc extracts with water (3 × 25 mL), drying the EtOAc fraction (Na_2SO_4), and removal of the solvent in vacuo yielded **19** (62 mg, 82%) as a white powder; mp 227–229 °C; IR (KBr): 3314 (NH), 3224 (NH), 1333 (SO) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.74 (d, J_{ortho} = 8.4 Hz, 2H, 4-aminosulfonylphenyl *H*-3, *H*-5), 7.39 (d, J_{ortho} = 8.4 Hz, 2H, 4-aminosulfonylphenyl *H*-2, *H*-6), 7.23–7.27 (m, 5H, phenyl hydrogens), 6.76 (s, 2H, NH_2). Anal. Calcd for $C_{14}H_{11}N_3O_3S$: C, 55.80; H, 3.68; N, 13.95. Found: C, 55.99; H, 3.59; N, 13.75.

5.10. 3-[4-(Azidosulfonyl)phenyl]-4-phenyl-1,2,5-oxadiazole (20)

Sodium azide (72 mg, 1.11 mmol) was added to the sulfonyl chloride **18** (0.11 g, 0.37 mmol) in THF (10 mL) and water (2 mL) at 25 °C with stirring. The reaction was allowed to proceed for 12 h with stirring at 25 °C, EtOAc (40 mL) was added to quench the reaction, the solids removed by filtration were discarded, the organic phase was washed with water (2 × 15 mL), the organic phase was dried (Na_2SO_4), and the solvent was removed in vacuo. The residue obtained was purified by silica gel column chromatography (hexanes–EtOAc, 90:10 v/v) to afford **20** (0.10 g, 86%) as a white semi-solid; IR (KBr): 3073 (CH), 2139 (N_3), 1595 (NO), 1434 (SO); ^1H NMR ($CDCl_3$): δ 8.07 (d, J_{ortho} = 8.4 Hz, 2H, 4-azidosulfonylphenyl *H*-3, *H*-5), 7.71 (d, J_{ortho} = 8.4 Hz, 2H, 4-azidosulfonylphenyl *H*-2, *H*-6), 7.46–7.51 (m, 5H, phenyl hydrogens). Anal. Calcd for $C_{14}H_9N_5O_3S$: C, 51.37; H, 2.77; N, 21.40. Found: C, 51.53; H, 2.45; N, 21.27.

5.11. In vitro cyclooxygenase (COX) inhibition assays

The ability of the test compounds listed in Table 1 to inhibit ovine COX-1 and COX-2 (IC_{50} value, μM) was determined using an enzyme immuno assay (EIA) kit (catalog no. 560101, Cayman Chemical, Ann Arbor, MI, USA) according to our previously reported method.[4]

5.12. In vitro nitric oxide release assay

In vitro nitric oxide release in phosphate buffer, in the presence or absence of L-cysteine, was determined by quantification of nitrite produced by the reaction of nitric oxide with oxygen and water using the Griess reaction. Nitric oxide release data acquired for the test compounds (**10**, **13a**,**b**, **16**, and **17**) using the reported procedures are listed in Table 2.[29]

5.13. Molecular modeling (docking) studies

Docking experiments were performed using INSIGHT II software Version 2000.1 (Accelrys Inc.) running on a Silicon Graphics Octane 2 R14000A workstation according to a previously reported method.[4]

5.14. Crystal structure data for the 4-[4-(methylsulfonyl)phenyl]-3-phenyl-1,2,5-oxadiazole-2-oxide (13a) and 3-[4-(methylsulfonyl)phenyl]-4-phenyl-1,2,5-oxadiazole-2-oxide (13b) mixture or regioisomers (3:1 ratio)

Molecular formula: $C_{15}H_{12}N_2O_4S$, formula weight: 316.33, crystal system: monoclinic, space group: $P2_1/c$ (no. 14) with unit cell dimensions a = 18.1969 (18) Å, b = 7.1642 (7) Å, c = 11.6272 (11) Å, β = 106.4720

(17)°, $V = 1453.6$ (2) Å^3, $Z = 4$, $\rho_{calcd} = 1.445$ g cm^{-3}, $\mu = 0.242$ mm^{-1}. A crystal fragment of approximate dimensions (mm^3) $0.49 \times 0.25 \times 0.04$ was mounted in a nonspecific orientation on a Bruker PLATFORM/SMART 1000 CCD diffractometer. All intensity measurements were performed using Mo Kα radiation ($\lambda = 0.71073$ Å) with a graphite crystal incident beam monochromator. The intensity data were collected at −80° using ω scans (0.2° scans, 45 s exposures). A total of 2970 independent reflections were collected to a maximum 2θ limit at 52.86°. The structure was solved by direct methods (SHELXS-86). Refinement of atomic parameters was carried out by using full-matrix least-squares on F^2 (SHELXL-93). Although the initial model refined was for **13a**, elongation of the thermal parameters for the phenyl and oxadiazole rings, plus the occurrence of a large residual electron-density peak near the 5-nitrogen of the 1,2,5-oxadiazole ring, suggested that the isomer **13b** was also present. The two isomers share the $MeSO_2$ group, but separate sets of positions for the phenyl and 1,2,5-oxadiazole 2-oxide groups were refined for both **13a** and **13b**, in 75:25 ratio. The final model gave agreement factors (R indices) of $R_1(F) = 0.0685$ (for 2432 data with $I \geqslant 2\sigma(I)$) and $wR_2(F^2) = 0.1982$ (for all 2970 unique data). Crystallographic data (excluding structure factors) have been deposited at the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 252139. Copies of the data can be obtained free of charge by application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: 44 01223 336033 or e-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.uk).

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