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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 4483–4487

Design and synthesis of new water-soluble tetrazolide derivatives of celecoxib and rofecoxib as selective cyclooxygenase-2 (COX-2) inhibitors

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Received 18 February 2006; revised 20 May 2006; accepted 12 June 2006
Available online 27 June 2006

Abstract—In an attempt to prepare a new water-soluble, parenteral COX-2 inhibitor, rofecoxib (**9**) and celecoxib (**13**) analogues were designed and synthesized for evaluation as selective cyclooxygenase-2 (COX-2) inhibitors with in vivo anti-inflammatory activity. In this experiment, respective SO_2Me and SO_2NH_2 hydrogen-bonding pharmacophores were replaced by a tetrazole ring. Molecular modeling (docking) studies showed that the tetrazole ring of these two analogues (**9** and **13**) was inserted deep into the secondary pocket of the human COX-2 binding site where it undergoes electrostatic interaction with Arg^{513}. The rofecoxib (**9**) and celecoxib (**13**) analogues exhibited a high in vitro selectivity (**9**, COX-1 IC_{50} = 3.8 nM; COX-2 IC_{50} = 1.8 nM; SI = 2.11; **13**, COX-1 IC_{50} = 4.1 nM; COX-2 IC_{50} = 1.9 nM; SI = 2.16) relative to the reference drug celecoxib (COX-1 IC_{50} = 3.7 nM; COX-2 IC_{50} = 2.2 nM; SI = 1.68) and also showed high aqueous solubility at pH higher than 7 and good anti-inflammatory activity in a carrageenan-induced rat paw edema assay. However, **9** and **13** had no significant damage on gastric mucosa.

Considering the number of surgeries, safe management of pains associated with surgery is a very important issue.[1,2] In the management of severe or moderately severe pain, parenteral treatment is preferred because of the rapid onset of action. Recent surveys have shown that postoperative pain is considered poorly managed, due to side-effect limitations of available injectable medications, such as opioids and nonsteroidal anti-inflammatory drugs (NSAIDs). In this regard, the most used nonnarcotic analgesic for these indications, ketorolac **1**, has been associated with a reduced risk of myocardial infarction among hospitalized patients attributed to its antiplatelet properties. Nevertheless, appearance of significant side effects limits its use (see Fig. 1).[3–5]

Inhibition of cyclooxygenase (COX), one of the key enzymes involved in the metabolism of arachidonic acid to prostaglandins, is the main target for NSAIDs. At the beginning of the 1990s two COX isoforms were discovered:[6] one (COX-1) constitutively present in many tissues such as stomach, kidney, and platelets, and the other (COX-2) cytokine-inducible and expressed mainly in a wide range of inflammatory cells. This scenario led to the recognition that selective COX-2 inhibitors could provide anti-inflammatory agents devoid of the undesirable effects associated with classical, nonselective NSAIDs.[7] Rofecoxib (**2**)[8] and celecoxib (**3**)[9] were the first COX-2 inhibitors to reach the market, followed by valdecoxib[10] and etoricoxib.[11] 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. For example, the clinical use of rofecoxib and valdecoxib was recently

Keywords: Cyclooxygenase (COX-2) inhibitors; Celecoxib; Parecoxib; Tetrazole; Parenteral.
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doi:10.1016/j.bmcl.2006.06.032

