Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 5273–5280

BIOORGANIC & MEDICINAL CHEMISTRY

Design and Synthesis of Novel Celecoxib Analogues as Selective Cyclooxygenase-2 (COX-2) Inhibitors: Replacement of the Sulfonamide Pharmacophore by a Sulfonylazide Bioisostere

Md. Jashim Uddin, P. N. Praveen Rao and Edward E. Knaus*

Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2N8

Received 5 June 2003; accepted 28 July 2003

Abstract—A group of celecoxib analogues in which the *para*-SO_2NH_2 substituent on the N^1-phenyl ring was replaced by a *para*-sulfonylazido (SO_2N_3) **4**, or a *meta*-SO_2N_3 **8**, substituent were designed for evaluation as selective cyclooxygenase-2 (COX-2) inhibitors. In vitro COX-1 and COX-2 inhibition studies showed that 4-[5-(4-methylphenyl)-3-trifluoromethyl-1*H*-pyrazol-1-yl]benzenesulfonyl azide (**4**) with a *para*-SO_2N_3 substituent was a selective COX-1 inhibitor. In contrast, 3-[5-(4-methylphenyl)-3-trifluoromethylpyrazol-1-yl]benzenesulfonyl azide (**8a**) having a *meta*-SO_2N_3 substituent (COX-1 $IC_{50} > 100\,\mu M$; COX-2 $IC_{50} = 5.16\,\mu M$; COX-2 selectivity index > 19.3) is a selective COX-2 inhibitor. A molecular modeling (docking) study showed that the SO_2N_3 group of **8a** inserts deep inside the secondary pocket of the COX-2 binding site. The SO_2N_3 moiety of **8a** can undergo a dual *H*-bonding interaction via one of its SO_2 oxygen-atoms, and an electrostatic (ion–ion) interaction via the terminal azido (N_3) nitrogen-atom, to the guanidino NH_2 of Arg^{513} in the secondary pocket of COX-2. These observations indicate that an appropriately positioned SO_2N_3 moiety is a novel alternative bioisostere to the traditional SO_2NH_2 and SO_2Me pharmacophores present in selective COX-2 inhibitors, that are only capable of *H*-bonding interactions with the COX-2 isozyme, for use in drug design.

Introduction

Development of the non-ulcerogenic selective COX-2 inhibitor celecoxib (Celebrex®, **1**)[1] provided a significant advancement in the treatment of rheumatoid arthritis and osteoarthritis.[2] The SO_2NH_2 pharmacophore present in celecoxib is believed to induce COX-2 selectivity by insertion into the secondary (2°) pocket of the COX-2 binding site that is absent in COX-1. This 2°-pocket in COX-2 is formed due to a conformational change at Tyr^{355} that is attributed to the presence of Ile^{523} in COX-1 relative to Val^{523} having a smaller side chain in COX-2.[3] Accordingly, the combined volume of the primary COX-2 binding site and its associated 2°-pocket (394 Å^3) is about 25% larger than the COX-1 binding site (316 Å^3).[4] It has been reported that replacement of His^{513} in COX-1 by Arg^{513} in COX-2 plays a key role in the hydrogen-bond network of the COX-2 binding site. Access of ligands to the 2-pocket of COX-2 is controlled by histidine (His^{90}), glutamine (Gln^{192}) and tyrosine (Tyr^{355}),[5] and interaction of Arg^{513} with the bound drug is a requirement for time-dependent inhibition of COX-2.[6] The SO_2NH_2 moiety of SC-558, an analogue of celecoxib (**1**) where Me is replaced by a Br substituent, interacts with His^{90}, Gln^{192} and Arg^{513}. One sulfonamide oxygen atom forms a *H*-bond to His^{90}, the other oxygen atom is linked by a *H*-bond to Arg^{513}, and the nitrogen atom is *H*-bonded to the carbonyl oxygen of Phe^{518}.[3] Recently we exploited, for the first time, the amino acid Arg^{513} to design selective COX-2 inhibitors having a dipolar azide (N_3) pharmacophore that can undergo an electrostatic (ion–ion) interaction with Arg^{513} in the COX-2 2°-pocket. In this regard, a molecular modeling study, where the azido compound **2**, namely 1-(4-azidophenyl)-5-(4-methylphenyl)-3-trifluoromethyl-1*H*-pyrazole was docked in the active site of the COX-2 isozyme, showed that (i) the terminal nitrogen atom of the azide substituent was inserted deep into the 2°-COX pocket, and (ii) the ligand–enzyme intermolecular and electrostatic binding energies for the azido compound **2** were significantly larger than that for celecoxib (**1**) in which the corresponding SO_2NH_2 pharmacophore is only capable of *H*-bonding (Fig. 1).[7]

*Corresponding author. Tel.: +1-780-492-5993; fax: +1-780-492-1217; e-mail: eknaus@pharmacy.ualberta.ca

0968-0896/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2003.07.005

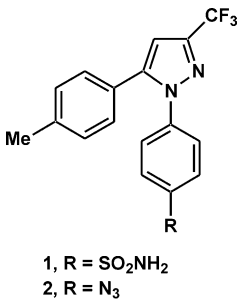


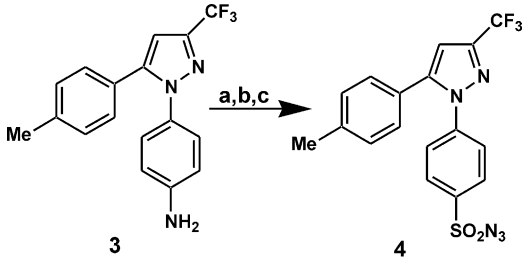
Figure 1. Structures for celecoxib (**1**) and 1-(4-azidophenyl)-5-(4-methylphenyl)-3-trifluoromethyl-1*H*-pyrazole (**2**).

As a part of our ongoing program to develop new drug design concepts,[8,9] we now report a novel dipolar sulfonylazide pharmacophore that has the potential to undergo dual *H*-bonding (sulfonyl oxygens) and electrostatic (ion–ion) interactions (azido) with amino acid residues lining the 2°-pocket of the COX-2 binding site. Accordingly, a group of celecoxib analogues having a SO_2N_3 moiety at the *ortho*-, *meta*-, or *para*-position of the N^1-phenyl ring were synthesized, their COX-1/COX-2 inhibition selectivity indices were determined, and some molecular modeling studies are described where target compounds are docked in the binding site of the COX-2 isozyme.

