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Synthesis and evaluation of 1,5-diaryl-substituted tetrazoles as novel selective cyclooxygenase-2 (COX-2) inhibitors

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ABSTRACT

A series of 1,5-diaryl-substituted tetrazole derivatives was synthesized via conversion of readily available diaryl amides into corresponding imidoylchlorides followed by reaction with sodium azide. All compounds were evaluated by cyclooxygenase (COX) assays in vitro to determine COX-1 and COX-2 inhibitory potency and selectivity. Tetrazoles **3a–e** showed IC_{50} values ranging from 0.42 to 8.1 mM for COX-1 and 2.0 to 200 μM for COX-2. Most potent compound **3c** (IC_{50} (COX-2) = 2.0 μM) was further used in molecular modeling docking studies.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most widely used therapeutics worldwide for the treatment of inflammation, pain, fever, and for the prevention of thrombosis.

The mechanisms of their pharmacological effects are based on inhibition of the catalytic domain of cyclooxygenase enzymes by sterically hindering the entrance of arachidonic acid as their physiological binder. This results in a reduced production of prostaglandins and thromboxanes, which contribute as important autocrine and paracrine mediators in many physiologic and pathophysiologic responses.[1–4]

Cyclooxygenases (COXs) are membrane-bound heme proteins which exist in two distinct isoforms, a constitutive form (COX-1) and an inducible form (COX-2). COX-1 and COX-2 share the same substrates, produce the same products and catalyze the same reaction using identical catalytic mechanisms. The X-ray crystal structure of both enzymes suggests that the proteins are very similar in their tertiary conformation.[5,6] The amino acids which serve as substrate binding pocket and catalytic site are nearly identical in both enzymes. The COX-1 enzyme is expressed in resting cells of most tissues, functions as a housekeeping enzyme, and is responsible for maintaining homeostasis (gastric and renal integrity) and normal production of eicosanoids. COX-2 is predominantly found in brain and kidney while being virtually absent in most other tissues. However, COX-2 expression is significantly upregulated as part of various acute and chronic inflammatory conditions. It is also well documented that COX-2 is overexpressed in numerous human cancers such as colorectal, gastric, and breast cancer.[7]

Recognition of the importance of COX-2 in inflammation and carcinogenesis has prompted the synthesis of various COX-2 selective inhibitors over the last two decades.

The close structural similarities of both COX isoforms represent a formidable challenge for the development of selective COX-2 inhibitors. In COX-1, the space of the selectivity pocket is reduced due to the presence of Ile523, while in COX-2 the presence of Val523. The presence of valine in COX-2 induces a conformational change, thereby forming an additional hydrophobic secondary internal pocket protruding off the primary binding site which is absent in COX-1.[8]

Over the last two decades a large number of compounds have been synthesized and investigated for selective COX-2 inhibition. Many selective COX-2 inhibitors belong to the class of diaryl-heterocyclic compounds. A common structural feature is the presence of two vicinal aryl rings attached to a central five- or six-membered carbocyclic or heterocyclic motif. Prominent examples including Celecoxib, Rofecoxib, Valdecoxib, Etoricoxib, and SC57666 are depicted in Figure 1.[9]

Selective COX-2 inhibitors as shown in Figure 1 demonstrate that a broad variety of 1,2-diaryl-substituted carbocycles and heterocycles containing a sulfonamide or methylsulfonyl pharmacophore are acceptable for binding to the cyclooxygenase active site in the COX-2 enzyme. Recent reviews on the current status of COX-2 inhibitors have further confirmed the flexibility of

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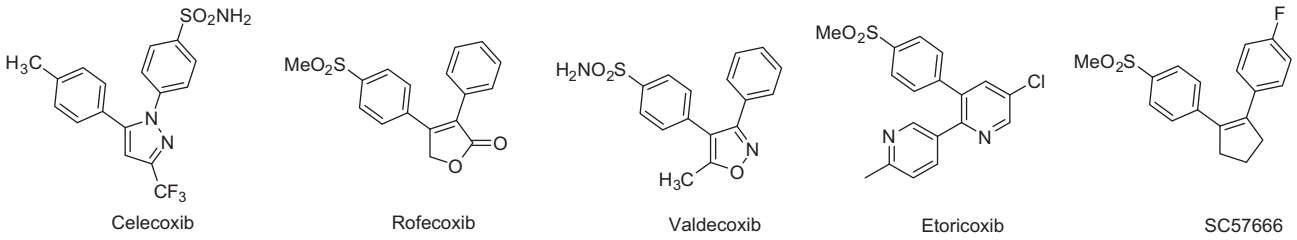