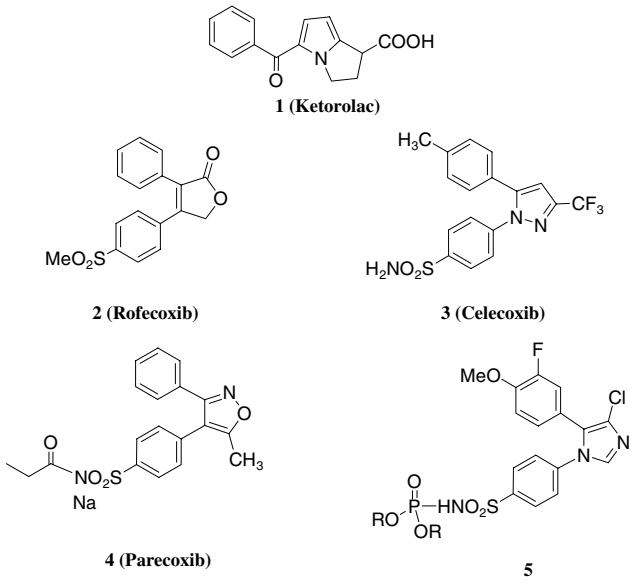


Figure 1. Representative examples of selective COX-2 inhibitors.

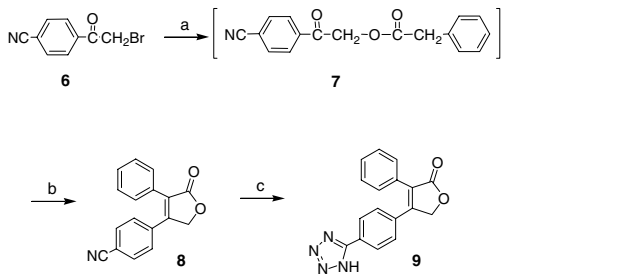
terminated due to adverse cardiovascular side effects associated with their use.[12,13] Parecoxib **4** (water-soluble prodrug of valdecoxib) is the only parenteral COX-2 inhibitor which has been marketed. Hence, design of new COX-2 inhibitors for parenteral treatment of acute pain is much required.

In general, COX-2 inhibitors of the diarylheterocycle class such as **2** and **3** possess modest aqueous solubility. This physicochemical characteristic restricts the dosing options available for this class of drug. In order to develop a COX-2 inhibitor for parenteral administration, all the attempts had been to prepare a prodrug of a sulfonamide-based inhibitor including base-treated acyl sulfonamide **4** and phosphoramidate derivatives **5**.[4,14]

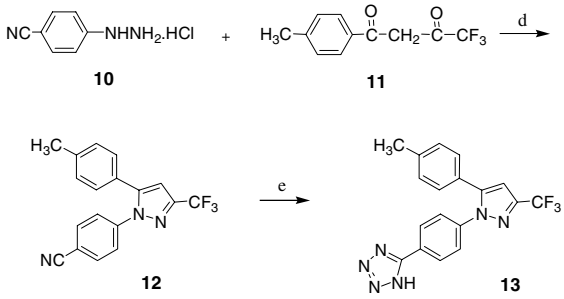
Recently, it has been reported that the SO_2NH_2 and SO_2Me hydrogen-bonding pharmacophore present in many selective COX-2 inhibitors could be replaced by dipolar azido group as a bioisoester.[15] A search for another bioisoester of SO_2NH_2 and SO_2Me which is water soluble envisaged tetrazolide ion as an interesting approximation, since high water solubility seemed to be achievable through the formation of alkaline salts, due to the predicted pK_a value of 5–6 for the NH group.

As a part of our ongoing program to design novel selective COX-2 inhibitors,[16] here we describe our results on the investigation of the use of tetrazolide salt as a new water-soluble pharmacophore for further development in the parenteral treatment of acute pain.

The synthetic reactions used for the synthesis of 4-[4-(5-tetrazolyl)phenyl]-3-phenyl-2(5*H*)-furanone (**9**) and 4-(4-methylphenyl)-1-[4-(5-tetrazolyl)phenyl]-3-(trifluoromethyl)-1*H*-pyrazole (**13**) are outlined in Schemes 1 and 2.



Scheme 1. Reagents and conditions: (a) $PhCH_2COOH$, Et_3N, CH_3CN, 25 °C, 20 min; (b) DBU, 25 °C, 20 min; (c) NaN_3, reflux, 48 h.



Scheme 2. Reagents and conditions: (d) EtOH, reflux, 20 h; (e) NaN_3, $ZnBr_2$, reflux, 48 h.

