Brief Articles

Design and Synthesis of Celecoxib and Rofecoxib Analogues as Selective Cyclooxygenase-2 (COX-2) Inhibitors: Replacement of Sulfonamide and Methylsulfonyl Pharmacophores by an Azido Bioisostere

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Received April 3, 2001

Celecoxib (**13**) and rofecoxib (**17**) analogues, in which the respective SO_2NH_2 and SO_2Me hydrogen-bonding pharmacophores were replaced by a dipolar azido bioisosteric substituent, were investigated. Molecular modeling (docking) studies showed that the azido substituent of these two analogues (**13**, **17**) was inserted deep into the secondary pocket of the human COX-2 binding site where it undergoes electrostatic interaction with Arg^{513}. The azido analogue of rofecoxib (**17**), the most potent and selective inhibitor of COX-2 (COX-1 $IC_{50} = 159.7\ \mu M$; COX-2 $IC_{50} = 0.196\ \mu M$; COX-2 selectivity index = 812), exhibited good oral antiinflammatory and analgesic activities.

Introduction

Many selective COX-2 inhibitors belong to a tricyclic group of compounds with a central ring possessing a diaryl stilbene-like structure with a sulfonyl (SO_2) group at the para position of one of the aryl rings, such as DuP-697 (**1**),[1] celecoxib (Celebrex) (**2**),[2] SC-588 (**3**),[2] rofecoxib (Vioxx) (**4**),[3] 3-(4-methylsulfonylphenyl)-4-phenyl-3-trifluoromethylisoxazole (**5**),[4] and 2,3-dimethyl-5-(4-methylsulfonylphenyl)-4-phenyl-4-isoxazoline (**6**),[5] as illustrated in Figure 1. The SO_2Me and SO_2NH_2 pharmacophores are believed to induce COX-2 selectivity by insertion into the secondary pocket of COX-2 which is absent in COX-1. The secondary pocket present in COX-2 has been attributed to the presence of isoleucine (Ile^{523}) in COX-1 relative to the smaller valine (Val^{523}) in COX-2.[6] Replacement of histidine (His^{513}) in COX-1 by arginine (Arg^{513}) in COX-2 has been reported to play a key role in the hydrogen-bond network of the COX active site. Histidine (His^{90}), glutamine (Gln^{192}), and tyrosine (Tyr^{355}) control the access of ligands into the secondary pocket.[7] The interaction of Arg^{513} with the bound ligand has been reported to be a requirement for the time-dependent inhibition of COX-2.[8] The presence of the Arg^{513} residue, to our knowledge, has not been exploited for the design of selective COX-2 inhibitors. Accordingly, we now describe the design, synthesis, cyclooxygenase inhibitory, analgesic and antiinflammatory activities, and some molecular modeling studies for the pyrazole regioisomers **10** and **13** and the furanone **17** that possess an azido group in place of the SO_2NH_2 and SO_2Me pharmacophores present in celecoxib and rofecoxib, respectively.

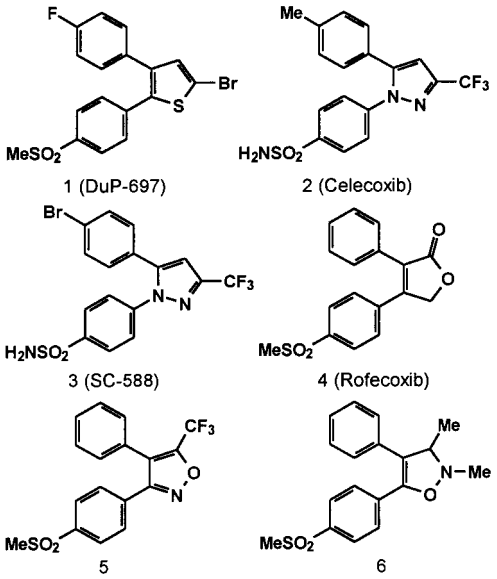

Figure 1. Representative examples of selective COX-2 inhibitors having a central five-membered heterocyclic ring.

