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Novel valdecoxib derivatives by ruthenium(II)-promoted 1,3-dipolar cycloaddition of nitrile oxides with alkynes – synthesis and COX-2 inhibition activity†

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Novel valdecoxib-based cyclooxygenase-2 inhibitors were synthesized in one step via 1,3-dipolar cycloaddition of nitrile oxides with a series of eleven aryl alkynes, six of them described for the first time. Application of Ru(II)-catalysis leads preferably to the formation of the 3,4-diaryl-substituted isoxazoles, while under thermal heating with base the 3,5-diaryl substitution pattern is favoured. The new the 3,4-diaryl-substituted isoxazoles possessing a small substituent (H and Me) displayed high COX-2 inhibition affinity (IC_{50} = 0.042–0.073 μ M) and excellent selectivity (COX-2 SI > 2000). In contrast, the 3,5-diaryl substituted compounds displayed almost no COX activity. The introduction of a 4-fluorophenyl substituent resulted in retained high COX-2 affinity, making these compounds together with the feasible one step reaction promising candidates for the development of fluorine-18 labelled radiotracers.

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1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a widely used class of therapeutic agents for the treatment of inflammation, fever and pain. The pharmacological effects of NSAIDs result from their capability to inhibit the enzyme family of cyclooxygenases (COX), which catalyses the biotransformation of arachidonic acid to prostanoids, and exists as two distinct isoforms, a constitutive form (COX-1) and an inducible form (COX-2).^{1,2} COX-1 derived prostaglandins control homeostatic functions, including gastric cytoprotection and homeostasis, while COX-2 is responsible for the synthesis of prostanoids involved in acute and chronic inflammatory

states.³ Inflammation is a common biological process shared by various diseases and elevated expression of COX-2 has been implicated in manifold pathological events, including rheumatoid arthritis, heart disease, stroke, and neurodegenerative disorders.⁴

Recently, it has become more and more obvious that COX-2 is also overexpressed in several human cancer entities and is assumed to be implicated in tumour inflammation and hypoxia.⁵ In consequence, expression of COX-2 has attracted considerable attention as a diagnostic marker and therapeutic target in oncology.^{6,7}

The therapeutic effect of NSAIDs is attributed to the selective inhibition of COX-2; however, undesired (*e.g.*, gastrointestinal) side effects may arise from the disruption of COX-1 by application of nonselective COX inhibitors.⁸ Due to the structural similarities of the COX-1 and the COX-2 enzymes, the development of selective COX-2 inhibitors (Coxibs) with no affinity towards COX-1 constitutes a persistent challenge in medicinal chemistry. In this context, the 3,4-diaryl substituted isoxazole scaffold is a frequently recurring pharmacophore found in a variety of NSAIDs/Coxibs,⁹ protein kinase inhibitors, and hypertensive agents. Valdecoxib is one of the well-known examples of 3,4-diaryl substituted isoxazoles derivatives commercialized in 2000 for clinical use,¹⁰ but withdrawn from the market in 2005 owing to its adverse cardiovascular side effects and skin reactions.¹¹ However, parecoxib¹² (Dynastat®), which was designed to be a water-soluble (>50 g L⁻¹ in normal saline, pK_a 4.9), parenterally safe prodrug form

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of valdecoxib (0.01 mol L^{-1} in normal saline, pK_a 9.8), is still in use for the management and treatment of acute pain.

Nevertheless, valdecoxib is a very potent and selective inhibitor of COX-2 ($\text{IC}_{50} = 0.005 \text{ }\mu\text{mol}$) with almost no inhibition of COX-1 ($\text{IC}_{50} = 150 \text{ }\mu\text{mol}$)¹³ and represents an interesting lead structure for the development of potential radiotracers for the functional imaging of COX-2 *via* positron emission tomography (PET). In the case of radiotracers the selectivity and affinity of drugs is of major importance, while pharmacological side effects can mainly be neglected, since only a few nanomoles of the drug are applied.

With our interest in imaging COX-2 functional expression *in vivo*, our ongoing efforts are focused on the development of novel radiolabeled COX-2 inhibitors as radioactive probes for the characterization of inflammatory and tumorigenic lesions.¹⁴ With this background, we aimed at the synthesis of novel fluorine-bearing valdecoxib derivatives having varying substituents (Fig. 1). While the substituent A represents an hydrogen or fluorine atom, the latter as surrogate for potential fluorine-18 labelling; the substituent B describes a number of functional groups. The sulfonamide or methylsulfonyl group C located in *para*-position at one of the phenyl substituents interacts effectively with the COX-2 side pocket through slow tight-binding kinetics,¹⁵ and should be generally preserved.