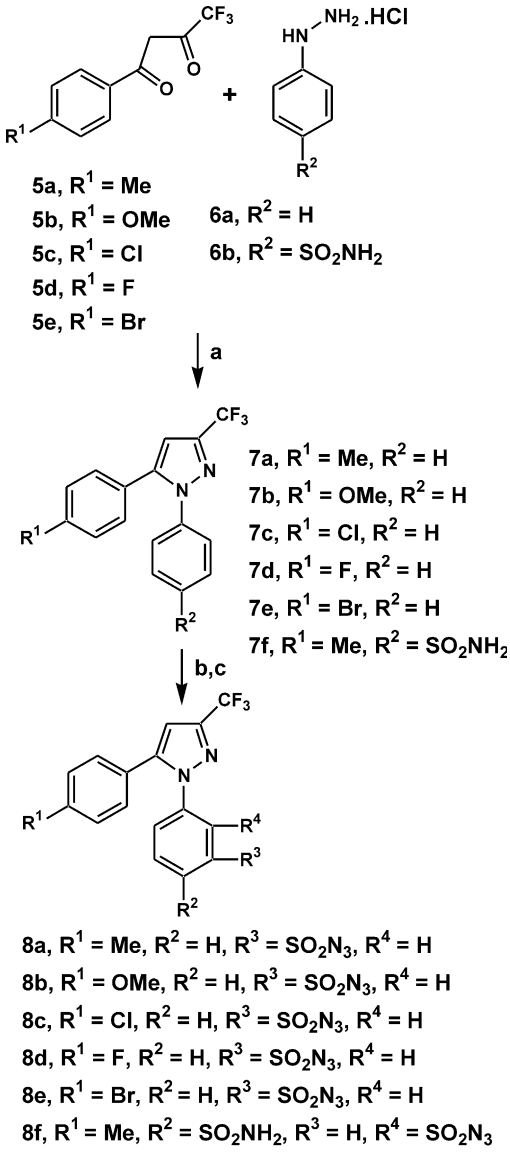
Chemistry

The diazotization of the previously reported[1,7] anilino compound **3** gave rise to the corresponding diazonium salt which on treatment with 37% w/v SO_2 in HOAc in the presence of $CuCl_2$ using a method described by Gilbert[10] yielded the intermediate sulfonyl chloride derivative. Subsequent azidation[11] of this SO_2Cl intermediate product using NaN_3 afforded the target product 4-[5-(4-methylphenyl)-3-trifluoromethyl-1*H*-pyrazol-1-yl]benezenesulfonyl azide (**4**, 22% yield) where the SO_2NH_2 pharmacophore of celecoxib has been replaced by a SO_2N_3 substituent (Scheme 1).

Condensation[1,7] of a 1-(*para*-substituted-phenyl)-4,4,4-trifluorobutane-1,3-dione **5a–e**[1] with either phenylhydrazine hydrochloride (**6a**), or 4-sulfonamidophenylhydrazine hydrochloride (**6b**),[12] afforded the respective pyrazole product **7a–f** in 39–92% yield (see Scheme 2). Reaction of the N^1-phenylpyrazoles (**7a–e**) with $ClSO_3H$ at 25 °C afforded the respective *meta*-benzenesulfonyl chloride in situ,[13] which on reaction with NaN_3, gave the respective *meta*-benzenesulfonyl azide product **8a–e** (46–70% yield). In contrast, similar in situ chlorosulfonation[13] and azidation of **7f** having a N^1-(4-sulfonamidophenyl) substituent yielded the corresponding N^1-(4-sulfonamido-2-sulfonylazidophenyl) product **8f** (45%).



Scheme 1. Synthesis of **4**. Reagents and conditions: (a) $NaNO_2$, concentrated HCl, 0–5 °C, 30 min (in dark); (b) 37% w/v SO_2 in HOAc, $CuCl_2$, 40–50 °C, 30 min (in dark); (c) NaN_3, acetone, H_2O, 0 °C, 3 h.



Scheme 2. Synthesis of **8a–f**. Reagents and conditions: (a) 95% EtOH, reflux, 24 h; (b) $ClSO_3H$, 25 °C, 1 h; (c) NaN_3, acetone, H_2O, 0 °C, 3 h.

Electrophilic chlorosulfonation of compound **7a,** and the related compounds **7b–e**, occurred at the *meta*-position of the N^1-phenyl ring system that is attributed to the electron-withdrawing effect of the protonated pyrazole ring. Chlorosulfonation at the *meta*-position of the N^1-phenyl ring is consistent with data obtained from a geometry optimized PM3 calculation[14] for **7a** which showed that the electron density was highest at the *meta*-position (−0.097) relative to the *ortho*- (−0.0065) and *para*- (−0.082) positons of the N^1-phenyl ring. In contrast, chlorosulfonation of compound **7f** having a sulfonamido substituent gave compound **8f** in which the chlorosulfonyl group was incorporated *ortho* to the

N^1-pyrazole nitrogen or *meta* to the sulfonamido substituent. This exclusive chlorosulfonation *meta* to the sulfonamide substituent of **7f** is due to the strong electron-withdrawing effect of the protonated sulonamido group rather than protonation of the pyrazole ring. The position at which this chlorosulfonation occurred is in agreement with electron densities data acquired from a PM3 calculation which showed the electron density was low (0.009) at the *ortho*-position, and high (−0.1000) at the *meta*-position, to the sulfonamido substituent.

Results and Discussion

The dipolar azido substituent has attracted our attention as a pharmacophore for use in drug design since it has the potential to undergo electrostatic (ion–ion) binding interactions with enzymes or pharmacological receptors. In this regard, covalent azides can exist as resonance hybrids between ionic species **A**, **B** and **C** (see Fig. 2).[15] Pauling eliminated **C** as a major contributor based on the *adjacent charge rule*.[16] Accordingly, 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**, Fig. 2). A structure determination for methyl azide (**D**) is also in good agreement with this prediction since the bond lengths from an electron diffraction study[17] are 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 azido group has a linear configuration that is consistent with the sp^3 hybridization indicated by the lack of nonbonded electron pairs on N_2.[15]

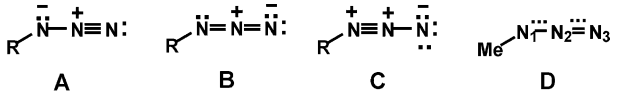
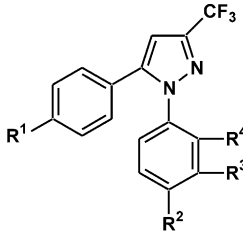


Figure 2. Resonance hybrid structures for the azido moiety.