Chemistry

Reaction of 4-nitrophenylhydrazine with 1-(4-methylphenyl)-4,4,4-trifluorobutane-1,3-dione[2] in EtOH afforded the cyclic pyrazoline-5-ol **7** which eliminated a molecule of water upon treatment with HOAc at reflux temperature to yield the pyrazole **8** (see Scheme 1). Reduction of the nitro group in the pyrazole **8** with hydrazine hydrate and 10% Pd/C, using a method reported by Penning et al.,[2] yielded the corresponding amino product **9**. Diazotization of **9**, and treatment of the diazonium salt with NaN_3, afforded the 1,3-regioisomer of celecoxib having an azido substituent in place of the SO_2NH_2 pharmacophore. In contrast, the 1,5-regioisomer was prepared by condensation of 4-nitro-

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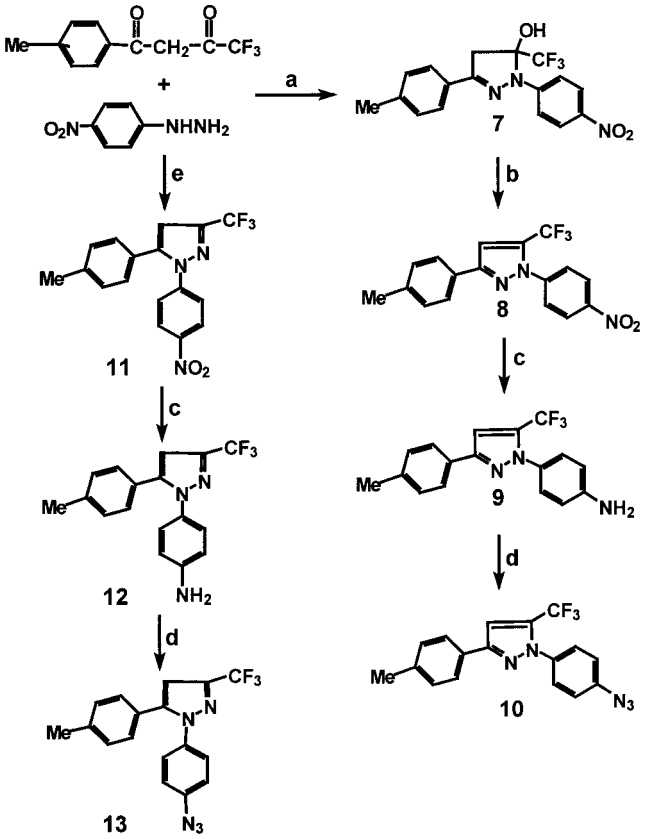
10.1021/jm010153c CCC: $20.00
Published on Web 08/03/2001

Scheme 1[a]



[a] Reagents and conditions: (a) EtOH, reflux, 20 h; (b) HOAc, reflux, 2 h; (c) $H_2NNH_2 \cdot xH_2O$, 10% Pd/C, reflux, 45 min; (d) $NaNO_2$/HCl and then NaN_3, 0−5 °C, 45 min; (e) EtOH, HCl, reflux, 20 h.

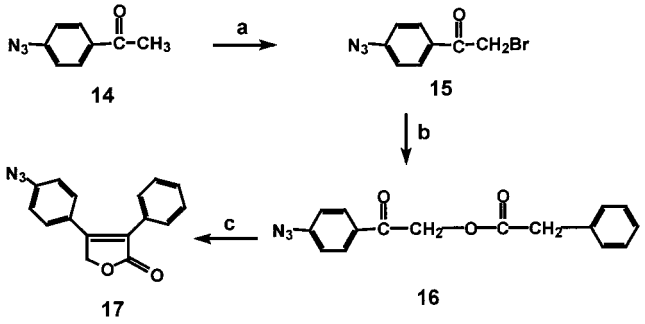
phenylhydrazine with 1-(4-methylphenyl)-4,4,4-trifluorobutane-1,3-dione in EtOH under acidic reaction conditions to yield the pyrazole **11** (76%). This reaction, performed under acidic reaction conditions, provided a superior yield relative to related reactions carried out under neutral reaction conditions.[2] The azido analogue of celecoxib **13** was prepared starting from the pyrazole **11** using the same reaction sequence used for the elaboration of the nitro compound **10** to the azido product **11**.

The azido analogue **17** of rofecoxib, where $MeSO_2$ is replaced by N_3, was prepared starting with the bromination of 4-azidoacetophenone **14**[9] using Br_2 according to a reported method.[10] The subsequent reaction of the bromo compound **15** with phenylacetic acid in the presence of $(Et)_3N$ gave 4-(4-azidophenyl)-3-phenyl-2(5*H*)furanone (**17**) that was formed via the intermediate ester **16** as illustrated in Scheme 2.