Accordingly, in this paper we describe the one step synthesis of a series of diaryl-substituted isoxazoles, derivatives of valdecoxib, by 1,3-dipolar cycloaddition of nitrile oxides with a panel of various substituted phenyl acetylenes. To determine their potential suitability, the COX-1 and COX-2 inhibitory activity of the new compounds was evaluated *in vitro*.

2. Results and discussion

2.1 Chemistry

Although several literature reports depict the synthesis of substituted isoxazoles, in the case of 3,4-diaryl substituted isoxazoles the question of selectivity in general is a crucial problem, whose solution depends mostly on the availability of the starting materials.¹⁶ As displayed in Scheme 1, different protocols for the synthesis of valdecoxib and analogues have been described. Among them, 1,3-dipolar cycloaddition of nitrile oxides with olefinic compounds followed by aromatization is often used¹⁷ (Scheme 1A). A different approach starting from ketones, involves the synthesis of enamines. 1,3-dipolar cycloaddition of enamine with aryl nitrile oxides

followed by treatment with HCl affords the corresponding 3,4-diaryl substituted isoxazole.¹⁸

Secondly and as displayed at Scheme 1B, 1,3-dipolar cycloaddition reaction of nitrile oxides with alkynylboronates followed by Pd-mediated coupling reactions offers a regio-controlled access to valdecoxib and analogues by furnishing isoxazole boronates.^{19–21} These readily prepared isoxazole boronates are useful precursors for the Suzuki coupling reactions using arylhalides in the presence of palladium catalysts.

In addition, the electrophilic cyclization followed by cross-coupling reaction constitutes an interesting alternative for the synthesis of diaryl-substituted isoxazoles^{22,23} (Scheme 1C). The iodine monochloride promoted electrophilic cyclization of O-methyl oximes displays an access to 4-iodoisoxazoles, which are useful precursors to synthesize 3,4-diaryl substituted isoxazoles through Suzuki cross-coupling with the corresponding boronic acid and palladium catalyst.^{24,25}

At Scheme 1D a concise synthesis of valdecoxib and its derivatives starting from deoxybenzoin derivatives is shown.²⁶

The 1,3-dipolar cycloaddition between benzonitrile oxides and phenylacetylenes is a well-known method for direct access to 3,5-diaryl substituted isoxazoles.^{17,27–29} However, the control of the regiochemistry to obtain the 3,4-regioisomer is, in this case, a considerable challenge.³⁰

2.1.1 Ruthenium(II) catalysed 1,3-dipolar cycloaddition of nitrile oxides with alkynes. Although the cycloaddition of phenylacetylenes and benzonitrile oxides is the most direct route for accessing 3,4-diaryl substituted isoxazoles, this method is rarely used for the synthesis of valdecoxib derivatives.³¹ The reasons for this are simple: the non-catalysed, thermal cycloaddition reactions of nitrile oxides with alkynes are neither chemo- nor regioselective. Furthermore, examples of reactions of nitrile oxides with internal alkynes are limited to highly activated alkynes (*e.g.*, acetylene dicarboxylate and related electron-deficient acetylenes). Non-activated, electron-rich, or sterically hindered acetylenes usually do not react.^{32,33} Copper(I) acetylides have been reported to react regioselectively with nitrile oxides to generate 3,5-disubstituted isoxazoles.^{28,34}

An extension of the 1,3-dipolar cycloaddition reaction of nitrile oxides and terminal or internal alkynes was recently described by Fokin *et al.*³⁵ They report on the use of ruthenium(II) complexes instead of copper(I) for the cycloaddition reaction, leading exclusively to the formation of the corresponding regiocomplementary 3,4-di- and 3,4,5-trisubstituted

Fig. 1 Design concept of novel derivatives with valdecoxib as lead compound.

A: 1,3-dipolar cycloaddition of nitrile oxide with enolates **B: 1,3-dipolar cycloaddition of nitrile oxide with alkynylboronates** **C: Coupling and Electrophilic cyclization reactions** **D: Condensation reactions** **Scheme 1** A–D Recent approaches used to synthesize 3,4-diaryl substituted isoxazoles.

isoxazoles, respectively. Whilst the natural regioselectivity of the cycloaddition reaction is delivering isoxazoles with the 3,5-substitution pattern, Ru(II) complex-catalysed 1,3-dipolar cycloaddition would provide a direct route for accessing 3,4-diaryl substituted isoxazoles (Fig. 2).