Figure 1. Chemical structures of selective COX-2 inhibitors.

various carbocycles or heterocycles as central core motifs for selective and potent COX-2 binding.[10]

Recently, our group has reported on the synthesis and characterization of various 1,5-diaryl 1,2,3-triazoles as a novel class of potent and selective COX-2 inhibitors.[11] As an extension to this work and our continuing interest in the design and synthesis of COX-2 inhibitors as molecular probes for molecular imaging of COX-2 expression in vivo, we want to explore the effect of various 1,2-diaryl-substituted tetrazoles upon COX-2 binding. To the best of our knowledge, 1,2-diaryl-substituted tetrazoles have not yet been studied as COX-2 inhibitors. Herein we describe the synthesis, in vitro COX-1 and COX-2 inhibitory activity, and molecular modeling docking experiments of a series of 1,5-diaryl-substituted tetrazoles as novel class of selective COX-2 inhibitors.

1,5-Disubstituted tetrazoles have found many applications in medicinal chemistry and drug development as important *cis*-amide isosteres. Important examples include glucokinase activators,[12] NAD(P)H oxidase inhibitors,[13] anti-migraine agents,[14] and hepatitis C virus serine protease NS3 inhibitors.[15]

Many different preparative methods for 1,5-disubstituted tetrazoles have been developed. Popular and frequently used methods consist of (1) reactions of imidoylchlorides with various azide sources,[16,17] (2) reactions of oximes, nitriles and nitrilium triflates with azides,[18] and (3) reactions of amidrazones with dinitrogen tetroxide or nitrous acid. Other methods involve various alkylation reactions of 5-substituted tetrazoles.[19]

1,5-Diaryl-substituted tetrazoles **3a–e** as novel COX-2 inhibitors were prepared based on the reaction of imidoylchlorides with sodium azide as the key step within the reaction sequence. The synthesis route is outlined in Figure 2.

The synthesis of tetrazoles **3a–e** commenced with the conversion of commercially available 4-(methylsulfonyl)benzoic acid **1** into amides **2a–e** through treatment of **1** with 1,1′-carbonyldiimidazole (CDI) to form the corresponding imidazolide derivative in situ. Imidazolide formation can easily be monitored by evolution of CO_2, which is the driving force of the reaction. Subsequent reaction of reactive imidazolide intermediate with various *para*-substituted anilines (R = H, Me, OMe, F, Cl) afforded desired amides **2a–e** in high chemical yields of 90–97%. Amides **2a–e** were treated with thionylchloride under reflux to prepare corresponding imidoylchloride intermediates. After removal of the excess of $SOCl_2$ and washing the crude reaction mixture with hexane, the residue was re-dissolved in dry DMF. Treatment of imidoylchlorides with a two-fold excess of NaN_3 gave desired 1,5-diaryl-substituted tetrazoles **3a–e** in good to very good 70–87% yield after purification using column chromatography. Tetrazoles **3a–e** were obtained as readily crystalline compounds, which were fully characterized using ^{1}H NMR, ^{13}C NMR and high resolution mass spectrometry.[20]

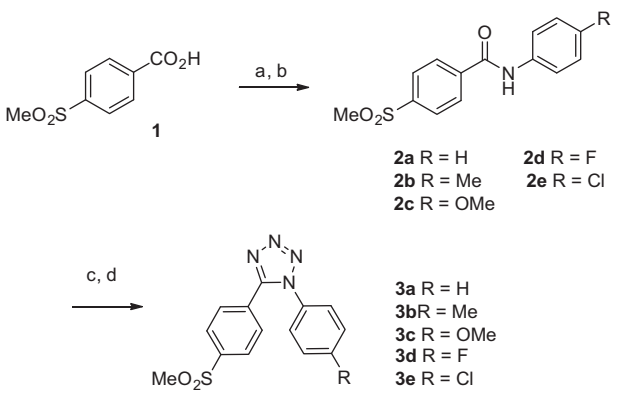

Figure 2. Reagent and conditions: (a) CDI, THF; (b) 4-substituted anilines; (c) $SOCl_2$, reflux, 2 h; (d) NaN_3, DMF, room temperature, 24 h (70–87%).

