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Novel Valdecoxib Derivates by Ruthenium-catalyzed 1,3-Dipolar Cycloaddition of Nitrile Oxides with Alkynes - Synthesis and COX-2 Inhibition Affinity.

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Ruthenium-Catalyzed 1,3-Dipolar Cycloaddition of Nitrile Oxides with Alkynes Builds Selective Valdecoxib-Derived Cyclooxygenase-2 Inhibitors.

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Abstract: A series of new valdecoxib-based cyclooxygenase-2 inhibitors has been synthesized in one step by 1,3-dipolar cycloaddition of benzonitrile oxides with aryl alkynes. The isomeric arrangement of the two aryl rings attached to the isoxazole can easily be steered by application of Ru(II)-catalysis, leading in most cases to exclusive formation of the 3,4-regioisomer. The 3,4-diaryl-substituted compounds possessing a small substituent (H and Me) displayed high COX-2 inhibition potency (IC_{50}=0.042–0.073 μM), which is in the same range as valdecoxib. The introduction of a fluorine substituent resulted in enhanced COX-2 affinity, making these compounds interesting candidates for the development of fluorine-18 labeled radiotracers.

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a widely used class of therapeutic agents for the treatment of inflammation, fever and pain.[1] The pharmacological effects of NSAIDs result from their capability to inhibit the enzyme family of cyclooxygenases (COX) .[2, 3, 4] The COX enzyme catalyzes the rate-limiting step of the biotransformation of arachidonic acid to prostanoids, and exists as two distinct isoforms, a constitutive form (COX-1) and an inducible form (COX-2).[5, 6] COX-1 derived prostaglandins control homeostatic functions, including gastric cytoprotection and hemostasis, while COX-2 is responsible for the synthesis of prostanoids involved in acute and chronic inflammatory states.[7] Inflammation is a common biological process shared by various diseases and elevated expression of COX-

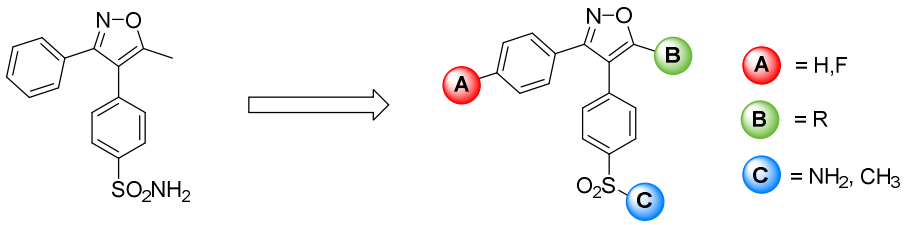
2 has been implicated in manifold pathological events, including rheumatoid arthritis, heart disease, stroke, and neurodegenerative disorders.[8, 9, 10, 11, 12]

Recently, it has become more and more obvious that COX-2 is also overexpressed in many human cancer entities and is assumed to be implicated in tumor inflammogenesis and hypoxia.[13, 14, 15, 16] In consequence, expression of COX-2 has attracted considerable attention as a diagnostic marker and therapeutic target in oncology.[17, 18, 19, 20, 21]

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.[22, 23, 24] 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-diarylisoxazole scaffold is a frequently recurring pharmacophore found in a variety of NSAIDs/Coxibs,[25] protein kinase inhibitors,[26] and hypertensive agents.[27] Valdecoxib [4-(5-methyl-3-phenyl-4-isoxazolyl)benzenesulfonamide] is one of the well-known examples of 3,4-diarylisoxazoles derivatives commercialized in 2000 for clinical use under the brand name Bextra®.[28, 29] However, owing to its adverse cardiovascular side effects and skin reactions it was withdrawn from the market in 2005.[30] On the other hand, parecoxib[31] (Dynastat®, *N*-[[4-(5-methyl-3-phenylisoxazol-4-yl)phenyl]sulfonyl]propanamide), which was designed to be a water-soluble (> 50 mg/mL in normal saline, pKa 4.9), parenterally safe prodrug form of valdecoxib (10 μmol/mL in normal saline, pKa 9.8), is still used for the management and treatment of acute pain.