The proposed mechanism of the Ru catalyst in this cycloaddition reaction is displayed in Scheme 2.³⁵ In the first step the displacement of the cyclooctadiene ligand from the [Cp*RuCl(cod)] catalyst by an alkyne and nitrile oxide produces the activated complex A, which mediates the oxidative coupling of a nitrile oxide and alkyne, resulting in ruthenacycle B. The oxidative coupling step controls the regio selectivity of the whole process. It appears that the new carbon–oxygen bond is formed between the more electronegative carbon cen-

tre of the alkyne and the oxygen atom of the nitrile oxide, which represents an unexpected mode of activation of nitrile oxides. Normally, their carbon centre is electrophilic and readily reacts with nucleophiles. Thus, coordination to the ruthenium atom effectively changes the polarity of the nitrile oxide. Ruthenacycle B undergoes reductive elimination giving C, and release of the isoxazole product, then completes the catalytic cycle. The observation that Cp*-based catalysts are especially active is consistent with the ability of the spectator ligands in such ruthenium complexes.³⁶

To evaluate the scope and limitations of this approach, activated and non-activated alkynes have been investigated. For this purpose we first prepared a series of *para*-substituted phenyl acetylenes 1a–k (Table 1) possessing the SO₂R (R =

Fig. 2 Synthesis of valdecixib derivatives by Ru-catalysed 1,3-dipolar cycloaddition of nitrile oxides with alkynes.

Scheme 2 Proposed mechanism of Ru(II)-catalysed 1,3-dipolar cycloaddition of nitrile oxides with alkynes.³⁵

NH₂, CH₃) pharmacophore for subsequent 1,3-dipolar cycloaddition reactions with nitrile oxides. The alkynes **1a**, **b** were synthesized from the corresponding 4-trimethylsilyl-substituted alkynes **1j** and **1k** respectively, by following literature procedures.^{37,38}

Other alkynes carrying a methyl- (**1c**), trifluoromethyl- (**1d**), chloro- (**1e**), formyl- (**1f**), methoxycarbonyl- (**1g**) and acetyl-substituent (**1h**) are novel; accordingly their synthesis had to be established. The 4-(prop-1-yn-1-yl)-benzenesulfonamide **1c** was synthesized from 4-bromobenzenesulfonamide by reaction with trimethylsilylacetylene

and subsequent methylation with methyl iodide (Scheme 4). The synthesis of 1-(methylsulfonyl)-4-(3,3,3-trifluoroprop-1-yn-1-yl)benzene **1d** started from 4-methylthiobenzaldehyde which was chloro-trifluoro-propenylated and after elimination of HCl transformed into the 4-trifluoroprop-1-yn-substituted methyl-phenyl-sulfane. Subsequent oxidation with Oxone® delivered the final methylsulfone **1d** in 18% yield over three steps (Scheme 3). 1-(Chloroethynyl)-4-(methylsulfonyl)-benzene **1e** could be obtained in 25% yield by reaction of 1-ethynyl-4-(methylsulfonyl)benzene **1b** with *N*-chlorosuccinimide in presence of Ag₂CO₃ and K₂CO₃ (Scheme 3). The formyl-, methoxycarbonyl-, acetyl-substituted alkynes **1f–h** were accessible by Sonogashira coupling of prop-2-yn-1-ol or but-3-yn-2-ol with 1-bromo-4-(methylsulfonyl)benzene followed by oxidation reactions in about 40% overall yield (Scheme 4). The benzoyl-substituted alkyne **1i** was formed *via* a Pd-mediated coupling reaction of **1b** with benzoyl chloride (Scheme 3).

Since nitrile oxides are generally unstable, with a tendency to dimerization, we decided to synthesize the corresponding hydroximoyl chlorides **2a** and **2b** which are storable and can be easily transformed *in situ* to the nitrile oxide by treatment with Et₃N. The hydroximoyl chlorides **2a** and **2b** are facile available by the reaction of the corresponding benzaldoximes with *N*-chlorosuccinimide.³⁹

With hydroximoyl chlorides **2a–b** and a panel of various *para*-substituted phenyl acetylenes **1a–k** in hand, we performed a ruthenium(II)-catalysed click reaction to prepare the desired 3,4-diaryl substituted isoxazoles **3**.

