Articles

Antiinflammatory 4,5-Diarylpyrroles. 2. Activity as a Function of Cyclooxygenase-2 Inhibition

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The antiinflammatory activity of a series of 2-substituted- and 2,3-disubstituted-4-(4-fluorophenyl)-5-[4-(methylsulfonyl)phenyl]-1*H*-pyrroles was previously shown by quantitative structure–activity relationship (QSAR) studies to be correlated with the molar refractivity and inductive field effect of the 2-substituent and the lipophilicity of the 3-substituent. The present study demonstrates that much of the antiinflammatory activity of these pyrroles could be correlated with the inhibition of the inducible isoform of cyclooxygenase (COX2). Additional QSAR studies have been used to identify the molecular parameters necessary for maximizing COX2 inhibition while simultaneously minimizing the inhibition of constitutively expressed cyclooxygenase-1. Such an effort should facilitate the discovery and development of selective COX inhibitors that should lead to safer nonsteroidal antiinflammatory drugs.

Introduction

With the discovery that cyclooxygenase (COX) existed in two isoforms (COX1 and -2) and that one form (COX2) could be induced by mitogens and inflammatory mediators,[1–4] we have reexamined some of our orally active antiinflammatory agents for their ability to inhibit COX1 and COX2. One such set of compounds was the 2-substituted- and 2,3-disubstituted-4-(4-fluorophenyl)-5-[4-(methylsulfonyl)phenyl]pyrroles.[5,6] The pyrroles (Figure 1) were of interest because at the time of their original synthesis and pharmacological evaluation, we did not fully understand the mechanism of action for this class of compounds. They were orally active in the rat adjuvant arthritis model (AA),[7–10] but none was active against bovine seminal prostaglandin synthase (BSV PGS),[11] and none was active in the rat carrageenan paw edema model (CAR).[12] The compounds were also inactive in the mouse phenylquinone writhing (PQW)[13,14] model of analgesia and were not ulcerogenic in the animal model.[6] Since CAR, PQW, and gastrointestinal (GI) toxicity were sensitive to COX inhibition,[15–17] we concluded that the pyrroles, as well as other diaryl heterocyclic systems (imidazoles[18] and thiophenes[13]), were not antiinflammatory through COX inhibition. Since the discovery of COX2 and the realization that its induction is caused by proinflammatory stimuli, the hypothesis has been set forth that the *in vivo* antiinflammatory efficacy of nonsteroidal antiinflammatory drugs (NSAIDs) is the result of the ability of these compounds to act as potent inhibitors of this isozyme specifically. There have been several reports of potent antiinflammatory activity in animal models for compounds that are now known to be selective COX2 inhibitors.[13,19] Until now, however, no one has demonstrated a direct correlation between COX2 inhibition and *in vivo* antiinflammatory efficacy within a single class of compounds. Such a demonstration would significantly bolster the validity of the above-mentioned hypothesis. The objective was to determine to what degree the diarylpyrroles inhibited both COX1 and COX2, determine if the inhibition of COX1 and/or COX2 could be correlated with AA activity; and generate quantitative structure–activity relationship (QSAR) equations that might help in understanding the mechanism of action of the compound and facilitate the discovery and optimization of new and safer antiinflammatory drugs.

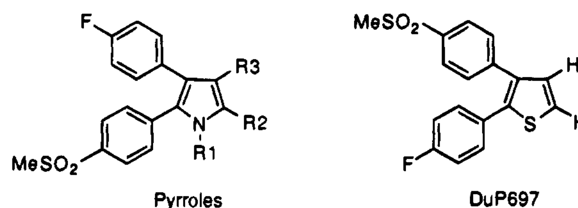

Figure 1. Antiinflammatory diaryl heterocycles.

Chemistry and Biology

The synthesis, chemical characterization, and oral rat adjuvant arthritis results for the compounds in this study have previously been reported.[5,6]

The anti-COX activities were obtained as a result of a screening program that used the methods described by Copeland et al.[20] All COX1 data were obtained with the ovine enzyme (Caymen), and the COX2 data were obtained with recombinant human COX2 that was produced via expression from baculovirus.[21] For selected compounds, the IC_{50} values were determined also for recombinant human COX1, expressed and purified as described by George et al.[21] In all cases tested, there was no significant difference in inhibitor potency between the human and ovine COX1. As pointed out by Copeland et al.,[20] the best measure of potency for a time dependent COX2 inhibitor is the second-order rate constant obtained by dividing k_{inact}, the maximal rate of inactivation, by K_i, the concentration of inhibitor that displays half-maximal inactivation kinetics. The kinetic