However, valdecoxib is a very potent and selective inhibitor of COX-2 (IC_{50} = 5 nmol) with almost no inhibition of COX-1 (IC_{50} =150 μmol)[32] and represents an interesting lead structure for the development of, for example, potential radiotracers for the functional imaging of COX-2 via positron emission tomography (PET). In the case of radiotracers, among other properties the selectivity of a drug is of highest importance, and further pharmacological side effects may be disregarded, since only a few nanomoles of the drug are applied. Since we have an ongoing interest in imaging COX-2 functional expression *in vivo,* our efforts are focused on the development of novel radiolabeled COX-2 inhibitors as radioactive probes for the characterization of inflammatory and tumorigenic lesions.[33] With this background, we aimed at the synthesis of novel fluorine-bearing valdecoxib-based derivatives with varying substituents (Figure 1) as a gateway for a potential fluorine-18 radiolabeled PET radiotracer.

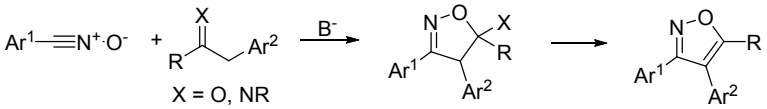
Figure 1. Valdecoxib as lead compound and design concept of novel derivatives



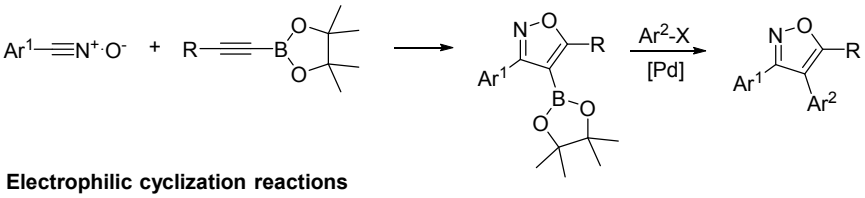
The phenylsulfonamide or methylsulfonyl group located in *para*-position at one of the phenyl substituents of the 3,4-diarylisoxazole interacts effectively with the COX-2 side pocket through slow tight-binding kinetics[34, 35] and should be generally preserved. Although several literature reports depict the synthesis of substituted isoxazoles, in the case of 3,4-diarylisoxazoles the question of selectivity in general is a crucial problem whose solution depends mostly on the availability of the starting materials.[36, 37] As displayed in Scheme 1, different protocols for the synthesis of valdecoxib and analogues have been described. Among them, the 1,3-dipolar cycloaddition of nitrile oxides with olefinic compounds followed by aromatization is often used.[38, 39, 40] Also, the 1,3-dipolar cycloaddition reaction of nitrile oxides with alkynyl boronates followed by Pd-mediated coupling reactions offer a regio-controlled access to valdecoxib and analogues.[41, 42, 43] In addition to cycloaddition-based methods, the electrophilic cyclization[44, 45] followed by cross-coupling reaction[46, 47] constitutes an interesting alternative for the synthesis of diarylisoxazoles. Furthermore, a concise synthesis of valdecoxib and derivatives starting from deoxybenzoin derivatives has also been reported.[28, 48] However, most of the above described synthetic methods involve the use of inconvenient or, from an industrial point of view, prohibitively unpleasant reagents such as butyl lithium, lithium diisopropylamide, diethyl chlorophosphate, and hexamethylphosphoramide. On the other hand, some of them suffer from lack of regioselectivity.[49] The 1,3-dipolar cycloaddition between benzonitrile oxides and phenylacetylenes can be used for direct access to diaryl-substituted isoxazoles. However, the control of the regiochemistry to obtain the 3,4-diarylsubstituted isomer is, in this case, a considerable challenge.[50] Nevertheless, an outstanding feature of the 1,3-cycloaddition of nitrile oxides with alkynes is that it delivers the 3,4-diarylisoxazoles in one step, making this approach very promising for radiotracer synthesis.