In a first set of reactions, we employed the Ru(II)-catalysed 1,3-dipolar cycloaddition reaction between hydroximoyl chlorides **2** and various phenyl acetylenes **1** to prepare

Table 1 Ruthenium(II)-catalysed 1,3-dipolar cycloaddition of nitrile oxides with alkynes^a

Alkyne	R ¹	R ²	Oxime	X	Time	3 : 4 ratio ^b	Yield 3 ^c	Yield 4 ^c
1a	H	NH ₂	2a	F	5 min	99 : 1	3a (74%)	4a (0)
1a	H	NH ₂	2b	H	5 min	98 : 2	3b (74%)	4b (0)
1b	H	CH ₃	2a	F	5 min	98 : 2	3c (65%)	4c (0)
1b	H	CH ₃	2b	H	5 min	99 : 1	3d (64%)	4d (0)
1c	CH ₃	NH ₂	2a	F	24 h	—	3e (0) ^d	4e (0) ^d
1d	CF ₃	CH ₃	2a	F	24 h	40 : 60	3f (29%)	4f (52%)
1e	Cl	CH ₃	2a	F	24 h	—	3g (0) ^d	4g (0) ^d
1f	CHO	CH ₃	2a	F	1 h	9 : 1	3h (57%)	4h (0)
1g	CO ₂ Me	CH ₃	2a	F	1 h	94 : 6	3i (59%)	4i (0)
1h	COMe	CH ₃	2a	F	24 h	85 : 15	3j (63%)	4j (0)
1i	COPh	CH ₃	2a	F	18 h	65 : 35	3k (58%)	4k (27%)
1j	TMS	NH ₂	2a	F	24 h	—	3l (0) ^d	4l (0) ^d
1k	TMS	CH ₃	2a	F	24 h	—	3m (0) ^d	4m (0) ^d

^a Reaction conditions: alkyne (1.0 mmol), hydroximoyl chloride (1.6 mmol), Et₃N (1.6 mmol), [Cp*RuCl(cod)] (15% mol) in 1,2-DCE at room temperature. ^b Determined by HPLC and ¹H-NMR analysis. ^c Isolated yield after purification by flash chromatography and recrystallization from ethyl ether. ^d No reaction under Ru-catalysis, thermal activation required.

Scheme 3 Synthesis routes for alkynes **1d**, **1e** and **1i**.

Scheme 4 Synthesis routes for alkynes **1c** and **1f-h**.

regioselective isoxazoles **3** possessing a 3,4-diaryl substitution pattern. In accordance with the literature, we chosen $[\text{Cp}^*\text{RuCl}(\text{cod})]$ as the most suitable ruthenium(II) complex for this reaction. Briefly, alkynes **1** were combined with hydroximoyl chlorides **2** (1.6 eq.) in the presence of 15 mol% of

$[\text{Cp}^*\text{RuCl}(\text{cod})]$ and 1.6 equivalents of Et_3N and stirred at room temperature for the period of time indicated in Table 1. Terminal alkynes **1a** and **1b** reacted fast and HPLC and $^1\text{H-NMR}$ analysis showed that the 3,4-disubstituted isoxazoles **3a-d** were the major regioisomers formed. The 3,4-substitution

pattern was confirmed by the characteristic down-field-shifted ^1H NMR signals (9.37–9.43 ppm) as typically observed for the 3,4-disubstituted isoxazoles (see Fig. S61 ESI†).

The electron-deficient alkynes (**1d**, **1f–1i**) were effective cycloaddition partners as well, forming under Ru(II) catalysis mixtures of 3,4-diaryl and 3,5-diaryl substituted isoxazoles; whereby the 3,4-regioisomers were mostly the major product (Table 1). It should be noted that significant longer reaction times (1–24 h) were needed to perform the cycloaddition. The methyl-, chloro- and trimethylsilyl-substituted alkynes **1c**, **1e**, **1j–k** did not react at r.t. and Ru(II) catalyses and required thermal induced reaction conditions to perform 1,3-dipolar cycloaddition (see 2.1.2 and Table 2).

2.1.2 Thermal induced 1,3-dipolar cycloaddition of nitrile oxides with alkynes. As described before, the Ru(II) -catalysed 1,3-dipolar cycloaddition reaction failed, with non-activated alkynes having a methyl (**1c**), or a chlorine substituent (**1e**), and also with the sterically hindered acetylenes bearing a TMS group **1j** and **1k**. With these alkynes, we applied classic thermal conditions (heating in the presence of a base) to synthesize the desired isoxazoles (Table 2). These reactions were not regioselective and in most cases a 20 : 80 ratio of the 3,4-diarylated to the 3,5-diarylated isomers was observed, indicating the favoured 3,5-substitution pattern under thermal conditions. Additionally, the thermal reaction conditions in the absence of the ruthenium(II) catalyst were performed for alkynes **1d**, **1f** and **1i**, and we observed no 3,4-regioisomers but exclusively the formation of the 3,5-diaryl substituted isoxazoles **4f**, **4h** and **4k**, respectively. Unlike the Ru(II) -promoted reaction, thermal cycloadditions required drastic reaction conditions in an aprotic solvent at elevated temperature for several hours, and the desired 3,4-diarylated isoxazoles **3e**, **3g** and **3l–o** were obtained in significantly lower chemical yields (4–11%) compared to compounds synthesized under ruthenium catalysis (29–74%).