Results and Discussion

Celecoxib and rofecoxib analogues, having an azido group in place of the respective SO_2NH_2 and SO_2Me pharmacophores, were investigated to determine whether the azido substituent is a suitable bioisostere with respect to selective COX-2 inhibition, and AI and analgesic activities. Structure−activity studies for the tricyclic class of selective COX-2 inhibitors have shown that a SO_2Me or SO_2NH_2 substituent at the para position of one aryl ring usually confers optimal COX-2 inhibitory potency.[11] In the 1,2-diarylcyclopentene class

Scheme 2[a]



[a] Reagents and conditions: (a) Br_2, $CHCl_3$, 25 °C, 2 h; (b) $PhCH_2COOH$, Et_3N, CH_3CN, 25 °C, 1 h; (c) Et_3N, CH_3CN, reflux, 8 h.

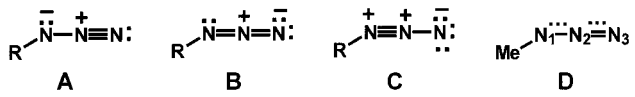


Figure 2. Azide resonance hybrid structures.

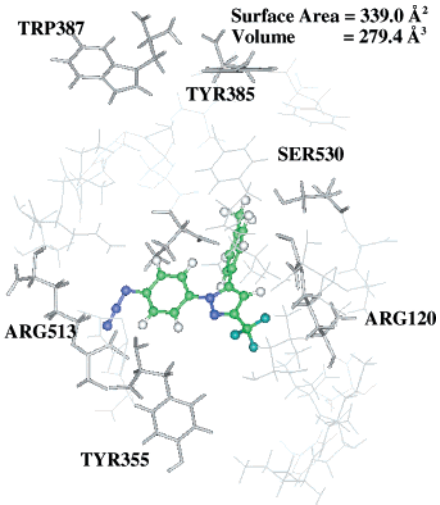


Figure 3. Docking the pyrazole **13** (ball-and-stick) in the active site of human COX-2 (line and stick) ($E_{intermolecular}$ = −46.49 kcal/mol). The *C*-atom of the CF_3 substituent is 10.26 Å from the phenolic *O*H of Tyr^{355}, but removed from Ser^{530} (*O*H) by 7.18 Å. The terminal *N*-atom of the azido substituent is about 4.52 Å inside the entrance to the secondary pocket (Val^{523}). The center of the N-1 phenyl ring is about 3.95 Å from the entrance to the secondary pocket (Val^{523}).

of compounds, replacement of SO_2Me by SO_2CF_3, COMe, PO(OH)Me, CO_2H, $PO(OH)_2$, or SO(=NH)Me abolished COX-2 inhibitory activity.[12] A similar replacement of SO_2Me by NO_2, which can dispose a pair of oxygen atoms such as SO_2, in the 1,5-diarylpyrazole class also abolished COX-2 inhibitory activity.[2]

The azido substituent is particularly attractive since it has the potential to undergo electrostatic (ion−ion) binding interactions with amino acid residues, particularly Arg^{513}, lining the secondary pocket of COX-2. Covalent azides can be viewed as resonant hybrids between structures **A**, **B**, and **C** (see Figure 2).[13] Pauling rejected **C** as a major contributor based on the *adjacent charge rule*.[14] The remaining hybrids **A** and **B** predict a 2.5 bond order for the N_2-N_3 bond and a 1.5 bond order for the N_1-N_2 bond (see **D**, Figure 2). This prediction was in very good agreement with a structure determination of methyl azide (**D**) where the bond

Table 1. Antiinflammatory and Analgesic Activities, in Vitro COX-1 and COX-2 Inhibition Data, and Molecular Volumes of 1-(4-Azidophenyl)-3-(4-methylphenyl)-5-trifluoromethylpyrazole (**10**), 1-(4-Azidophenyl)-5-(4-methylphenyl)-3-trifluoromethylpyrazole (**13**), and 4-(4-Azidophenyl)-3-phenyl-2(5*H*)furanone (**17**)

	AI activity[a]		analgesic activity[b]			IC_{50}, μM[d]		
compd	% inhibition at 3 h	% inhibition at 5 h	% inhibition at 30 min	% inhibition at 60 min	vol. (Å^3)[c]	COX-1	COX-2	selectivity index (COX-1/COX-2)
10					278.3	9.88	2.63	3.74
13	46.6 ± 4.4	16.9 ± 2.7	60.9 ± 9.4	63.1 ± 1.2	279.4	>100	1.55	64.55
17	42.9 ± 1.0	27.5 ± 4.6	46.7 ± 1.3	60.6 ± 1.6	242.1	159.72	0.196	812.4
celecoxib	79.9 ± 1.9[e]	58.2 ± 1.8[f]	31.7 ± 9.6	62.0 ± 7.3	298.4	22.9	0.0507	404
rofecoxib					262.5	26.0[g]	0.34[g]	76.5