In a recent study we reported that the dipolar azido (N_3) substituent present in the azido compound **2** undergoes an electrostatic (ion–ion) interaction with Arg^{513} in the COX-2 2°-pocket. This observation, together with the fact that the azido compound **2** is a selective COX-2 inhibitor, indicates that the dipolar azido substituent is a pharmacophore of the *H*-bonding SO_2NH_2 moiety present in celecoxib (**1**).[7] A comparison of substituent volumes, calculated by PM3 geometry optimization using the Alchemy 2000 program, showed the azido substituent (25.5 Å^3) is smaller than the traditional SO_2NH_2 (44.8 Å^3) and SO_2Me (49.7 Å^3) pharmacophores present in selective COX-2 inhibitors, or a SO_2N_3 (58.6 Å^3) substituent. A SO_2N_3 substituent has the potential to undergo dual *H*-bonding (sulfonyl oxygens) and electrostatic (ion-ion) interactions (dipolar N_3) suggesting that the SO_2N_3 moiety could serve as a new and alternative COX-2 pharmacophore to the SO_2NH_2 and SO_2Me pharmacophores.

Replacement of the SO_2NH_2 moiety of celecoxib (**1**) by a SO_2N_3 substituent gave the target compound 4-[5-(4-methylphenyl)-3-trifluoromethyl-1*H*-pyrazol-1-yl]benzenesulfonylazide (**4**). In vitro COX inhibition studies showed that **4** inhibited COX-1 selectively (COX-1 IC_{50} = 3.3 μM; COX-2 IC_{50} > 100 μM) (see data in Table 1). A molecular modeling study was therefore performed where **4** was docked in the active site of the murine COX-2 isozyme in order to explain its failure to inhibit COX-2. This docking experiment showed **4** binds in the center of the enzyme active site with the phenylsulfonylazide moiety oriented towards the mouth of the COX-2 channel such that the SO_2N_3 group is in close proximity with Arg^{120} and Tyr^{355} (see Fig. 3). The linear N_3 group is involved in extensive electrostatic interaction with the side chains of Arg^{120} and Tyr^{355}. Llorens and coworkers have shown the importance of the perturbation of the hydrogen bonding network

Table 1. In vitro COX-1 and COX-2 inhibition data and molecular volumes of 4-[5-(4-methylphenyl)-3-trifluoromethyl-1*H*-pyrazol-1-yl]benzenesulfonyl azide **4**, 3-[5-(4-substituted-phenyl)-3-trifluoromethyl-1*H*-pyrazol-1-yl]benzenesulfonyl azide **8a**, **8b** and **8d**, and 5-sulfonamido-2-[5-(4-methylphenyl)-3-trifluoromethyl-1*H*-pyrazol-1-yl]benzenesulfonyl azide **8f**



Compd	R^1	R^2	R^3	R^4	Vol (Å^3)[a]	IC_{50} (μM)[b]		SI[c]
						COX-1	COX-2	
4	Me	SO_2N_3	H	H	312.6	3.3	>100	<0.033
8a	Me	H	SO_2N_3	H	312.6	>100	5.16	>19.36
8b	OMe	H	SO_2N_3	H	321.3	>500	>100	>5.00
8d	F	H	SO_2N_3	H	300.4	38.1	>100	<0.38
8f	Me	SO_2NH_2	H	SO_2N_3	357.9	0.59	178.81	0.0032
1	Me	SO_2NH_2	H	H	279.4	22.9	0.0507	404

[a]The volume of the molecule, after minimization using the MM3 force field, was calculated using the Alchemy 2000 program.
[b]The in vitro test compound concentration required to produce 50% inhibition of COX-1 or COX-2. The result (IC_{50}, μM) is the mean of two determinations.
[c]Selectivity Index (SI) = COX-1 IC_{50}/COX-2 IC_{50}.

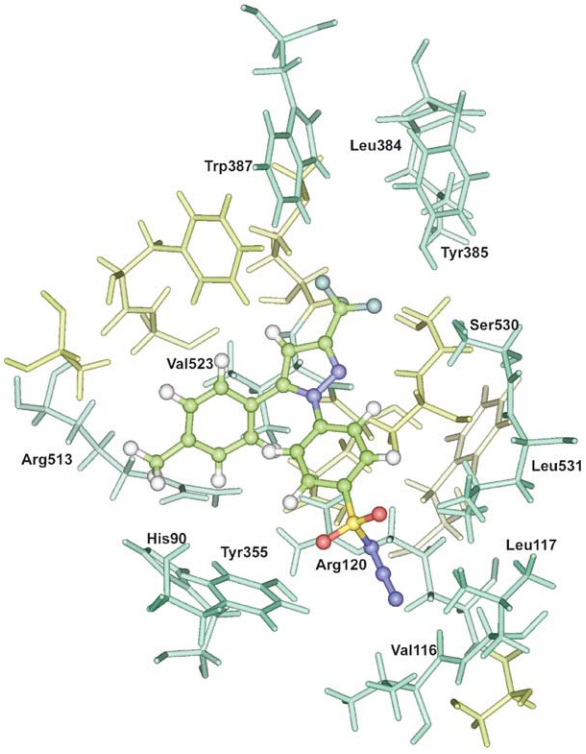


Figure 3. Docking the pyrazole **4** (ball and stick) in the active site of murine COX-2.

involving Arg^{120}, Glu^{524}, Tyr^{355} and His/Arg^{513} at the mouth of the channel by different ligands and their effect on COX inhibition.[5] The SO_2N_3 terminal *N*-atom is located about 4.93 Å from the O*H* of Tyr^{355}, a SO_2 *O*-atom of the SO_2N_3 substituent is about 4.30 Å away from the O*H* of Tyr^{355}, and the C-5 phenyl ring is oriented toward Val^{523}. The C-3 CF_3 substituent is oriented towards a hydrophobic pocket comprised of Leu^{384}, Tyr^{385} and Trp^{387}, and it is positioned about 3.32 Å from the O*H* of Tyr^{385} and about 4.92 Å from the O*H* of Ser^{530}. The N^2-atom of the central pyrazole ring is about 5.88 Å away from the O*H* of Ser^{530}. Recent studies have shown the importance of ionic interactions involving the CO_2H group of NSAIDs with Arg^{120} and its critical role in COX-1 inhibition.[18,19] The in vitro COX inhibition data showing that **4** is a selective COX-1 inhibitor are consistent with this docking experiment which indicates that the dipolar N_3 moiety of the SO_2N_3 substituent is interacting with Arg^{120} at the mouth of the primary binding site, like the CO_2H group of NSAIDs, rather than insertion into the 2°-pocket of the COX-2 isozyme near Val^{523} that is required for selective COX-2 inhibition.