The identity of the 3,4- and 3,5-diaryl substituted regioisomers **3f**, **3h–k** and **4f**, **4h–k** was established by NMR-based analyses as exemplified for **3f** and **4f** (see Fig. S62 ESI†). The signal of the trifluoromethyl group in the ^{13}C NMR spectrum, identified as a quartet is much more down-field-shifted in the 3,4-diaryl-substituted isoxazole **3f** than in **4f**.

The determination of the regiochemistry of the cycloaddition by ^1H -NMR analysis was difficult in the case of the diaryl-trimethylsilyl-substituted isoxazoles **3l–3o**, and **4l–4o**, therefore the purified trisubstituted compounds **4l–o** were subjected to protodesilylation to obtain the corresponding di-substituted isoxazoles **4a–d** having a proton instead of the trimethylsilyl group (Scheme 5). Protodesilylation was performed with CsF in good yield (74–90%). ^1H -NMR analysis showed a characteristic signal between 7.77 ppm and 7.86 ppm, which is indicative for the proton in isoxazole rings containing a 3,5-substitution pattern, as visible for **4a** (ESI†). The minor 3,4-diaryl-5-trimethylsilyl substituted regioisomer **3l** was subjected to the same reaction conditions and afforded exclusively the compound **3a**.

Further evidence confirming the supposed 3,4-substitution pattern was possible in case of the trimethylsilyl substituted compounds **3l** and **3m** for which crystals suitable for X-ray structural analysis could be obtained. Fig. 3 displays the molecular structures as obtained by X-ray diffraction analysis of **3l** and **3m** that undoubtedly confirmed the composition of both compounds. Both crystallize in the triclinic space group $P\bar{1}$, **3l**: $a = 9.705(6)$ Å, $b = 9.854(6)$ Å, $c = 11.726(8)$ Å, $\alpha = 65.43(3)^\circ$, $\beta = 68.51(3)^\circ$, $\gamma = 76.92(2)^\circ$, $V = 945(1)$ Å³, $Z = 2$, $R1 = 4.87\%$; **3m**: $a = 7.6192(5)$ Å, $b = 10.3601(7)$ Å, $c = 13.1271(9)$ Å, $\alpha = 102.578(4)^\circ$, $\beta = 92.847(4)^\circ$, $\gamma = 101.855(4)^\circ$, $V = 984.9(1)$ Å³, $Z = 2$, $R1 = 5.77\%$; for more details of the X-ray structure analysis see ESI†. Even though the two molecules have a very similar structure, their packing in the crystal is different with respect to the orientation to each other. All bond lengths

Table 2 Thermal induced 1,3-dipolar cycloaddition of nitrile oxides with alkynes

Alkyne	R ¹	R ²	Oxime	X	Reaction conditions	Time	3 : 4 ratio ^a	Yield 3 ^b	Yield 4 ^b
1c	CH ₃	NH ₂	2a	F	KF, 1,2-DME	18 h	37 : 63	3e (4%)	4e (8%)
1d	CF ₃	CH ₃	2a	F	Et ₃ N, toluene	18 h	—	3f (0)	4f (62%)
1e	Cl	CH ₃	2a	F	Et ₃ N, toluene	18 h	18 : 82	3g (8%)	4g (37%)
1f	CHO	CH ₃	2a	F	Et ₃ N, toluene	5 h	—	3h (0)	4h (45%)
1i	COPh	CH ₃	2a	F	Et ₃ N, toluene	18 h	—	3k (0)	4k (85%)
1j	TMS	NH ₂	2a	F	KF, 1,2-DME	18 h	17 : 83	3l (11%)	4l (59%)
1k	TMS	CH ₃	2a	F	KF, 1,2-DME	18 h	22 : 78	3m (7%)	4m (66%)
1j	TMS	NH ₂	2b	H	KF, 1,2-DME	18 h	16 : 84	3n (5%)	4n (65%)
1k	TMS	CH ₃	2b	H	KF, 1,2-DME	18 h	11 : 89	3o (4%)	4o (70%)

^a Determined by HPLC and ^1H -NMR analysis. ^b Isolated yield after purification by flash chromatography and recrystallization from ethyl ether.

Scheme 5 Protodesilylation of isoxazoles 4I-o and 3I.

Fig. 3 Molecular structures of compounds 3I (left) and (3m) (right) in the crystal (ORTEP plot, ellipsoids at the 50% probability level at 123 K).

within the molecules fall within those ranges which are expected for the respective bonds. Intermolecular contacts are dominated by Van-der-Waals interactions. Only 3I shows a weak hydrogen bond contact.