[a] Inhibitory activity on carrageenan-induced rat paw edema; the result is the mean value ± SEM using four animals following a 50 mg/kg oral dose of the test compound. [b] Inhibitory activity in the rat 4% NaCl-induced abdominal constriction assay; the result is the mean value ± SEM using four animals following a 50 mg/kg ip dose of the test compound. [c] The volume of the molecule, after minimization using the MM3 force field, was calculated using the Alchemy 2000 program. [d] The result (IC_{50}, μM) is the mean of two determinations. [e] ID_{50} = 10.8 mg/kg po dose. [f] ID_{50} = 40.8 mg/kg po dose. [g] Data taken from the literature for inhibition of purified human recombinant COX-1 and COX-2.[19]

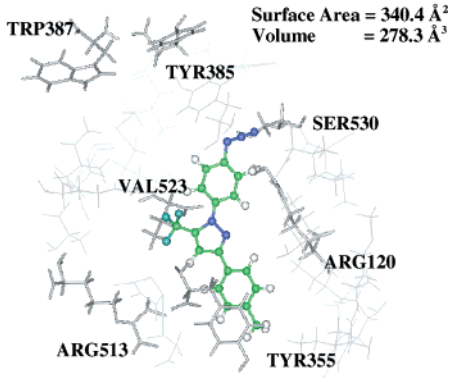


Figure 4. Docking the pyrazole (**10**) (ball-and-stick) in the active site of human COX-2 (line and stick) ($E_{intermolecular}$ = −34.12 kcal/mol). The *C*-atom of the CF_3 substituent is 12.25 Å from the phenolic *O*H of Tyr^{355}, but removed from the Ser^{530} (*O*H) by 8.56 Å. The terminal *N*-atom of the azido substituent is about 10.30 Å outside the entrance to the secondary pocket (Val^{523}). The center of the *N*-1 phenyl ring is about 6.46 Å outside the entrance to the secondary pocket (Val^{523}).

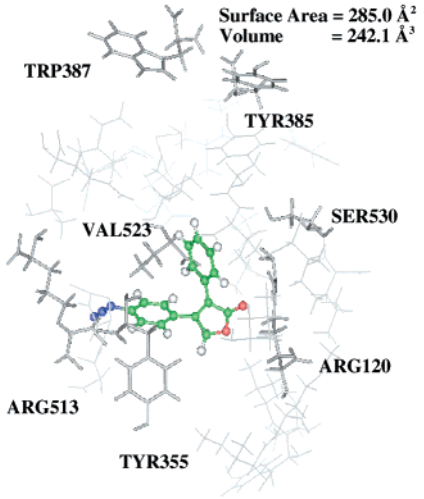


Figure 5. Docking the furanone **17** (ball-and-stick) in the active site of human COX-2 (line and stick) ($E_{intermolecular}$ = −49.6 kcal/mol). The *O*-atom of the CO substituent is 13.51 Å from the phenolic *O*H of Tyr^{355}, but removed from Ser^{530} (*O*H) by 4.03 Å. The terminal *N*-atom of the azido substituent is about 4.52 Å inside the entrance to the secondary pocket (Val^{523}). The center of the C-4 phenyl ring is about 4.11 Å from the entrance to the secondary pocket (Val^{523}).

lengths from electron diffraction studies[15] were as follows: N_2-N_3 = 1.12 Å, N_1-N_2 = 1.24 Å, $C-N_1$ = 1.47 Å ,and the $C-N_1-N_2$ bond angle was 120°. The linear configuration of the azido group is in agreement with the sp^3 hybridization indicated by the lack of nonbonded electron pairs on N_2.[13] The azido group is slightly smaller in size [MR (molar refractivity) = 10.20] than a SO_2Me (MR = 13.49) or SO_2NH_2 (MR = 12.28) substituent, but more lipophilic (π = 0.46) relative to the more polar SO_2Me (π = −1.63) and SO_2NH_2 (π = −1.82) subsituents[16] which have the potential to improve absorption and provide a more rapid onset of action.[11]