The tetrazole analogue of rofecoxib **9**, where SO_2Me is replaced by tetrazole ring, was prepared starting from 2-bromo-1-(4-cyanophenyl)ethanone **6**. Reaction of bromo compound with phenylacetic acid in the presence of Et_3N, then DBU, via the intermediate ester **7**, gave 4-(4-cyanophenyl)-3-phenyl-2(5*H*)furanone **8**.[17] Reaction of **8** with NaN_3 in the presence of $ZnBr_2$ gave required rofecoxib analogue as illustrated in Scheme 1.[18]

Reaction of 4-cyanophenylhydrazine hydrochloride **10** with 1-(4-methylphenyl)-4,4,4-trifluorobutane-1,3-dione **11** in EtOH afforded the pyrazole **12**.[9] Reaction of pyrazole **12** with NaN_3 in the presence of $ZnBr_2$ leads to the formation of the celecoxib derivative **13**, having a tetrazole ring in place of SO_2NH_2 pharmacophore (see Scheme 2).[18,19]

The tetrazole substituent is particularly attractive since it has the potential to undergo hydrogen-bonding interactions with amino acid residues, particularly Arg^{513}, lining the secondary pocket of COX-2. Moreover, the tetrazole ring is similar in size [MR (molar refractivity) = 1.48] to SO_2Me (MR = 1.51) or SO_2NH_2 (MR = 1.42) substituent.

Docking 4-[4-(5-tetrazolyl)phenyl]-3-phenyl-2(5*H*)-furanone (**9**) (as tetrazolide ion) in the active site of human COX-2 (1CX2 PDB file)[20,21] showed that the tetrazole ring was inserted into the secondary COX-2 pocket about 6.31 Å from Val^{523}. One of the N-atoms of the tetrazole ring forms hydrogen bond with the amine hydrogen (guanidine group) of Arg^{513} (2.1 Å) and another nitrogen forms hydrogen bond with His^{90} (2.5 Å) and

is positioned about 3.7 Å from Phe[518] as shown in Figure 2. The other nitrogen of tetrazole ring also forms a weak hydrogen bond with Gln[192] (3.7 Å).

These molecular modeling studies correlate well with in vitro enzyme inhibition data determined by colorimetric COX (ovine) inhibitor screening assay (Table 1).[22–24] In this regards, the rofecoxib analogue **9** showed potent and selective inhibition of COX-2 relative to the reference drug celecoxib (as shown in Table 1).

Similar docking of the celecoxib analogue **13** showed that it binds in the primary binding site such that one of the N-atoms of the tetrazole ring forms hydrogen bonds with the amine hydrogen of Arg[513] (2.4 Å) and His[90] (2.5 Å), and another nitrogen forms hydrogen bond with Phe[518] (3.9 Å). The other nitrogen is positioned about 3.6 Å from Gln[192] (see Fig. 3). The tetrazole analogue of celecoxib **13** exhibited potent and selective inhibition of COX-2 relative to the reference drug celecoxib (as shown in Table 1).

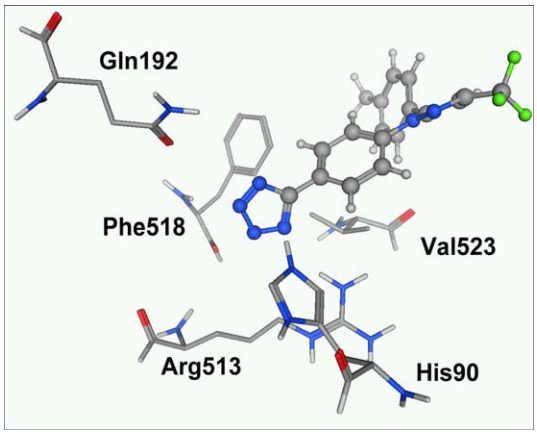


Figure 3. Docking of the 4-(4-methylphenyl)-1-[4-(5-tetrazolyl)phenyl]-3-(trifluoromethyl)-1*H*-pyrazole (**13**) (ball and stick) in the active site of human COX-2 (line and stick). The center of the tetrazole ring is about 6.4 Å outside of the entrance to the secondary pocket (Val[523]).