2.2 *In vitro* COX-1/COX-2 inhibitory activity

Structure–activity relationship (SAR) studies have revealed that mono- and bicyclic compounds possessing two vicinal aryl moieties on the central heterocyclic ring system represent one major class of selective COX-2 inhibitors.⁴⁰ The vicinal 1,2-diaryl substitution pattern results in high affinity towards COX-2; however, examples of 1,3-diaryl-substituted isoindole or pyrimidine derivatives showing a *meta*-substitution pattern for the aryl rings, have also been reported to be highly affine towards COX-2.⁴¹

Based on 1,3-dipolar cycloaddition reactions between nitrile oxides and various phenyl acetylenes **1**, we prepared two classes of compounds possessing a central isoxazole moiety; the vicinal 3,4-diaryl-substituted isoxazoles **3** and 3,5-diaryl-

substituted isoxazoles **4**. Compounds **3** are structural closely related to traditional selective COX-2 inhibitors *e.g.* valdecoxib and parecoxib (see Fig. 1). For determining the resulting combined different steric and electronic effects upon COX-1 and COX-2 inhibitory affinity and COX isoenzyme selectivity, all isolated compounds were subjected to an *in vitro* COX Fluorescent Inhibitor Screening Assay (Cayman Chemicals; Item nr: 700100). Valdecoxib as lead compound was used as the reference. The determined *in vitro* enzyme inhibition data, along with the calculated COX-2 selectivity index (COX-2 SI) and calculated lipophilicity values (cLogP) of the new 3,4- and 3,5-diaryl-substituted isoxazoles, are summarized in Table 3.

As expected, valdecoxib proved to be a potent and selective COX-2 inhibitor in the performed enzyme inhibitory assay; the COX-2 inhibitory activity was determined by us with an IC₅₀ value of 0.05 μM (see Fig. S63, ESI†), a value that is by a factor of 10 higher than reported (0.005 μM).¹³ This difference is owed to the fact that in our work a fluorescence-based assay was used as oppose to the ³H-based radioligand

Table 3 *In vitro* COX-1 and COX-2 enzyme inhibition data of 3,4- and 3,5-diaryl-substituted isoxazoles

	R ¹	R ²	X	COX-1 IC ₅₀ (μM) ^a	COX-2 IC ₅₀ (μM) ^a	COX-2 SI ^b	cLog P ^c
Valdecoxib	CH ₃	NH ₂	H	>100 (150) ^d	0.050 ± 0.002 (0.005) ^d	>2000	2.73
3a	H	NH ₂	F	>100	0.042 ± 0.033	>2400	2.68
3b	H	NH ₂	H	>100	0.048 ± 0.002	>2100	2.51
3c	H	CH ₃	F	>100	0.057 ± 0.006	>1800	2.85
3d	H	CH ₃	H	>100	0.247 ± 0.028	>400	2.69
3e	CH ₃	NH ₂	F	>100	0.073 ± 0.008	>1400	2.90
3f	CF ₃	CH ₃	F	>100	8.145 ± 0.191	>12	3.99
3g	Cl	CH ₃	F	10.07	0.351 ± 0.026	28.7	3.65
3h	CHO	CH ₃	F	>100	13.777 ± 0.50	>7	2.88
3i	CO ₂ Me	CH ₃	F	>100	>100	—	2.92
3j	COMe	CH ₃	F	>100	>100	—	2.99
3k	COPh	CH ₃	F	>100	>100	—	4.55
3l	TMS	NH ₂	F	>100	0.93 ± 1.70	>110	5.19
3m	TMS	CH ₃	F	>100	1.87 ± 1.24	>54	5.36
3n	TMS	NH ₂	H	>100	0.13 ± 0.001	>770	5.02
3o	TMS	CH ₃	H	>100	4.17 ± 3.35	23.9	5.20
4a	H	NH ₂	F	>100	81.2 ± 5.10	>1.2	2.87
4b	H	NH ₂	H	>100	10.79 ± 0.01	>9.3	2.71
4c	H	CH ₃	F	>100	>100	—	3.05
4d	H	CH ₃	H	>100	13.55 ± 5.05	>7.4	2.88
4l	TMS	NH ₂	F	>100	38.16 ± 0.01	>2.6	5.19
4m	TMS	CH ₃	F	>100	>100	—	5.36
4n	TMS	NH ₂	H	>100	11.05 ± 1.31	>9.0	5.02
4o	TMS	CH ₃	H	>100	>100	—	5.20

^a Values are means of two determinations with standard deviation. ^b *In vitro* COX-2 selectivity index (IC₅₀ COX-1/IC₅₀ COX-2). ^c cLog P values have been calculated based on molinspiration predictions (www.molinspiration.com). ^d Literature value.¹³

assay from the literature. However, useful information is provided by comparing the results of all compounds measured by this procedure.