Tetrazoles **3a–e** were evaluated in a fluorescence-based COX assay[21] to determine the different steric and electronic effects upon COX-1 and COX-2 inhibitory potency and selectivity. Compounds **3a–e** possess a tricyclic scaffold containing a central heterocyclic ring system with two vicinal aryl substituents as typically found in numerous selective and potent COX-2 inhibitors. One of the aryl rings bears a methylsulfonyl (SO_2Me) group, which was shown to be an important pharmacophore to confer COX-2 selectivity and potency. Thus, 1,5-diaryl-substituted tetrazoles **3a–e** are structurally related to several selective COX-2 inhibitors depicted in Figure 1, and to our previously prepared series of 1,5-diaryl-substituted 1,2,3-triazoles.[11] Potent and selective COX-2 inhibitor Celecoxib was used as reference compound in the COX assay. The determined enzyme inhibitory data, the respective COX-2 selectivity index (COX-2 SI), and calculated lipophilicity values (Log $P_{o/w}$) are summarized in Table 1.

In our enzyme inhibitory assay, Celecoxib showed high COX-2 inhibitory potency and selectivity with IC_{50} values of 0.03 μM for COX-2 and 9.4 μM for COX-1, which is in the same range as previously reported in the literature. All prepared tetrazoles **3a–e** are selective COX-2 inhibitors as reflected by the COX-2 SI values ranging from 4.5 (**3e**) to 237 (**3d**).

Compounds **3c** and **3d** showed high COX-2 SI values of 210 and 237, respectively, which is in the same order of magnitude as determined for reference compound Celecoxib (COX-2 SI = 313). However, inhibitory potency of all compounds **3a–e** is much lower

Table 1
COX-1 and COX-2 enzyme inhibitory data

	R	IC_{50}[a] (μM)		COX-2 SI[b]	Log $P_{o/w}$[c]
		COX-1	COX-2		
Celecoxib		9.4	0.03	313	3.0
3a	H	3700	200	18.5	2.3
3b	Me	8100	45	180	2.8
3c	OMe	420	2.0	210	2.2
3d	F	640	2.7	237	2.4
3e	Cl	500	110	4.5	2.9

[a] Values are means of two determinations.
[b] In vitro COX-2 selectivity index (IC_{50} COX-1/IC_{50} COX-2).
[c] Log $P_{o/w}$ values have been calculated based on ACDLabs predictions.

compared to Celecoxib, ranging from 2.0 μM for compound **3c** (R = OMe) to 200 μM for compound **3a** (R = H). The determined IC_{50} values of all tetrazoles are also significantly lower in direct comparison to our previously prepared set of 1,4-substituted 1,2,3-triazoles as novel selective and potent COX-2 inhibitors, which displayed IC_{50} values in the submicromolar range (0.03–0.36 μM).[11] Compound **3c** (R = OMe) is the most potent COX-2 inhibitor possessing the lowest IC_{50} value (2.0 μM) within the series of tetrazoles. This is about 10 times higher compared with the corresponding OMe-substituted analog within the 1,2,3-triazole series (IC_{50} = 0.17 μM).[11] However, tetrazole compound **3c** shows much higher selectivity towards COX-2 (COX-2 SI = 210) compared to the corresponding 1,2,3-triazole compound (COX-2 SI = 4.9).[11] Thus, changing the 1,2,3-triazole central heterocycle to a tetrazole heterocycle seems to have a detrimental effect upon COX-2 inhibitory potency but enhances COX-2 selectivity.

Within the series of all studied tetrazoles, compounds **3c** and **3d** containing an electron-donating 4-OMe and a strongly electron-withdrawing 4-F group showed the highest COX-2 potency and selectivity. No systematic effect of the chemical nature of various *para*-substituents (R = H, Me, OMe, F, Cl) within tetrazoles **3a–e** upon COX-2 selectivity and inhibitory potency profile could be observed.

Replacement of the central 1,2,3-triazole ring with a tetrazole heterocyle resulted in a slight increase of lipophilicity as expressed by the calculated Log *P* values of 2.2–2.9 for tetrazole-containing compounds **3a–e**. Previously reported 1,2,3-triazoles containing a comparable substitution pattern showed Log *P* values of 1.7–2.4.[11]

The binding of the most potent tetrazole compound **3c** within the active site of the COX-2 enzyme was further studied through docking experiments using the known crystal structure of active site of murine COX-2.[22] The result is depicted in Figure 3.

Compound **3c** binds to binding pocket of COX-2 through the formation of various distinct H-bonds and hydrophobic interaction with selected amino acid residues of the binding pocket. The docking of compound **3c** into the COX-2 binding pocket resulted in a calculated binding free energy $\Delta G = -10.01$ kcal/mol.