Scheme 1. Recent approaches used to synthesize 3,4-diarylisoxazoles

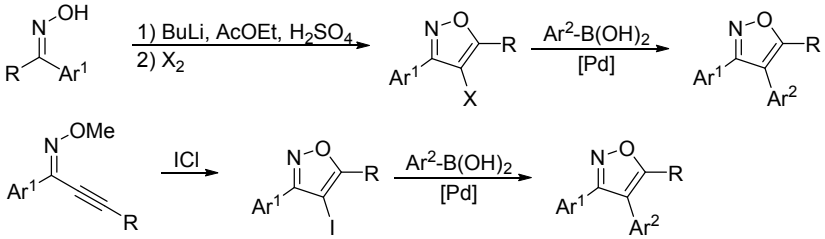
1,3-dipolar cycloaddition of nitrile oxide with olefinic compounds



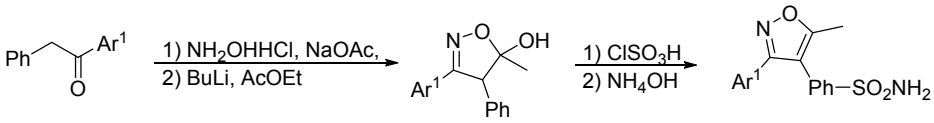
1,3-dipolar cycloaddition of nitrile oxide with alkynylboronates



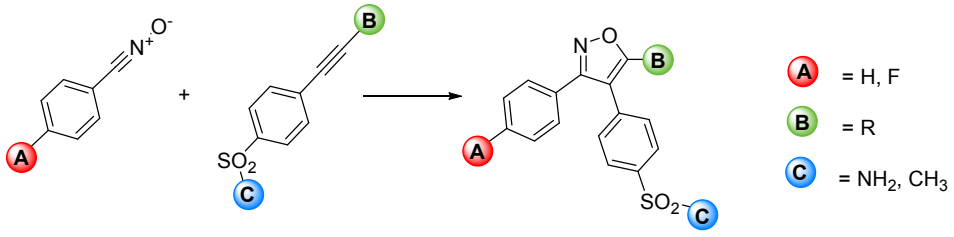
Electrophilic cyclization reactions



Condensation reactions



This work: 1,3-dipolar cycloaddition of nitrile oxide with alkynes



Accordingly, in this paper we describe the one step synthesis of a series of diaryl substituted isoxazoles, analogues to valdecoxib, by 1,3-dipolar cycloaddition of nitrile oxides with a panel of various substituted phenyl acetylenes. The SO_2Me or SO_2NH_2 group at one aryl ring as COX-2 pharmacophore should be preserved, while the other phenyl ring should, in the majority of cases, bear a fluorine substituent for optional fluorine-18 labeling. To determine their potential suitability, the COX-1 and COX-2 inhibitory activity of the new compounds was analyzed *in vitro* by using a fluorescence-based assay.