The first series of compounds (3a–o) containing the common vicinal substitution pattern of the aryl moieties showed interesting results with regard to the inhibitory activity, depending on the substituents at position 5 of the isoxazole. All compounds of this series displayed no inhibitory potency against the constitutive form of cyclooxygenase (COX-1) (>100 μM), with one exception, compound 3g (IC₅₀ COX-1 = 10 μM).

Regarding the inhibition against COX-2, a more differentiated picture within compounds 3a–o was observed (Table 3). Compounds 3a–3e containing an H or CH₃ substituent displayed a nanomolar COX-2 inhibitor potency (0.042–0.247 μM), compound 3g with a chloro- and 3l as well as 3n bearing a TMS-substituent showed submicromolar affinity (0.13–0.93 μM). Compounds 3i–k containing carbonyl substituents CO₂Me, COMe and COPh showed no inhibitory potency against COX-2 (>100 μM) and compounds 3f and 3h (containing electron-withdrawing groups CF₃ and CHO) as well as compounds 3m and 3o (with a bulky TMS group) displayed only very low inhibition of the COX-2 enzyme as reflected by IC₅₀ values in a micro molar range (1.87 μM to 13.77 μM).

In conclusion, compounds 3a–c and 3e containing an H or CH₃ substituent are 2- to 22-fold more potent COX-2 inhibitors compared to compounds 3d, 3g, 3l and 3n having an Cl or TMS group. Compounds 3a–c and 3e containing an H or CH₃ substituent behave as particularly highly potent COX-2 inhibitors, and it can be concluded that the inhibitory activity determined for COX-2 is in the same range as valdecoxib.

Within this series, compounds 3a–c and 3e also showed the highest COX-2 selectivity. The SI value of >2400 makes 3a the most COX-2 selective compound within all isoxazole containing compounds studied, and even more selective than valdecoxib (SI > 2000). According to these data, increasing size and electron-withdrawing properties of the substituents at position 5 in the isoxazole ring, as found for the CF₃, COR, and TMS group in compounds 3f, 3h–k, 3m and 3o, decreases their COX-2 inhibitory potency.

Compounds 3a–d and 3l–o showed interesting results regarding inhibitory activity depending on the substituents at the *para*-position of the aryl rings. The structure–activity relationship study of compounds 3a–e indicated that the order of COX-2 inhibitory potency was in the same range comparing a fluoro with a hydrogen substituent (3a vs. 3b and valdecoxib vs. 3e). This result suggests that the presence of electron-withdrawing groups like fluorine favours selective and potent

inhibition of COX-2. This observation is interesting with regard to former investigations which we have made with vicinal diaryl-substituted (dihydro)pyrrolo[3,2,1-*hi*]indoles where a *para*-fluoro substituent did not result in enhanced or steady COX-2 affinity.⁴²

Compounds **3a–d** and **3l–o** possess the typical SO₂R COX-2 relevant substituent in the *para*-position of one of the aryl rings attached to the central isoxazole unit with R = NH₂ or CH₃. Comparison of the IC₅₀ values for these compounds suggests that compounds **3a**, **3b**, **3l**, and **3n** bearing a SO₂NH₂ group as pharmacophore are more selective and potent COX-2 inhibitors than the compounds **3c**, **3d**, **3m**, and **3o** with a SO₂Me group (compare **3a** vs. **3c**, **3b** vs. **3d**, **3l** vs. **3m**, and **3n** vs. **3o**).

The second class of compounds (**4**) contains the uncommon 3,5-disubstitution pattern of the aryl moieties. All these compounds displayed no inhibitor activity towards COX-1 (IC₅₀ >100 μM). Additionally, these substances showed only very low or no inhibitory potency against COX-2 in the range of 10.79 μM to >100 μM. Direct comparison of compounds **3** and compounds **4** further confirms the favourable vicinal diaryl substitution pattern with regard to potent COX-2 inhibition. Changing the 3,5-diaryl substitution pattern in compounds **4d** and **4l–o** to the vicinal diaryl substitution design in compounds **3d** and **3l–o** is accompanied by a 24- to 85-fold increase in COX-2 inhibition affinity. Highly affine compound **3b** is 225-fold more potent than its corresponding 3,5-diaryl-substituted counterpart **4b**. The positive effect of the vicinal diaryl substitution on COX-2 inhibition potency was even more pronounced for comparing compounds **3a** versus **4a** and **3c** versus **4c** (see Table 3). To summarize, in the case of the aryl-substituted isoxazoles the 3,5-disubstitution pattern does not result in potent inhibitors of COX-2.