It was anticipated that relocation of the SO_2N_3 substituent from the *para*- to the *meta*-position of the N^1-phenyl ring of compound **4** may result in a more suitable orientation within the COX-2 binding side that could provide selective COX-2 inhibition. In this regard, in vitro COX inhibition studies showed that 3-[5-(4-methylphenyl)-3-trifluoromethylpyrazol-1-yl)benzenesulfonylazide (**8a**) is a selective COX-2 inhibitor (COX-2 $IC_{50} = 5.16\,\mu M$; COX-1 $IC_{50} > 100\,\mu M$) with a COX-2 selectivity index > 19 (see data in Table 1). The selective in vitro COX-2 inhibition exhibited by **8a** is consistent with observations from a molecular modeling experiment where **8a** was docked in the active site of the COX-2 isozyme (see Fig. 4). Compound **8a** binds in the center of the active site with the phenylsulfonyl azide moiety oriented towards the 2°-pocket region. The terminal *N*-atom of the *m*-SO_2N_3 is inserted about 4.25 Å deep inside the entrance to the 2°-pocket of COX-2 (Val^{523}) and about 5.20 Å removed (within electrostatic ion-ion interaction distance) from the NH_2 of Arg^{513}. A SO_2 oxygen atom of the SO_2N_3 group is about 2.74 Å away (within *H*-bonding distance) from the NH_2 of Arg^{513}. The C-3 CF_3 substituent is positioned about 5.63 Å from the NH_2 of Arg^{120} and the N^2-nitrogen atom of the central pyrazole ring is located about 3.11 Å from the O*H* of Tyr^{355}. The center of the C-5 phenyl ring is about 5.87 Å from the *O*H of Ser^{530} with the Me group at the *para*-position orienting in a hydrophobic region surrounded by Leu^{384}, Tyr^{385} and Trp^{387}. Molecular modeling studies of 1,5-diarylpyrazoles have shown that stereoelectronic effects of substituents at the *para*-position of the C-5 phenyl ring are determinants of cyclooxygenase inhibition where *H*-bonding, and electron-withdrawing, substituents exhibit diminished COX-1 and COX-2 binding affinity.[20] Accordingly, among this group of celecoxib analogues **8**, compound **8a** with a SO_2N_3 moiety at the *meta*-position of the N^1-phenyl ring and a *para*-Me substituent on the C-5 phenyl ring was the most potent and selective COX-2 inhibitor. Introduction of a *para*-OMe substituent on the C-5 phenyl ring abolished both COX-1 and COX-2 inhibitory activity, and compound **8d** having a 5-(4-fluorophenyl) substituent selectively inhibited COX-1 (COX-1 $IC_{50} = 38.1\,\mu M$; COX-2 IC_{50}

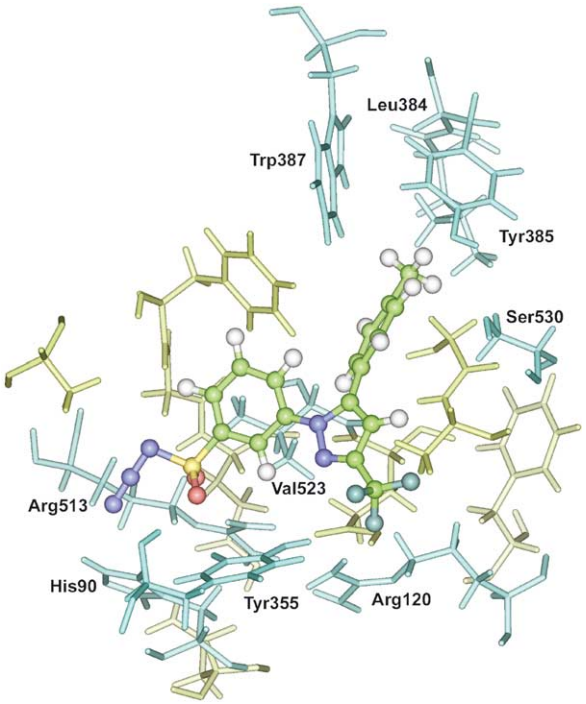


Figure 4. Docking the pyrazole **8a** (ball and stick) in the active site of murine COX-2.

$>100\,\mu M$). Introduction of a SO_2N_3 substituent at the *ortho*-position of the N^1-phenyl ring of celecoxib produced **8f** which was a more selective inhibitor of COX-1 ($IC_{50}=0.59\,\mu M$) than COX-2 ($IC_{50}=178.8\,\mu M$).

Conclusions

It has been demonstrated that the *meta*-sulfonylazido analogue **8a** of celecoxib (**1**) is a selective COX-2 inhibitor. Molecular modeling (docking) studies showed that the sulfonylazido (SO_2N_3) group of **8a** inserts deep inside the 2°-pocket of the COX-2 isozyme where it can undergo both *H*-bonding via one of its SO_2 oxygen-atoms, and an electrostatic ion–ion interaction via its terminal azide nitrogen atom, with the guanidino NH_2 group of Arg^{513}. These results indicate that a suitably positioned sulfonylazido moiety is a potentially novel dual *H*-bonding/electrostatic pharmacophore for the design of potent COX-2 inhibitors with a high COX-2 selectivity index.

Experimental

Melting points were determined using a Buchi capillary apparatus and are uncorrected. Infrared (IR) spectra were recorded as films on NaCl plates with a Nicolet 550 Series II Magna FT-IR spectrometer. ^1H NMR, ^{13}C NMR and ^{19}F NMR spectra were recorded on a Bruker AM-300 spectrometer, where *J* (coupling constant) values are estimated in Hz. ^{13}C NMR spectra were acquired using the *J* modulated spin echo technique where methyl and methine carbons appear as positive peaks and methylene and quaternary carbon resonances appear as negative peaks. Elemental Analysis (EA) were performed for C, H and N (Micro-analytical Service Laboratory, Department of Chemistry, University of Alberta, Canada). Silica gel column chromatography was performed using Merck silica gel 60 ASTM (70–230 mesh). Compounds **3**,[1,7] **5a–e**,[1] **6b**,[12] and **7f**[1] were prepared using literature methods. All other reagents were purchased from the Aldrich Chemical Company (Milwaukee, WI, USA) and used without further purification.