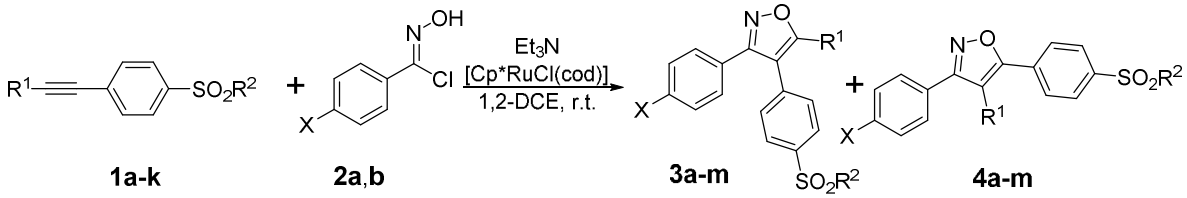
2. Chemistry

Although the cycloaddition of phenylacetylenes and benzonitrile oxides is the most direct route for accessing 3,4-diarylisoxazoles, this method is rarely used.[51] The reasons for this are simple: the non-catalyzed, thermal cycloaddition reactions of nitrile oxides with alkynes are neither chemo- nor regioselective. On the other hand, these reactions are elapsed by low yields as a consequence of the high reactivity of nitrile oxides (the propensity to dimerize of these compounds is well documented) and the low reactivity of the alkyne counterpart. In fact, even when thermal cycloaddition reactions of nitrile oxides with alkynes are successful, they favor the formation of the 3,5-disubstituted isomer. 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.[52, 53] Copper(I) acetylides have been reported to react regioselectively with nitrile oxides to generate 3,5-disubstituted isoxazoles.[54, 55] An extension of the 1,3-dipolar cycloaddition reaction of nitrile oxides and terminal or internal alkynes was recently described by Fokin.[56] They report on the use of ruthenium(II) complexes instead of copper(I) for the cycloaddition reaction, which led exclusively to the formation of the corresponding regiocomplementary 3,4-di- and 3,4,5-trisubstituted isoxazoles, respectively. Whilst the natural regioselectivity of the cycloaddition reaction provides isoxazoles with the 3,5-substitution pattern, Ru(II) complex-catalyzed 1,3-dipolar cycloaddition would provide a direct route for accessing 3,4-diarylsubstituted isoxazoles. We therefore decided to exploit ruthenium(II)-catalyzed 1,3-dipolar cycloaddition reactions for the construction of 3,4-diarylsubstitued isoxazoles, as potential COX-2 inhibitors. To evaluate the scope and limitations of this approach, activated and non-activated alkynes have been investigated.

For this purpose we first prepared various para-substituted phenyl acetylenes **1** (Table 1) possessing the SO_2R (R = NH_2, CH_3) pharmacophore for subsequent 1,3-dipolar cycloaddition reactions with nitrile oxides. The syntheses of a series of alkynes **1,** via Sonogashira coupling starting from 4-bromobenzenesulfonamide or 1-bromo-4-(methylsulfonyl)benzene, were accomplished by following modified literature procedures (for experimental details, see Experimental section/Supporting info). Since nitrile oxides are generally unstable, with a tendency to dimerization, we decided to synthesize the corresponding hydroximoyl chlorides **2** which are storable and can be easily transformed *in situ* to the nitrile oxide by treatment with Et_3N.

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

Table 1: Ruthenium-catalyzed reactions of nitrile oxides with alkynes.[a]



1a-k **2a,b** **3a-m** **4a-m**

	Alkyne R^1	Alkyne 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)	**4e** (0)
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)	**4g** (0)
1f	CHO	CH_3	**2a**	F	1 h	9:1	**3h** (57)	**4h** (0)
1g	CO_2Me	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)	**4l** (0)
1k	TMS	CH_3	**2a**	F	24 h	-	**3m** (0)	**4m** (0)

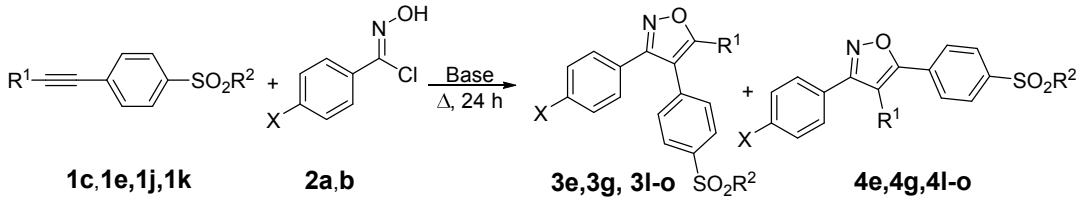
a) Reaction conditions: alkyne (1.0 mmol), hydroximoyl chloride (1.6 mmol), Et_3N (1.6 mmol), [Cp*RuCl(cod)] (15% mol) in 1.2-DCE at room temperature. b) Determined by HPLC and ^{1}H-NMR analysis. c) Isolated yield after purification by flash chromatography and recrystallization form ethyl ether.