Docking 1-(4-azidophenyl)-5-(4-methylphenyl)-3-trifluoromethylpyrazole (**13**) in the active site of human COX-2 (1CX2 PDB file), showed that the terminal *N*-atom of the azido group was inserted into the secondary COX-2 pocket about 4.52 Å from Val^{523}, and about 3.15 Å from the center of the guanidino group of Arg^{513} (see Figure 3**)**. This orientation of the pyrazole **13** within the COX-2 active site provides an intermolecular energy between the enzyme and pyrazole **13** of about −46.19 kcal/mol, where the electrostatic component accounts for about 12% of this total energy. In comparison, the celecoxib (**2**) docked complex showed an intermolecular energy between the enzyme and celecoxib of about −45.16 kcal/mol where 2.6% was due to an electrostatic component. This difference is likely due to a greater electrostatic interaction between the dipolar azido group in pyrazole **13** and the charged guanidino moiety of Arg^{513} in the secondary COX-2 pocket. Similar docking of the 1-(4-azidophenyl)-3-(4-methylphenyl)-5-trifluoromethylpyrazole regioisomer (**10**) in the active site of human COX-2 showed that the azido substituent did not insert into the secondary pocket since the ligand **10** extended parallel to the longitudinal axis of the hydrophobic primary COX-2 channel (cavity), in a manner characteristically observed for nonselective COX-2 inhibitors[17] as illustrated in Figure 4.

These molecular modeling studies correlate well with in vitro enzyme inhibition data. In this regard, the 1,5-pyrazole regioisomer **13** showed selective inhibition of COX-2 [COX-1 IC_{50} > 100 μM; COX-2 IC_{50}= 1.5 μM; selectivity index (SI) ≈ 64], whereas the 1,3-regioisomer **10** showed a modest COX-2 selectivity ≈ 4. These results are similar to those described for other studies[2,18] utilizing compounds not having a 1,2-diarylstilbene-like structure. Docking the rofecoxib analogue **17**, in which

the SO_2Me moiety was replaced by an azido substituent, in the active site of human COX-2 showed a similar interaction between the azido group and the COX-2 secondary pocket amino acid residues similar to that observed for the pyrazole **13** (see Figure 5). The intermolecular energy between the ligand **17** and the enzyme was −49.6 kcal/mol with the electrostatic component comprising 5.8% of the total energy. In contrast, the rofecoxib (**4**) docked complex showed an intermolecular energy of −42.16 kcal/mol where only 1.2% was due to an electrostatic component. The higher electrostatic component for the azido compound **17** (5.8%), relative to that for rofecoxib **4** (1.2%), is attributed to the fact that the $MeSO_2$ moiety present in rofecoxib undergoes H-bonding to the imidazole N*H* of $Hist^{90}$ in the secondary pocket. In contrast, the azido moiety in **17** undergoes an electrostatic interaction with Arg^{513} in the secondary COX-2 pocket. The lower contribution of the electrostatic energy to the total intermolecular energy in the case of the furanone **17**, relative to the pyrazole **13**, can be attributed to the observed hydrogen bonding interaction between the *O*-atom of the C=*O* in the furanone structure and residues lining the primary COX-2 channel, particularly Arg^{120}. The azido analogue of refocoxib **17** exhibited a potent and selective inhibition of COX-2 (COX-2 $IC_{50} = 0.196\ \mu M$; COX-1 $IC_{50} = 159.7\ \mu M$; SI ≈ 812). The molecular volumes of the selective COX-2 inhibitors **13** (279.4 $Å^3$) and **17** (242.1 $Å^3$) are moderately smaller than that for the selective COX-2 inhibitors celecoxib (298.4 $Å^3$) and rofecoxib (262.5 $Å^3$).

The azido analogues of celecoxib **13** and rofecoxib **17** exhibited good AI and analgesic activities (see Table 1).

Conclusions

In conclusion, the dipolar azido group is a bioisostere of the SO_2NH_2 and SO_2Me hydrogen-bonding pharmacophores present in many selective COX-2 inhibitors, and the azido analogues **13** and **17** may be useful biochemical agents for photoaffinity labeling of the COX-2 enzyme.

Experimental Section

Cyclooxygenase Inhibition Studies. All compounds described herein were tested for their ability to inhibit COX-1 and COX-2 using a COX-(ovine) inhibitor screening kit (catalog no. 560101, Cayman Chemical, Ann Arbor, MI) using the method previously reported.[4]

Antiinflammatory Assay. The test compounds were evaluated using the in vivo rat carrageenan-induced foot paw edema model reported previously.[20]

Analgesic Assay. Analgesic activity was determined using the 4% sodium chloride-induced writhing (abdominal constriction) assay as described previously.[21]

Acknowledgment. We are grateful to the Canadian Institutes of Health Research (Grant MOP-14712) for financial support of this research and to C.-A. McEwen for technical assistance in performing the antiinflammatory and analgesic assays.

Supporting Information Available: Experimental procedures for the preparation of compounds **7**−**13** (Scheme 1) and **15**−**17** (Scheme 2) and their IR and NMR (^1H, ^{13}C, ^{19}F) spectroscopic data. This material is available free of charge on the Internet at http://pubs.acs.org.

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