Synthesis of 4-[5-(4-methylphenyl)-3-trifluoromethyl-1*H*-pyrazol-1-yl]benzenesulfonyl azide (4). Sodium nitrite (0.42 g, 4.94 mmol) was added slowly to a solution of the amine **3**[7] (0.14 g, 0.44 mmol) in concentrated HCl (5 mL) at 0 °C with stirring. This mixture was stirred for 30 min at 0–5 °C in the dark and the 4-[5-(4-methylphenyl)-3-trifluoromethyl-1*H*-pyrazol-1-yl]benzenediazonium chloride produced in situ was added slowly with stirring to a solution of 37% w/v SO_2 in HOAc (3.7 mL, 21.34 mmol), $CuCl_2$ (0.19 g, 2.11 mmol), KCl (0.51 g, 6.84 mmol), benzene (2 mL) and 1,4-dioxane (4 mL) at 5–10 °C. The reaction mixture was stirred for 30 min at 40–50 °C in the dark at which time nitrogen gas evolution was complete, the mixture was cooled to 25 °C, water (12 mL) was added, and the mixture was extracted with benzene (3 × 15 mL). The combined organic extracts were washed with 10% w/v aqueous NaOH (20 mL), the organic extract was dried (Na_2SO_4), and the solvent was removed in vacuo to yield the crude 4-[5-(4-methylphenyl)-3-trifluoromethyl-1*H*-pyrazol-1-yl]-benzenesulfonyl chloride as a brown syrup.[10] Dissolution of this syrup in acetone (2 mL) and then drop wise addition to a stirred solution of NaN_3 (0.04 g, 0.61 mmol) in water (2 mL) at −10 °C to initiate the azidation reaction.[11] The reaction was allowed to proceed for 3 h at 0 °C with stirring. After warming to 25 °C, the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography using *n*-hexane–EtOAc (2:1, v/v) as eluent to afford the benzenesulfonyl azide **4** (0.04 g, 22%) as a white solid; mp 130–132 °C; IR (film) ν3100, 3020 (CH_{arom}), 2138 (N_3), 1168, 1335 (SO_2) cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ 7.34 (d, 2H, $J=8.8$ Hz, benzenesulfonyl azido H-2, H-6), 7.26 (d, 2H, $J=8.8$ Hz, benzenesulfonyl azido H-3, H-5), 7.16 (d, 2H, $J=8.5$ Hz, 4-methylphenyl H-2, H-6), 7.10 (d, 2H, $J=8.5$ Hz, 4-methylphenyl H-3, H-5), 6.72 (s, 1H, pyrazole H-4), 2.37 (s, 3H, CH_3); ^{13}C NMR (75 MHz; $CDCl_3$) δ 144.83 (pyrazole C-5), 144.34 (q, $J_{CCF}=38.4$ Hz, pyrazole C-3), 139.32 (benzenesulfonyl azido C-4), 137.82 (4-methylphenyl C-4), 134.14 (benzenesulfonyl azido C-1), 129.50, 129.21 and 128.65 (benzenesulfonyl azido C-2, C-6; 4-methylphenyl C-3, C-5 and C-2, C-6), 126.56 (benzenesulfonyl azido C-3, C-5), 126.00 (4-methylphenyl C-1), 121.19 (q, $J_{CF}=269.1$ Hz, CF_3), 105.59 (pyrazole C-4), 21.31 (CH_3). Anal. calcd for $C_{17}H_{12}F_3N_5O_2S$: C, 50.12; H, 2.97; N, 17.19. Found: C, 50.21; H, 2.83; N, 17.28.

General procedure for synthesis of 1-phenyl-5-(4-methylphenyl)-3-trifluoromethyl-1*H*-pyrazole (7a)

Phenylhydrazine hydrochloride **6a** (1.66 g, 11.48 mmol) was added to a solution of the 4,4,4-trifluoro-1-(4-methylphenyl)butane-1,3-dione **5a**[1] (2.60 g, 11.29 mmol) in 95% ethanol (100 mL) with stirring and the reaction was allowed to proceed at reflux for 24 h. After cooling to 25 °C, the solvent was removed in vacuo. Water (40 mL) was added to the residue that was extracted with EtOAc (3 × 50 mL), the combined organic extracts were dried (Na_2SO_4) and the solvent was removed in vacuo to give a brown syrup. Purification of this product by silica gel column chromatography using *n*-hexane–EtOAc (1:1, v/v) as eluent afforded **7a** (1.92 g, 55%) as a yellow syrup; IR (film) ν 3137, 3055 (CH_{arom}) cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ 7.30–7.38 (m, 5H, N^1-phenyl hydrogens), 7.12 (d, 2H, $J=8.5$ Hz, 4-methylphenyl H-2, H-6), 7.14 (d, 2H, $J=8.5$ Hz, 4-methylphenyl H-3, H-5), 6.73(s, 1H, pyrazole H-4), 2.36 (s, 3H, CH_3). Anal. calcd for $C_{17}H_{13}F_3N_2$: C, 63.74; H, 4.68; N, 8.74. Found: C, 64.18; H, 4.41; N, 8.44.

Compounds **7b–e** were prepared using a similar procedure where **5b–e** were used in place of **5a**, and **7f** was obtained from the reaction of **5a** with **6b** according to a literature method.[1] Physical and spectral data for **7b–e** are listed below.

5-(4-Methoxyphenyl)-1-phenyl-3-trifluoromethyl-1*H*-pyrazole (7b). Brown syrup (92%); IR (film) ν 3137, 3070 (CH_{arom}) cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ

7.31–7.38 (m, 5H, N^1-phenyl hydrogens), 7.15 (d, 2H, $J = 9.0$ Hz, 4-methoxyphenyl H-2, H-6), 6.85 (d, 2H, $J = 9.0$ Hz, 4-methoxyphenyl H-3, H-5), 6.70 (s, 1H, pyrazole H-4), 3.81 (s, 3H, OCH_3); ^{13}C NMR (75 MHz; $CDCl_3$) δ 159.40 (4-methoxyphenyl C-4), 144.49 (pyrazole C-5), 142.77 (q, $J_{CCF} = 38.4$ Hz, pyrazole C-3), 132.95 (N^1-phenyl C-1), 129.23 (4-methoxyphenyl C-1), 128.74, 128.68, 128.54 and 126.77 (4-methoxyphenyl C-2, C-6, N^1-phenyl C-2, C-6; C-3, C-5; C-4), 121.28 (q, $J_{CF} = 269.1$ Hz, CF_3), 114.17 (4-methoxyphenyl C-3, C-5), 105.08 (pyrazole C-4), 55.48 (OCH_3). Anal. calcd for $C_{17}H_{13}F_3N_2O$: C, 64.15; H, 4.12; N, 8.80. Found: C, 64.39; H, 4.01; N, 8.45.