In a first set of reactions, we employed the Ru(II)-catalyzed 1,3-dipolar cycloaddition reaction between hydroximoyl chlorides **2** and various phenyl acetylenes **1** to prepare regioselective isoxazoles **3** possessing a 3,4-diaryl substitution pattern. In accoedance with the literature, we chose [Cp*RuCl(cod)] as the most suitable ruthenium complex for this reaction. Briefly, alkynes **1** were combined with hydroximoyl chlorides **2** (1.6 eq.) in the presence of 15 mol% of [Cp*RuCl(cod)] and 1,6 equivalents of Et_3N and stirred at room temperature for the period of time indicated (Table 1). Terminal alkynes **1a** and **1b** reacted fast and HPLC and ^{1}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 high-field-shifted ^{1}H NMR signals (9.37 Hz-9.43 ppm) as typically observed for the 3,4-disubstitued isoxazoles. Internal electron-deficient alkynes (**1d**, **1f-1i**) were effective cycloaddition partners as well. For compounds **1d**, **1f** and **1i**, the thermal reaction conditions (heating to 80ºC) in the absence of the ruthenium catalyst were also

performed, and we observed exclusively the formation of the 3,5-diarylsubstituted regioisomers **4f**, **4h** and **4k**, respectively. By way of contrast the major 3,4-regioisomers **3h-k** were obtained when [Cp*RuCl(cod)] was used. The identity of isoxazoles **3f**, **3h-k** was established by ^{1}H NMR analysis-based comparison to the corresponding isomers **4f**, **4h-k**.

Unfortunately, the Ru(II)-catalyzed 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 the 3,5-diarylated isomers **4e**, **4g** and **4l-o** were found to be the major products. Unlike the Ru(II)-catalyzed reaction, thermal cycloadditions required drastic reaction conditions in an aprotic solvent at elevated temperature for several hours, and the desired 3,4-diaryl isoxazoles **3e**, **3g** and **3l-o** were obtained in significantly lower chemical yields (4-11%) compared to compounds synthesized under ruthenium catalysis (29-74%).

Table 2: Thermal 1,3-dipolar cycloaddition reactions of nitrile oxides with alkynes.



1c,**1e**,**1j**,**1k** **2a**,**b** **3e**,**3g**, **3l-o** **4e**,**4g**,**4l-o**

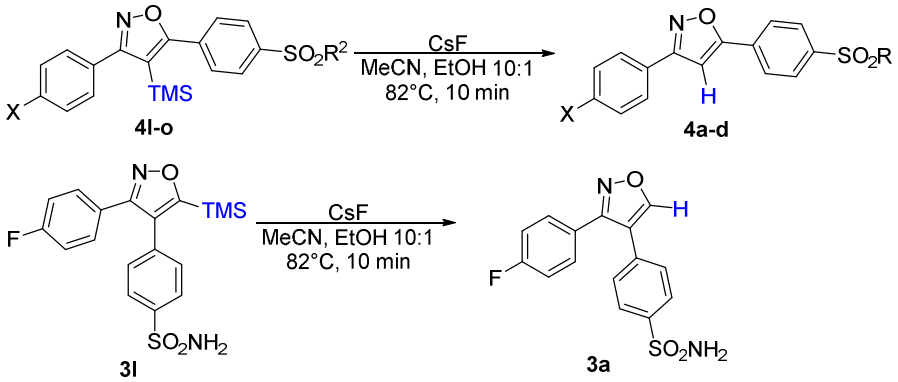
	Alkyne R^1	Alkyne R^2	Oxime	X	Reaction conditions	3:4 Ratio[a]	Yield 3 (%)[b]	Yield 4 (%)[b]
1c	CH_3	NH_2	**2a**	F	KF (5 eq.), 1,2-DME	37:63	**3e** (4)	**4e** (8)
1e	Cl	CH_3	**2a**	F	Et_3N (4 eq.), toluene	18:82	**3g** (8)	**4g** (37)
1j	TMS	NH_2	**2a**	F	KF (3 eq.), 1,2-DME	17:83	**3l** (11)	**4l** (59)
1k	TMS	CH_3	**2a**	F	KF (3 eq.), 1,2-DME	22:78	**3m** (7)	**4m** (66)
1j	TMS	NH_2	**2b**	H	KF (3 eq.), 1,2-DME	16:84	**3n** (5)	**4n** (65)
1k	TMS	CH_3	**2b**	H	KF (3 eq.), 1,2-DME	11:89	**3o** (4)	**4o** (70)