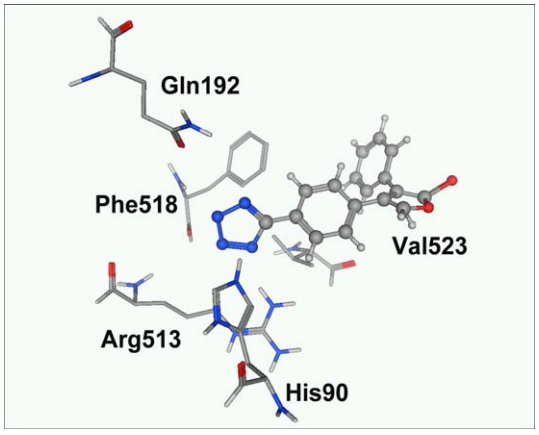


Figure 2. Docking the 4-[4-(5-tetrazolyl)phenyl]-3-phenyl-2(5*H*)furanone (**9**) (ball and stick) in the active site of human COX-2 (line and stick). The center of the tetrazole ring is about 6.3 Å outside of the entrance to the secondary pocket (Val[523]).

In vivo pharmacological evaluation of compounds **9** and **13** was carried out to assess their potential anti-inflammatory activity through parenteral injection in the rat carrageenan-induced paw edema assay.[4,24–26] They were intravenously administered at 3 mg/kg to rats, 10 min before edema induction by injection of λ-carrageenan. Paw volume was then measured at different times after injection and the percentage of inhibition was calculated. Rofecoxib analogue **9** and celecoxib analogue **13** both have shown anti-inflammatory activity with moderate to good activity range (Table 1). In this regard, rofecoxib analogue **9** reduced inflammation by 34% and 28%, and celecoxib analogue **13** decreased inflammation by 40% and 35% at 3 and 5 h postdrug administration, respectively, relative to the reference drug

Table 1. Anti-inflammatory, in vitro COX-1 and COX-2 inhibition data, and gastric toxicity studies of 4-[4-(5-tetrazolyl)phenyl]-3-phenyl-2(5*H*)furanone (**9**) and 4-(4-methylphenyl)-1-[4-(5-tetrazolyl)phenyl]-3-(trifluoromethyl)-1*H*-pyrazole (**13**)

Compound	IC_{50}[a] (nM)		Selectivity index (COX-1/COX-2)	AI activity[b]		Gastric damage[c]
	COX-1	COX-2		% inhibition at 3 h	% inhibition at 5 h	
9	3.8	1.8	2.11	34.1 ± 10.8	28.0 ± 12.7	0
13	4.1	1.9	2.16	40.2 ± 5.9	34.7 ± 8.5	0
Celecoxib	3.7	2.2	1.68	NA[d]	NA	NA
Parecoxib	NA	NA	NA	62.8 ± 6.9	42.0 ± 7.9	0[f]
Diclofenac	NA	NA	NA	NA	NA	22.8 ± 4.7[e] 51.5 ± 14.0[f]

[a] Values are means of two determinations and deviation from the mean is <10% of the mean value.
[b] Inhibitory activity on carrageenan-induced rat paw edema. The results are expressed as means ± SEM (n = 4–6) following a 3 mg/kg iv dose of the test compound.
[c] Nonsteroidal anti-inflammatory drug-induced gastric damage in rats. Compounds **9** and **13** (20 mg/kg) and diclofenac sodium (20 and 40 mg/kg) were administrated intravenously 4 h before rats were killed. Visible gastric lesions were scored and the sum score was determined. The results are expressed as means ± SEM (n = 8). $^{*}P < 0.05$, $^{***}P < 0.001$ compared to compounds **9** and **13**.
[d] NA, not applicable.
[e] 20 mg/kg iv dose.
[f] 40 mg/kg iv dose.

parecoxib (63% and 42% reduction in inflammation at 3 and 5 h postdrug administration, respectively), administered at the same dose.