5-(4-Chlorophenyl)-1-phenyl-3-trifluoromethyl-1*H*-pyrazole (7c). Yellow syrup (66%); IR (film) ν 3136, 3044 (CH_{arom}) cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ 7.32–7.40 (m, 3H, N^1-phenyl H-3, H-4, H-5), 7.29–7.31 (m, 4H, N^1-phenyl H-2, H-6, 4-chlorophenyl H-2, H-6), 7.15 (d, 2H, $J = 8.5$ Hz, 4-chlorophenyl H-3, H-5), 6.75 (s, 1H, pyrazole H-4). Anal. calcd for $C_{16}H_{10}ClF_3N_2$ requires C, 58.72; H, 3.20; N, 8.55. Found: C, 58.98; H, 3.39; N, 8.57.

5-(4-Fluorophenyl)-1-phenyl-3-trifluoromethyl-1*H*-pyrazole (7d). Brown syrup (58%); IR (film) ν 3145, 3065 (CH_{arom}) cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ 7.20–7.42 (m, 7H, N^1-phenyl hydrogens, 4-fluorophenyl H-2, H-6), 7.07 (d, 2H, $J_{HCCF} = 8.5$ Hz of d, $J_{HCCH} = 8.5$ Hz, 4-fluorophenyl H-3, H-5), 6.76 (s, 1H, pyrazole H-4). Anal. calcd for $C_{16}H_{10}F_4N_2$: C, 62.75; H, 3.29; N, 9.15. Found: C, 62.72; H, 3.06; N, 9.09.

5-(4-Bromophenyl)-1-phenyl-3-trifluoromethyl-1*H*-pyrazole (7e). Brown solid (39%); mp 92–94 °C; IR (film) ν 3116, 3050 (CH_{arom}) cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ 7.46 (d, 2H, $J = 8.8$ Hz, 4-bromophenyl H-3, H-5), 7.25–7.40 (m, 5H, N^1-phenyl hydrogens), 7.09 (d, 2H, $J = 8.8$ Hz, 4-bromophenyl H-2, H-6), 6.76 (s, 1H, pyrazole H-4). Anal. calcd for $C_{16}H_{10}BrF_3N_2$: C, 52.34; H, 2.75; N, 7.63. Found: C, 52.30; H, 2.60; N, 7.22.

General procedure for synthesis of 3-[5-(4-methylphenyl)-3-trifluoromethylpyrazol-1-yl]benzenesulfonyl azide (8a)

Chlorosulfonic acid (3.3 mL, 49.64 mmol) was added drop wise to the ice-cold 1-phenyl-5-(4-methylphenyl)-3-trifluoromethyl-1*H*-pyrazole **7a** (3.0 g, 9.92 mmol) with vigorous stirring. The cooling bath was removed, the mixture was stirred for 1 h at 25 °C, and then poured into crushed ice (50 g) very slowly. Extraction with EtOAc (3 × 60 mL), washing the combined organic extracts with water, and removal of the solvent in vacuo afforded a brown syrup.[12] This intermediate product, 3-[5-(4-methylphenyl)-3-trifluoromethylpyrazol-1-yl]benzenesulfonyl chloride, was dissolved in acetone (10 mL) and added drop wise to a stirred solution of NaN_3 (1.0 g, 15.38 mmol) in water (10 mL) at −10 °C.[11] The reaction was allowed to proceed for 3 h at 0 °C, the solvent was removed in vacuo, and water (30 mL) was added to the residue. Extraction with EtOAc (3 × 40 mL), drying the combined organic extracts (Na_2SO_4), and removal of the solvent in vacuo gave a yellow syrup which was purified by silica gel column chromatography using *n*-hexane–EtOAc (2:1, v/v) as eluent to give **8a** (2.48 g, 61%) as pale yellow needles; mp 65–67 °C; IR (film) ν 3133, 3083 (CH_{arom}), 2125 (N_3), 1183, 1356 (SO_2) cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ 7.89 (d, 1H, $J = 1.53$ Hz, benzenesulfonyl azido H-2), 7.28–7.44 (m, 7H, benzenesulfonyl azido H-4, H-5, H-6, 4-methylphenyl H-2, H-3, H-5, H-6), 6.86 (s, 1H, pyrazole H-4), 2.67 (s, 3H, CH_3); ^{13}C NMR (75 MHz; $CDCl_3$) δ 143.38 (q, $J_{CCF} = 38.4$ Hz, pyrazole C-3), 142.12 (pyrazole C-5), 139.12, 138.63 (benzenesulfonyl azido C-1 and C-3), 137.55 (4-methylphenyl C-4), 134.11, 133.49 (benzenesulfonyl azido C-2 and C-6), 129.45, 129.15 (4-methylphenyl C-2, C-6, C-3, C-5), 127.87 (4-methylphenyl C-1), 125.68 (benzenesulfonyl azido C-4, C-5), 121.02 (q, $J_{CF} = 269.1$ Hz, CF_3), 105.91 (pyrazole C-4), 20.26 (CH_3); δ_F (282 MHz; $CDCl_3$) 99.40 (s, CF_3). Anal. calcd for $C_{17}H_{12}F_3N_5O_2S$: C, 50.12; H, 2.97; N, 17.19. Found: C, 50.34; H, 2.81; N, 16.93.

Compounds **8b–f** were prepared using a similar procedure where **7b–f** were used in place of **7a**. The physical and spectral data for **8b–f** are listed below.