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

The determination of the regiochemistry of the cycloaddition by ^{1}H-NMR analysis was difficult in the case of the trimethylsilyl-substituted isoxazoles **3l**, **4l** – **3o**, **4o,** therefore the pure 3,5-diarylsubstituted regioisomers **4l-o** were subjected to protodesilylation to obtain the corresponding disubstituted isoxazoles **4a-d** having a proton instead of the trimethylsilyl group. Protodesilytation was performed with CsF in

good yield (74-90%) (Scheme 2) and ^{1}H-NMR analysis showed a characteristic signal between 7.77 Hz and 7.86 ppm, which is indicative of the proton in isoxazole rings containing a 3,5 substitution pattern. The minor 3,4-diarylsubstituted regioisomer **3l** was subjected to the same reaction conditions and afforded exclusively the compound **3a**. After the chromatographic separation of the regioisomers, **3a-o** and **4a-o** were obtained as solids.

Scheme 2. Protodesilylation of isoxazoles **4l-o** and **3l**.



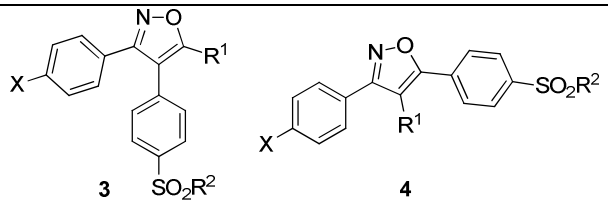
3. Results and discussion

Structure-activity relationship (SAR) studies have revealed that tricyclic compounds possessing two vicinal aryl moieties on the central heterocyclic ring system represent one major class of selective COX-2 inhibitors.[57] Moreover, the SO_2R pharmacophore at the *para*-position of one of the aryl rings has been shown to frequently confer optimal COX-2 selectivity and potency.[24] In most cases the 1,2-diaryl substitution pattern results in high affinity towards COX-2; however, examples of diaryl-substituted isoindole or pyrimidine derivatives showing an unusual 1,3-substitution pattern for the aryl rings, have also been reported to be highly affine towards COX-2.[58]

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 3,4-diaryl-substituted isoxazoles **3** and 3,5-diaryl-substituted isoxazoles **4**. Compounds **3** possessing the preferred two vicinal aryl moieties, were successfully obtained through Ru(II) complex-catalyzed reaction. This class of compounds is structurally related to traditional selective COX-2 inhibitors consisting of a heterocyclic central ring scaffold with two vicinal aryl substitutents.[55] On the other hand, compounds **4** exhibit a comparable molecule geometry based on 1,3-diaryl-substituted heterocycles. To determine the resulting combined different steric and electronic effects upon COX-1 and COX-2 inhibitory potency and COX

isoenzyme selectivity, all isolated compounds were subjected to an *in vitro* COX Fluorescent Inhibitor Screening Assay (item Nr 700100, Cayman Chemicals, USA). 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 3,4- and 3,5-diaryl-substituted isoxazoles, are summarized in Table 3.