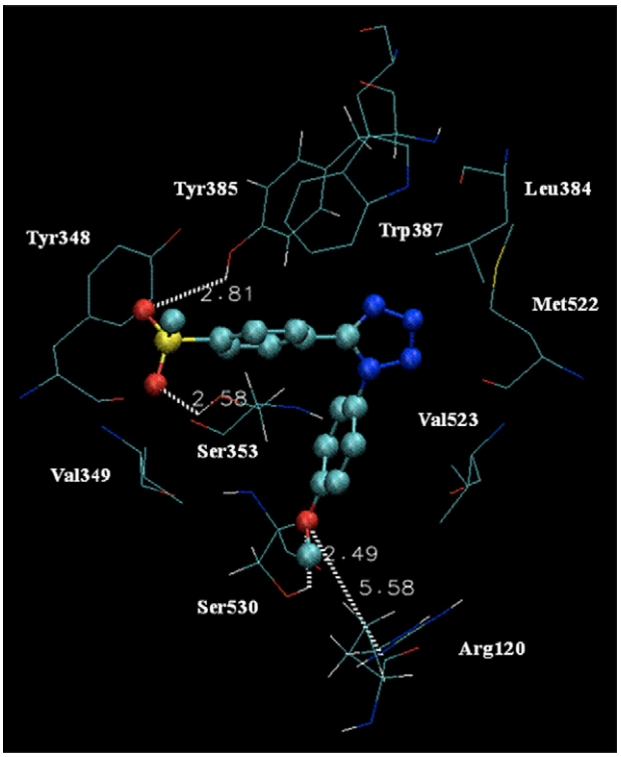

Figure 3. Docked position of tetrazole **3c** with the active site of murine COX-2. Hydrogen atoms have been removed to improve clarity except for amino acids involved in hydrogen bonding.

The presence of Val523 in the COX-2 isoenzyme results in the formation of a secondary hydrophobic binding pocket which accommodates the 4-MeO-phenyl substituent of compound **3c**. The oxygen of the methoxy-group is involved in a H-bond with the hydroxyl-group of Ser530. The measured distance of 2.49 Å is in the range typically found for H-bonding interactions. Compound **3c** is further stabilized in the binding pocket through two additional H-bonds between the oxygen atoms of the methylsulfonyl group of the other phenyl ring with the 4-OH-group of Tyr385 and the OH-group of Ser353. The measured distances of 2.81 Å and 2.58 Å agree with H-bonding interactions. Trp387, Leu384, Met522, and Val523 form a hydrophobic environment within the COX-2 binding pocket which surrounds both phenyl rings attached to the central tetrazole moiety of compound **3c**. The found H-bonding interaction of the OMe-group in **3c** with Ser530, and to a lesser extent with the guanidine group of Arg120 (distance = 5.58 Å), would also explain the observed high potency of compound **3d** (R = F) towards COX-2 (IC_{50} = 2.7 μM) within the series of compounds studied (Table 1). Fluorine is a very strong H-bond acceptor capable of forming comparable H-bonding interactions with Ser530 and Arg120 as shown for compound **3c**.

The found H-bonding and hydrophobic interactions of the docking experiment are in good agreement with the determined COX-2 inhibitory activity of compound **3c** and they may also provide a reasonable explanation for the comparable inhibitory activity of compound **3d**.

In summary, we have prepared and evaluated a series of 1,5-diaryl-substituted tetrazoles as novel selective cyclooxygenase-2 (COX-2) inhibitors. However, the determined COX-2 inhibitory potency of compounds **3a–e** is almost an order of magnitude lower in comparison with our previously prepared 1,4-diaryl substituted 1,2,3-triazoles.[11]