To establish their GI safety profile, these compounds were evaluated in the acute gastric toxicity study.[26] Intravenous administration of 20 mg/kg of diclofenac sodium produced marked, visible, hemorrhagic gastric lesions with more petechiae 4 h after its administration. In contrast, compounds **9** and **13** were without any effect at a high dose (20 mg/kg, iv) as shown in Table 1.

As indicated above, an adequate aqueous solubility was of critical importance in order to develop an intravenous dosage form. The in situ preparation of the potassium salt (by adding 1 equiv of KOH) provided a solubility of 125 and 100 mg/mL in water for compounds **9** and **13**, respectively.[4,27]

The results of this investigation show that: (i) rofecoxib (**9**) and celecoxib (**13**) analogues, having a tetrazole ring in place of the respective SO_2Me and SO_2NH_2 pharmacophores provide potent and selective inhibition of the COX-2 isozyme, (ii) molecular modeling studies indicate the tetrazole ring inserts deep into the COX-2 secondary pocket, forming hydrogen bonds with Arg^{513}, His^{90}, Gln^{192}, and (iii) the tetrazole ring is a suitable water-soluble replacement with respect to selective COX-2 inhibition and AI activity for parenteral treatment of acute pain.

Acknowledgments

This work was supported by grants from the INSF (Iran National Science Foundation).

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19. Spectral data for compounds **8**, **9**, **12** and **13**. 4-(4-Cyanophenyl)-3-phenyl-2(5*H*)-furanone (**8**). Yield 45%. Mp 156–158 °C. IR (KBr): 2228 (CN), 1752 (CO) cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.65 (d, $J = 8.8$ Hz, 2H), 7.43 (d, $J = 8.8$ Hz, 2H), 7.42–7.37 (m, 5H), 5.19 (s, 2H). MS *m/z* (%) 261 (M^+, 90), 232 (30), 217 (13), 204 (100), 176 (9), 131 (15). Anal. Calcd for $C_{17}H_{11}NO_2$: C, 78.15; H, 4.24; N, 5.36. Found: C, 78.36; H, 4.10; N, 5.49.
4-[4-(5-Tetrazolyl)phenyl]-3-phenyl-2(5*H*)-furanone (**9**). Yield 60%. Mp 256–258 °C. IR (KBr): 3452 (NH), 1731 (CO) cm^{-1}. ^1H NMR (DMSO-d_6) δ 8.03 (d, $J = 8.4$ Hz, 2H), 7.59 (d, $J = 8.4$ Hz, 2H), 7.47–7.42 (m, 3H), 7.40–7.37 (m, 2H), 5.43 (s, 2H). MS *m/z* (%) 304 (M^+, 100), 292 (44), 281 (18), 273 (18), 219 (14), 204 (10), 178 (12). Anal. Calcd for $C_{17}H_{12}N_4O_2$: C, 67.10; H, 3.97; N, 18.41. Found: C, 66.98; H, 3.81; N, 18.59.
1-(4-Cyanophenyl)-5-(4-methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazole (**12**). Yield 50%. Mp 107–109 °C. IR (KBr): 2228 (CN) cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.65 (d, $J = 8.8$ Hz, 2H), 7.46 (d, $J = 8.8$ Hz, 2H), 7.19 (d, $J = 8$ Hz, 2H), 7.11 (d, $J = 8$ Hz, 2H), 6.74 (s, 1H), 2.78 (s, 3H). MS *m/z* (%) 327 (M^+, 100), 313 (5), 306 (12), 292 (16), 275 (13). Anal. Calcd for $C_{18}H_{12}F_3N_3$: C, 66.05; H, 3.70; N, 12.84. Found: C, 66.22; H, 3.88; N, 12.67.
4-(4-Methylphenyl)-1-[4-(5-tetrazolyl)phenyl]-3-(trifluoromethyl)-1*H*-pyrazole (**13**). Yield 51%. Mp 220–222 °C. IR (KBr): 3420 (NH) cm^{-1}. ^1H NMR (DMSO-d_6), δ 8.11 (d, $J = 8.4$ Hz, 2H), 7.58 (d, $J = 8.4$ Hz, 2H), 7.38–7.08 (m, 5H), 2.32 (s, 3H). MS *m/z* (%) 370 (M^+, 76), 341 (95), 327 (100), 321 (19), 306 (25), 292 (12), 273 (15), 258 (14). Anal. Calcd for $C_{18}H_{13}F_3N_6$: C, 58.38; H, 3.54; N, 22.69. Found: C, 58.29; H, 3.69; N, 22.82.
20. Docking studies were performed using MOE software version 2003.02 (CCG Inc.). The coordinates of the X-ray