3-[5-(4-Methoxyphenyl)-3-trifluoromethylpyrazol-1-yl]-benzenesulfonyl azide (8b). Yellow solid (70%); mp 121–123 °C; IR (film) ν 3141, 3067 (CH_{arom}), 2142 (N_3), 1165, 1355 (SO_2) cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ 7.86 (d, 1H, $J = 2.1$ Hz, benzenesulfonyl azido H-2), 7.29–7.43 (m, 6H, benzenesulfonyl azido H-5, H-6, 4-methoxyphenyl H-2, H-3, H-5, H-6), 7.03 (d, 1H, $J = 8.8$ Hz, benzenesulfonyl azido H-4), 6.79 (s, 1H, pyrazole H-4), 4.05 (s, 3H, OCH_3); ^{13}C NMR (75 MHz; $CDCl_3$) δ 157.04 (4-methoxyphenyl C-4), 143.19 (q, $J_{CCF} = 38.5$ Hz, pyrazole C-3), 142.00 (pyrazole C-5), 138.61 (benzenesulfonyl azido C-1), 136.08 (benzenesulfonyl azido C-2), 130.49 (benzenesulfonyl azido C-6), 129.39 (4-methoxyphenyl C-2, C-6), 129.00 (benzenesulfonyl azido C-4), 128.03 (benzenesulfonyl azido C-3), 125.61 (benzenesulfonyl azido C-5), 121.93 (4-methoxyphenyl C-1), 119.1 (q, $J_{CF} = 269.0$ Hz, CF_3), 112.61 (4-methoxyphenyl C-3, C-5), 105.71 (pyrazole C-4), 56.67 (OCH_3). Anal. calcd for $C_{17}H_{12}F_3N_5O_3S$: C, 48.02; H, 2.86; N, 16.46. Found: C, 48.34; H, 2.79; N, 16.06.

3-[5-(4-Chlorophenyl)-3-trifluoromethylpyrazol-1-yl]benzenesulfonyl azide (8c). Yellow syrup (46%); IR (film) ν 3110, 3084 (CH_{arom}), 2131 (N_3), 1150, 1355 (SO_2) cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ 7.98 (d, 1H, $J = 2.1$ Hz, benzenesulfonyl azido H-2), 7.16–7.70 (m, 7H, chlorophenyl hydrogens, benzenesulfonyl azido H-4, H-5, H-6), 6.88 (s, 1H, pyrazole H-4); ^{13}C NMR (75 MHz; $CDCl_3$) δ 143.52 (q, $J_{CCF} = 39.5$ Hz, pyrazole C-3), 141.09 (pyrazole C-5), 138.24, 137.08 (benzenesulfonyl azido C-1, C-3), 133.14 (4-chlorophenyl C-4), 128.73 (4-chlorophenyl C-1), 134.69, 132.55, 131.04, 129.57, 129.35, 125.58 (benzenesulfonyl azido and chlorophenyl C_{arom}–H), 120.84 (q, $J_{CF} = 269.1$ Hz, CF_3), 106.26 (pyrazole C-4). Anal. calcd for $C_{16}H_9ClF_3N_5O_2S$: C, 44.92; H, 2.12; N, 16.37. Found: C, 45.02; H, 2.15; N, 16.68.

3-[5-(4-Fluorophenyl)-3-trifluoromethylpyrazol-1-yl]benzenesulfonyl azide (8d). Pale yellow solid (54%); mp 123–125 °C; IR (film) ν 3100, 3050 (CH_{arom}), 2125 (N_3), 1380, 1150 (SO_2) cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ 7.91 (d, 1H, $J = 2.1$ Hz, benzenesulfonyl azido H-2), 7.06–7.16 (m, 4H, aryl hydrogens), 7.18–7.32 (m, 3H, aryl hydrogens), 6.85 (s, 1H, pyrazole H-4); ^{19}F NMR (282 MHz; $CDCl_3$) (proton decoupled spectrum) δ 104.0 (s, 3F, CF_3), 50.2 (s, 1F, F-C_6H_4–). Anal. calcd for $C_{16}H_9F_4N_5O_2S$: C, 46.72; H, 2.21; N, 17.0. Found: C, 46.65; H, 2.23; N, 17.21.

3-[5-(4-Bromophenyl)-3-trifluoromethylpyrazol-1-yl]benzenesulfonyl azide (8e). Yellow syrup (63%); IR (film) ν 3165, 3100 (CH_{arom}), 2140 (N_3), 1380, 1180 (SO_2) cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ 7.97 (dt, 1H, $J = 7.9$, 1.8, 1.8 Hz, benzenesulfonyl azido H-6), 7.84 (dd, 1H, $J = 1.8$, 1.8 Hz, benzenesulfonyl azido H-2), 7.62 (dd, 1H, $J = 7.9$, 7.9 Hz, benzenesulfonyl azido H-5), 7.54 (dt, 1H, $J = 7.9$, 1.8, 1.8 Hz, benzenesulfonyl azido H-4), 7.40 (d, 2H, $J = 8.5$ Hz, 4-bromophenyl H-2, H-6), 7.26 (d, 2H, $J = 8.5$ Hz, 4-bromophenyl H-3, H-5), 6.89 (s, 1H, pyrazole H-4); ^{13}C NMR (75 MHz; $CDCl_3$) δ 143.63 (q, $J_{CCF} = 38.4$ Hz, pyrazole C-3), 141.17 (pyrazole C-5), 138.92 and 138.31 (benzenesulfonyl azido C-1, C-3), 129.29 (4-bromophenyl C-1), 121.24 (4-bromophenyl C-4), 136.16, 134.52, 131.39, 129.63, 129.39, 125.65 (C_{arom}–H), 120.86 (q, $J_{CF} = 268.0$ Hz, CF_3), 106.25 (pyrazole C-4). Anal. calcd for $C_{16}H_9BrF_3N_5O_2S$: C, 40.69; H, 1.92; N, 14.83. Found: C, 40.88; H, 1.84; N, 14.64.