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20. *5-(4-(Methylsulfonyl)phenyl)-1-phenyl-1H-tetrazole* ***3a***: mp 174.5 °C; ^{1}H NMR (DMSO-d_6) δ 8.03 (d, J = 8.4 Hz, 2H), 7.79 (d, J = 7.8 Hz, 2H), 7.66–7.60 (m, 5H), 3.28 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 152.6, 142.9, 133.7, 130.8, 129.96, 129.94, 128.3, 127.4, 126.0, 43.0; HR-MS (m/z) [M+Na]$^+$ calcd for $C_{14}H_{12}N_4NaO_2S$, 323.0573; found 323.0575.
5-(4-(Methylsulfonyl)phenyl)-1-(p-tolyl)-1H-tetrazole ***3b***: mp 179.3 °C; ^{1}H NMR (DMSO-d_6) δ 8.12 (d, J = 7.8 Hz, 2H), 7.88 (d, J = 8.4 Hz, 2H), 7.57 (d, J = 8.4 Hz, 2H), 7.50 (d, J = 7.8 Hz, 2H), 3.37 (s, 3H), 2.44 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 152.6, 142.9, 140.8, 131.3, 130.4, 129.9, 128.4, 127.4, 125.8, 43.0, 20.8; HR-MS (m/z) [M+Na]$^+$ calcd for $C_{15}H_{14}N_4NaO_2S$, 337.0730; found 337.0729.
1-(4-Methoxyphenyl)-5-(4-(methylsulfonyl)phenyl)-1H-tetrazole ***3c***: mp 189.5 °C; ^{1}H NMR (DMSO-d_6) δ 8.03 (d, J = 9.0 Hz, 2H), 7.79 (d, J = 9.0 Hz, 2H), 7.54 (d, J = 9.0 Hz, 2H), 7.13 (d, J = 9.0 Hz, 2H), 3.84 (s, 3H), 3.27 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 160.6, 152.7, 142.9, 129.8, 128.4, 127.6, 127.4, 126.4, 115.0, 55.6, 43.0; HR-MS (m/z) [M+Na]$^+$ calcd for $C_{15}H_{14}N_4NaO_3S$, 353.0679; found 353.0680.
1-(4-Fluorophenyl)-5-(4-(methylsulfonyl)phenyl)-1H-tetrazole ***3d***: mp 205.0 °C ^{1}H NMR (DMSO-d_6) δ 8.04 (d, J = 8.4 Hz, 2H), 7.79 (d, J = 8.4 Hz, 2H), 7.70 (dd, $J_{H,H}$ = 8.7 Hz, $^4J_{F,H}$ = 4.5 Hz, 2H), 7.48 (dd, $J_{H,H}$ = 8.7 Hz, $^3J_{F,H}$ = 8.7 Hz, 2H), 3.28 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 162.9 (d, $J_{F,C}$ = 249 Hz), 152.8, 142.9, 130.1 (d, $^4J_{F,C}$ = 3.6 Hz), 129.9, 128.6 (d, $^3J_{F,C}$ = 9.3 Hz), 128.2, 127.5, 117.01 (d, $^2J_{F,C}$ = 23 Hz), 43.1; HR-MS (m/z) [M+Na]$^+$ calcd for $C_{14}H_{11}FN_4NaO_2S$, 341.0479; found 341.0481.
1-(4-Chlorophenyl)-5-(4-(methylsulfonyl)phenyl)-1H-tetrazole ***3e***: mp 207.7 °C; ^{1}H NMR (DMSO-d_6) δ 8.05 (d, J = 7.8 Hz, 2H), 7.80 (d, J = 9.0 Hz, 2H), 7.70 (d, J = 9.0 Hz, 2H), 7.65 (d, J = 8.4 Hz, 2H), 3.28 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 152.7, 143.0, 135.5, 132.6, 130.0, 129.2, 128.2, 127.9, 127.5, 43.1; HR-MS (m/z) [M+Na]$^+$ calcd for $C_{14}H_{11}ClN_4NaO_2S$, 357.0183; found 357.0183.
21. *In vitro cyclooxygenase (COX) inhibition assay*: The ability of compounds **3a–e** and Celecoxib to inhibit ovine COX-1 and recombinant human COX-2 was determined using a COX fluorescence inhibitor assay (catalog number 700100, Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's assay protocol. Compounds **3a–e** were assayed in concentrations ranging from 10^{-9} M to 10^{-3} M. PRISM5 software was used for the calculation of IC_{50} values.
22. The crystal structure of COX-2 inhibitor diclofenac bound to murine COX-2 enzyme was obtained from the RCSB Protein Data Bank (PDB identifier 1PXX). For the docking studies we used only chain A of 1PXX. Docking of compound **3c** was performed using AutoDock 4. The following protocol was used for the docking studies: application of the Lamarckian genetic algorithm with 150 individuals in the population; a maximum of 25×10^6 energy evaluations; a mutation rate of 0.02; a crossover rate of 0.80, and the elitism value of 1. For the local search, pseudo-Solis and Wets algorism was applied using a maximum of 300 iterations per local search. The probability of performing local search on individuals in the populations was set to 0.6. The maximum number of consecutive successes or failures before doubling or halving the local search step was set to 4. 100 independent docking runs were performed. Results differing by 2.0 Å in positional root–mean–square deviation were clustered.