2.3 Lipophilicity calculations

Range of lipophilicity is a crucial criterion in drug development and especially in the design of radiotracers. With increasing lipophilicity small-molecule drugs tend towards unspecific binding to plasma proteins and membranes. For the hydrogen-bearing isoxazoles **3a–d** a favourable lipophilicity was calculated (cLogP = 2.51–2.85); however, the replacement of the hydrogen atom at position 5 with more lipophilic substituents (Cl, CF₃, CPh, TMS), as done in compounds **3f**, **g** and **3k–o** resulted in a significant increase in lipophilicity by 1–2 orders of magnitude (cLogP = 3.99–5.20). On account not only of their low COX-2 affinity but also their lipophilicity range the CPh- and TMS-substituted compounds would represent unfavourable candidates for the further design of radiotracers. Conversely, the promising results as regard COX-2 inhibition selectivity and affinity, and the comparatively low lipophilicity of compounds **3a** and **3c**, make these 3,4-diaryl substituted isoxazoles interesting candidates for further development as potential fluorine-18 labelled COX-2 inhibitors.

3. Conclusion

We have prepared by ruthenium(II) promoted 1,3-dipolar cycloaddition a series of new compounds on the basis of the valdecoxib lead structure displaying a central isoxazole scaffold, with two aryl substituents and one additional substituent on the heterocycle. An outstanding feature of this approach is that it delivers the 3,4-diaryl substituted isoxazoles in one step in yields of up to 74%, making this approach very promising for radiotracer synthesis.

In general, compounds having a vicinal diaryl substitution pattern showed a COX-2 inhibition, whilst their corresponding 3,5-diaryl-substituted counterparts turned out to be inactive. The 3,4-diaryl substituted compounds possessing a small substituent at position 5 of the isoxazole ring (**3a–c** and **3e**) displayed highest COX-2 inhibition potency with IC₅₀ values of 0.042–0.073 μM, being in the same range as found for valdecoxib (0.050 μM). Isoxazoles bearing bulky or electron-withdrawing groups showed significantly lower affinity towards COX-2.

Interestingly, the introduction of a fluorine substituent by application of a fluoro-substituted nitrile oxide **2a** resulted in remaining high COX-2 affinity. This finding, along with their ease in synthesis through versatile Ru(II)-catalysed click chemistry make compounds **3a** and **3c** to interesting candidates for the design of potent fluorine-18 labelled PET tracers. That might be put into practice by application of 1,3-dipolar cycloaddition with 4-[¹⁸F]fluorobenzonitrile oxide, in analogy to the work of Zlatopolskiy *et al.*⁴³ As extension, further analogues of **3a** and **3c** might be explored, having the fluorine atom in *ortho* or *meta* position of the benzene ring with view on COX-2 affinity and radiolabelling; an approach that is challenging since 2- and 3-[¹⁸F]fluorobenzonitrile oxides are not described so far.

4. Experimentals

4.1 Chemistry

Synthesis procedures, NMR data and spectra of all new synthesized compounds are available free of charge as ESI.†

4.2 X-ray crystallography

The crystallographic data were collected using an Apex-II CCD diffractometer (Bruker), with Mo Kα radiation (λ = 0.71073 Å). The structures were solved using SHELXS-97 and refined against F² on all data by full-matrix least-squares with SHELXL-97.⁴⁴ All non-hydrogen atoms were refined anisotropically; all hydrogen atoms bonded to carbon atoms were placed on geometrically calculated positions and refined using a riding model. Details of the X-ray structure analysis of compounds **3l** and **3m** and copies of the ¹H and ¹³C NMR spectra of all compounds are available as ESI.† Crystallographic data for compounds **3l** and **3m** were deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-1483007 (compound **3m**), CCDC-1483008 (compound **3l**).

4.3 COX inhibition assay

COX-1 and human COX-2 was determined using the fluorescence-based COX assay “COX Fluorescent Inhibitor Screening Assay Kit” (catalogue number 700100; Cayman Chemicals, USA) according to the manufacturer's instructions. All compounds were assayed in a concentration range of 0.01–100 μ M, as described elsewhere.⁴⁵ Two technical replicates were performed for each inhibitor concentration. The mean of these two values was used for estimating the IC₅₀ values using a nonlinear logistic regression fitting procedure (sigmoidal dose–response model) with Origin® Software. Exemplary, dose–response curves for valdecocib and **3a** are shown in the ESI† (Fig. S63).

Conflicts of interest

No potential conflicts of interest were disclosed. No prior subsequent publication.

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