crystal structure of the selective COX-2 inhibitor SC-558 bound to the murine COX-2 enzyme were obtained from the RCSB Protein Data Bank (1cx2). The ligand molecules were constructed using the Builder module and were energy optimized. The purpose of docking is to search for favorable binding configuration between the small flexible ligands and the rigid protein. Protein residues with atoms greater than 7.5 Å from docking box were removed for efficiency. Searching is conducted within a specified 3D docking box using simulated annealing based on Monte Carlo method and MMFF94 molecular mechanics force fields for 8000 iterations.

21. MOE. Chemical Computing Group Inc., Montreal, Que., Canada, 2003.02, see http://www.chemcomp.com.
22. Cyclooxygenase (COX) activity was determined using arachidonic acid (AA) as substrate and *N*,*N*,*N*′,*N*′-tetramethylphenylenediamine (TMPD) as a cosubstrate, as previously studied.[23] The reaction mixture (200 μL) contained 0.5 μM heme, 0.05 mM TMPD, 0.1 mM AA, and 36 units of COX-2 enzyme (57 units for COX-1, Sigma Co.) in 0.1 M Tris/HCl (pH 8.1). The oxidation of substrate was measured at 25 °C by monitoring the increase of absorbance at 630 nm. The absorption due to the spontaneous oxidation of TMPD was subtracted from the initial rate of oxidation observed in the presence of AA. The inhibition of the studied compounds (**9** and **13**) was determined after preincubation for 5 min with the enzyme in the presence of heme, and the reaction was started by adding AA and TMPD. The mixture was incubated for further 5 min and the absorbance was measured on a strip reader. For synthesized compounds (**9** and **13**) 10 μL of scalar dilutions of the inhibitors in DMSO was added. Celecoxib, a potent and selective COX-2 inhibitor, has been used as a reference drug.
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24. Male Sprague–Dawley rats (120–150 g) were fasted with free access to water at least 16 h prior to experiments. Edema was produced by injecting 0.1 mL of a solution of 1% λ-carrageenan in the hindpaw. Paw volume was measured by water displacement with a plethysmometer (UGO BASILE) before, 3 and 5 h after treatment. The compounds were administered by intravenous route as a solution in PBS (1 mL/kg) 10 min before carrageenan injection and after being hydrated with H_2O (5 mL). The percentage were calculated by the following equation: anti-inflammatory activity (%) = $(1 - D/C) \times 100$, where *D* represents the difference in paw volume before and after drug was administered to the rats, and *C* stands for the difference of volume in the control groups.
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27. Equilibrium solubilities were obtained by adding solid compounds directly to an aqueous medium, followed by adding 1 equiv of KOH and stirring at room temperature for 24 h. Suspensions then were filtered and the remaining concentration in the solution was measured spectrophotometrically at 306 and 256 nm wavelengths for compounds **9** and **13**, respectively.