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]	cLogP[c]
valdecoxib	CH_3	NH_2	H	> 100 (150)[d]	0.050 (0.005)[d]	> 2000	2.73
3a	H	NH_2	F	> 100	0.042	> 2400	2.68
3b	H	NH_2	H	> 100	0.048	> 2100	2.51
3c	H	CH_3	F	> 100	0.057	> 1800	2.85
3d	H	CH_3	H	> 100	0.247	> 400	2.69
3e	CH_3	NH_2	F	> 100	0.073	> 1400	2.90
3f	CF_3	CH_3	F	> 100	8.145	> 12	3.99
3g	Cl	CH_3	F	10.07	0.351	28.7	3.65
3h	CHO	CH_3	F	> 100	13.777	> 7	2.88
3i	CO_2Me	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	> 110	5.19
3m	TMS	CH_3	F	> 100	1.87	> 54	5.36
3n	TMS	NH_2	H	> 100	0.13	> 770	5.02
3o	TMS	CH_3	H	> 100	4.17	23.9	5.20
4a	H	NH_2	F	> 100	81.2	> 1.2	2.87
4b	H	NH_2	H	> 100	10.79	> 9.3	2.71
4c	H	CH_3	F	> 100	> 100	-	3.05
4d	H	CH_3	H	> 100	13.55	> 7.4	2.88
4l	TMS	NH_2	F	> 100	38.16	> 2.6	5.19
4m	TMS	CH_3	F	> 100	> 100	-	5.36
4n	TMS	NH_2	H	> 100	11.05	> 9.0	5.02
4o	TMS	CH_3	H	> 100	> 100	-	5.20

a) Values are means of two determinations. b) *In vitro* COX-2 selectivity index (IC_{50} COX-1/IC_{50} COX-2).
c) cLogP values have been calculated based on molinspiration predictions (www.molinspiration.com) d) Literature value. Ref. 30.

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 IC_{50} value of 0.05 μM, a value that is by a factor of 10 higher than reported (0.005 μM)[30]. This difference is owed to the fact that in this work a fluorescence-based assay was used as oppose to the ^{3}H-based radioligand assay from the literature. However, useful information is provided by comparing the results of all compounds measured in this assay.

The first series of compounds (**3a–o**) containing the common vicinal substitution pattern of the aryl moieties showed interesting results with reagard 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** (IC_{50} COX-1 = 10 μM).

Regarding the inhibition against COX-2, a more differentiated picture within compounds **3a–o** was observed. Compounds **3a-3e** containing an H or CH_3 substituent displayed a nanomolar COX-2 inhibitor potency (0.042–0.247 μM), compound **3g** with a chloro-atom and **3l** and **3n** bearing a TMS-substituent showed submicromolar affinity (0.13 – 0.93 μM). Compounds **3i-k** containing carbonyl substituents CO_2Me, COMe and COPh showed no inhibitory potency against COX-2 (>100 μM) and compounds **3f** and **3h** (containing electron-withdrawing groups CF_3 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 IC_{50} values in a micro molar range (1.87 μM to 13.777 μM).

In conclusion, compounds **3a-c** and **3e** containing an H or CH_3 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 CH_3 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 compound **3a** the most COX-2 selective compound within all studied isoxazole containing compounds, 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 CF_3, COR, and TMS group in compounds **3f**, **3h-k**, **3m** and **3o,** decrease 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-d** indicated that the order of COX-2 inhibitory potency was F > H (compare **3a** *vs*. **3b** and **3c** *vs*. **3d**). The same observation can be made for one pair of the trimethylsilyl-substituted isoxazoles **3m** *vs.* **3o** but not for **3l** *vs.* **3n**. The order as regards COX-2 selectivity followed the same order (F > H). This result suggests that the presence of electron-withdrawing groups like fluorine favors selective and potent inhibition of COX-2. Several reports in the literature cocerning the study of tricyclic compounds possessing two vicinal aryl moieties on the central heterocyclic ring system support this observation.[55] This finding, that a fluorine substituent enhances the COX-2 affinity and selectivity of the 3,4-

diarylisoxazole moiety, is an interesting item for the development of, in particular, fluorine-18 based radiotracers.