5-Sulfonamido-2-[5-(4-methylphenyl)-3-trifluoromethylpyrazol-1-yl]benzenesulfonyl azide (8f). White solid (45%); mp 176–178 °C; IR (film) ν 3400, 3300 (NH_2), 3150, 3100 (CH_{arom}), 2135 (N_3), 1350, 1150 (SO_2) cm^{-1}; ^1H NMR (300 MHz; DMSO-d_6) δ 7.97 (1H, s, benzenesulfonyl azido H-6), 7.92 (2H, two overlapping d, $J = 8.5$ Hz, benzenesulfonyl azido H-3, H-4), 7.59–7.62 (4H, m, 4-methylphenyl hydrogens), 7.53 (2H, s, NH_2), 7.46 (1H, s, pyrazole H-4), 2.61 (3H, s, CH_3); ^{13}C NMR (75 MHz; DMSO-d_6) δ 144.25 (pyrazole C-5), 142.85 (benzenesulfonyl azido C-5), 142.15 (q, $J_{CCF} = 37.3$ Hz, pyrazole C-3), 140.52 (benzenesulfonyl azido C-2), 138.83 (4-methylphenyl C-4), 136.37 (benzenesulfonyl azido C-1), 135.20 (benzenesulfonyl azido C-6), 133.67 (benzenesulfonyl azido C-4), 129.11 (benzenesulfonyl azido C-3), 126.92 (4-methylphenyl C-1), 126.82 and 126.09 (4-methylphenyl C-2, C-3, C-5, C-6), 121.10 (q, $J_{CF} = 268.0$ Hz, CF_3), 107.15 (pyrazole C-4), 19.53 (CH_3). Anal. calcd for $C_{17}H_{13}F_3N_6O_4S_2$: C, 41.97; H, 2.69; N, 17.28. Found: C, 42.33; H, 2.65; N, 17.06.

Molecular modeling (docking) studies

Docking studies were performed using Insight II software (Version 97) running on a Silicon Graphics Indigo 2 workstation.[21] The coordinates for the X-ray crystal structure of the selective COX-2 inhibitor SC-558 bound to the murine COX-2 enzyme was obtained from the RCSB Protein Data Bank (1cx2) and hydrogens were added. The ligand molecules were constructed using the Builder module and were energy minimized for 1000 iterations reaching a convergence of 0.01 kcal/mol Å. The energy minimized ligands were superimposed on SC-558 in the PDB file 1cx2 after which SC-558 was deleted. The resulting structure was subjected to docking using the Affinity command in the Docking module of Insight II by defining subsets of the enzyme such that residues within 10 Å of the ligand were allowed to relax, while the rest of the enzyme residues were fixed. The ESFF (force field) was employed for all docking purposes. The optimized ligand-enzyme complex was further subjected to molecular dynamics simulation at a constant temperature of 300 K for over 1000 fs with a time step of 1 fs.

Volume determination

The Alchemy 2000 program[14] was used to calculate the molecular volume ($Å^3$) of compounds **4** and **8** after minimization using PM3.

In vitro cyclooxygenase inhibition studies

The compounds listed in Table 1 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, USA) using the method previously reported.[22]

Acknowledgements

We are grateful to the Canadian Institutes of Health Research (MOP-14712) for financial support of this research, to the Alberta Heritage Foundation for Medical Research for a postdoctoral fellowship to one of us (MJU), and to Rx&D-HRF/CIHR for a graduate scholarship to one of us (PR).

References and Notes

1. Penning, T. D.; Talley, J. J.; Bertenshaw, S. R.; Carter, J. S.; Collins, P. W.; Docter, S.; Graneto, M. J.; Lee, L. F.; Malecha, J. W.; Miyashiro, J. M.; Rogers, R. S.; Rogers, D. J.; Yu, S. S.; Anderson, G. D.; Burton, E. G.; Cogburn, J. N.; Gregory, S. A.; Koboldt, C. M.; Perkins, W. E.; Seibert, K.; Veenhuizen, A. W.; Zhang, Y. Y.; Isakson, P. C. *J. Med. Chem.* **1997**, *40*, 1347.
2. Goldstein, J. L.; Silverstein, F. E.; Agrawal, N. M.; Hubbard, R. C.; Kaiser, J.; Maurath, C. I.; Verburg, K. M.; Geis, G. S. *Am. J. Gastroenterol.* **2000**, *95*, 1681.
3. Kurumbail, R. G.; Stevens, A. M.; Gierse, J. K.; McDonald, J. J.; Stegeman, R. A.; Pak, J. Y.; Gildehaus, D.; Miyashiro, J. M.; Penning, T. D.; Seibert, K. P.; Isakson, C.; Stallings, W. C. *Nature* **1996**, *384*, 644.
4. Luong, C.; Miller, A.; Barnett, J.; Chow, J.; Ramesha, C.; Browner, M. F. *Nat. Struct. Biol.* **1996**, *3*, 927.
5. Llorens, O.; Perez, J. L.; Palomer, A.; Mauleon, D. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2779.
6. Garavito, R. M.; DeWitt, D. L. *Biochim. Biophys. Acta* **1999**, *1441*, 278.
7. Habeeb, A. G.; Rao, P. N. P.; Knaus, E. E. *J. Med. Chem.* **2001**, *44*, 3039.
8. Habeeb, A. G.; Rao, P. N. P.; Knaus, E. E. *J. Med. Chem.* **2001**, *44*, 2921.

9. Rahim, M. A.; Rao, P. N. P.; Knaus, E. E. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 2753.
10. Gilbert, E. E. *Synthesis* **1969**, 3.
11. Leffler, J. E.; Tsuno, Y. *J. Org. Chem*. **1962**, *28*, 902.
12. Soliman, R. *J. Med. Chem*. **1979**, *22*, 321.
13. Hashimoto, H.; Imamura, K.; Haruta, J.-I.; Wakitani, K. *J. Med. Chem*. **2002**, *45*, 1511.
14. *Alchemy 2000* (program), Version 2.0; Tripos Inc.: St. Louis, MO, USA.
15. Lieber, E.; Curtice, J.; Rao, C. *Chem. Ind*. **1966**, 586.
16. Pauling, L. In *The Nature of the Chemical Bond*, 3rd ed.; Cornell University Press: Ithaca, NY, 1960.
17. Livingston, R.; Rao, R. R. *J. Phys. Chem*. **1960**, *64*, 756.
18. Greig, G. M.; Francis, D. A.; Falgueyret, J. P.; Ouellet, M.; Percival, M. D.; Roy, P.; Bayly, C.; Mancini, J. A.; O'Neill, G. P. *Mol. Pharmacol*. **1997**, *52*, 829.
19. Selinsky, B. S.; Gupta, K.; Sharkey, C. T.; Loll, P. J. *Biochemistry* **2001**, *40*, 5172.
20. Price, M. L. P.; Jorgensen, W. L. *J. Am. Chem. Soc*. **2000**, *122*, 9455.
21. *Insight II,* Version 97 and *Discover,* Version 2.98; Molecular Simulations, Inc: San Diego, CA, USA, 1997.
22. Habeeb, A. G.; Rao, P. N. P.; Knaus, E. E. *Drug Dev. Res*. **2000**, *51*, 273.