Compounds **3a-d** and **3l-o** possess the typical SO_2R COX-2 relevant substituent in the *para*-position of one of the aryl rings attached to the central isoxazole unit with R = NH_2 or CH_3. Comparison of the IC_{50} values for these compounds suggests that compounds **3a**, **3b**, **3l**, and **3n** bearing a SO_2NH_2 group as pharmacophore are more selective and potent COX-2 inhibitors than the compounds **3c**, **3d**, **3m**, and **3o** with a SO_2Me 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 (IC_{50} > 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 favorable 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 pattern 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 .

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 favorable lipophilicity was calculated (cLogP = 2.51-2.85); however, the replacement of the hydrogen atom with different substituents, as done in compounds **3f-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 COPh- and TMS-substituted compounds **3k-o** would represent unfavorable 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-diarylisoxazoles interesting candidates for further development as potential fluorine-18 labeled COX-2 inhibitors.

4. Conclusion

We have prepared a series of new compounds on the basis of a valdecoxib lead structure displaying a central isoxazole scaffold, with two aryl substituents and one additional substituent on the heterocycle, as novel selective COX-2 inhibitors. The synthesis was performed in one step by 1,3-dipolar cycloaddition of benzonitrile oxides with aryl alkynes in yields of up to 74%. The geometric arrangement of the two aryl rings attached to the isoxazole moiety can easily be steered by application of Ru(II)-catalysis, leading in most cases to exclusive formation of the 3,4-regioisomer. In general, compounds having a vicinal diaryl substitution pattern showed high 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 (H and Me) displayed significantly higher COX-2 inhibition potency than was determined for compounds containing bulky or electron-withdrawing groups. The highest COX-2 inhibition potency was determined for compounds **3a-c** and **3e** with IC_{50} values of 0.042–0.073 μM, being in the same range as that found in valdecoxib (0.050 μM). Interestingly the introduction of a fluorine substituent resulted in enhanced COX-2 affinity. This circumstance along with their ease in synthesis through versatile Ru(II)-catalyzed click chemistry make these compounds interesting candidates for the further development of potent fluorine-18 labeled radiotracers for imaging COX-2 by PET, e.g., by application of 1,3-dipolar cycloaddition with 4-[^{18}F]fluorobenzonitrile oxide as [^{18}F]fluoride bearing moiety.

Associated Content

Supporting Information

Synthesis procedures, NMR data and spectra of all new synthesized compounds are available free of charge via the Internet at http://pubs.acs.org.

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Disclosure of potential conflicts of interest

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

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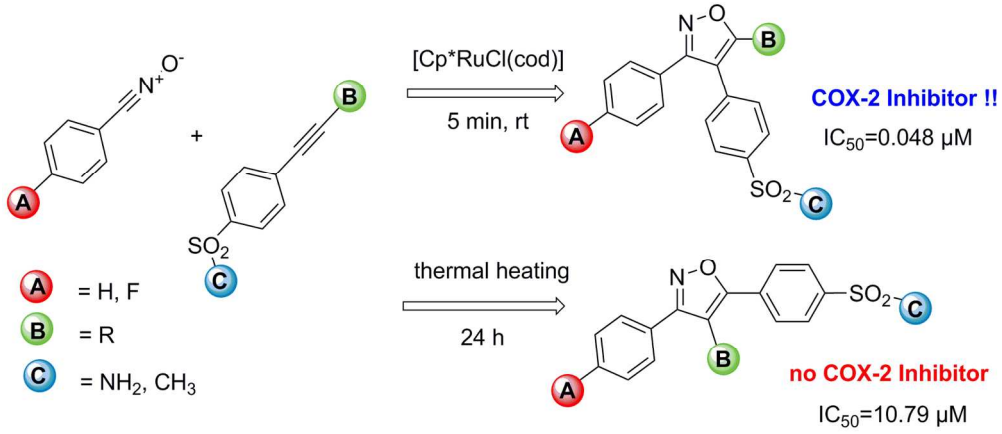

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