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analysis of this kind is too cumbersome to be performed routinely for a large set of inhibitors, as studied here. If, however, the dose–response measurements of enzyme activity are performed with a reasonably long preincubation of inhibitor with the enzyme (in the case of these inhibitors, ca. 2 min), then the observed IC_{50} values will reflect the influences of both k_{inact} and K_i as described in eqs 2–4. For a time dependent inhibitor, the remaining enzymatic activity after a given preincubation time (t) is given by eq 1:

$$\%\text{control} = \frac{100}{1 + \left(\frac{IC_{50}}{[I]}\right)^n} = Ae^{(-k_{obs}t)} \quad (1)$$

Here k_{obs} is the observed first-order rate constant describing the diminution of enzymatic activity with preincubation time at a given inhibitor concentration, [I], and A is the extrapolated percent control activity at time zero (i.e., with no preincubation time). The observed rate constant is related to k_{inact} and K_i by the hyperbolic equation:

$$k_{obs} = \frac{k_{inact}[I]}{K_i + [I]} \quad (2)$$

Substituting this equality into eq 1 and rearranging we obtain

$$IC_{50} = \frac{\left[\frac{100}{Ae^{-(k_{inact}[I]/K_i+[I]t)}} - 1\right]^{1/n}}{[I]} \quad (3)$$

Thus, as long as the preincubation time used is long compared to the observed rate constant for inactivation, the IC_{50} values measured should provide a good estimate of the relative inhibitor potency for COX2. On the basis of the reported kinetics for the related 4,5-diarylthiophenes (Figure 1)[13,22] and kinetic measurements for selected 4,5-diarylpyrroles, we determined that a 2 min preincubation of compound with the enzyme would meet these criteria (R. A. Copeland, unpublished results).

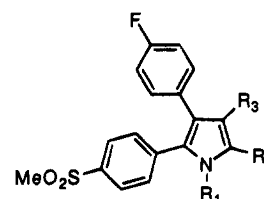
Standard multiple linear regression (MLR) techniques were used to determine the concentration that produced a 50% inhibition of enzyme activity expressed in micromolar units (IC_{50}, μM).[20] Each compound and standard was assayed at least twice for COX inhibitory activity, and the standard deviation in the reported IC_{50}s values in Table 1 has been found to be less than 20%. For the purposes of these QSAR studies and on the basis of previous pharmacokinetic data,[6] we have assumed that the bioavailability of these compounds was similar, except where noted.

Computer Methods and Statistics

Statistical analyses were conducted using JMP v3.0.2 by SAS Institute, Cary, NC. Computer-generated ClogP and CMR were obtained using MedChem Software v3.0, Pomona College, Claremont, CA. Statistical methods were in accord with Havilcek and Crain[23] and Dowdy and Wearden.[24]

QSAR studies used the following parameters: computer-calculated log P (ClogP) and molar refractivity (CMR or log CMR) for the molecule, proton nuclear magnetic resonances for the pyrrole proton (NH) in parts per million (NH ppm), substituent parameters π, MR, Swain–Lupton constants $\mathcal{R}$ and $\mathcal{F}$, and Sterimol parameters.[25,26] These parameters are listed in Table 2. The following statistical measures were used, n, the number of samples in the regression; r, correlation coefficient; SE, standard error of the regression; F-ratio; and the probability of finding a greater F-ratio. In the regression equations, the number in parentheses is the standard error of the estimate for the coefficient.

Table 1. Structural and Biological Data for Compounds **1–20**



compd	R1	R2	R3	AA ED_{50}, μM/kg[a]	IC_{50}, μM[b] COX1	IC_{50}, μM[b] COX2	COX1/COX2
1	H	H	H	>317.0	373.00	12.67	29.4
2	H	SCN	H	10.47	219.00	72.00	3.0
3	H	SMe	H	4.89	36.00	34.50	1.0
4	H	Br	H	2.66	132.50	40.00	3.3
5	H	$COCF_3$	H	47.16	580.00	298.20	2.0
6	H	I	H	49.86	>300	92.30	>3.2
7	H	SO_2Me	H	93.02	>300	267.00	>1.1
8	H	Cl	H	1.43	94.00	42.00	2.2
9	Me	Br	H	4.16	515.67	35.50	14.5
10	H	CN	H	3.82	nd	64.40	
11	H	NO_2	H	24.97	nd	63.00	
12	ᵗBoc	Br	H	32.00	>300	>300	
13	H	I	I	72.82	127.00	>300	<0.4
14	H	Br	Cl	9.98	nd	nd	
15	H	Cl	Cl	3.90	20.00	69.00	0.3
16	H	Cl	Br	7.93	44.00	88.00	0.5
17	COMe	Br	H	82.95	300.00	300.00	1.0
18	Me	Br	Br	8.54	766.67	55.67	13.8
19	Me	Cl	Cl	12.55	650.00	25.00	26.0
20	Me	H	H	>303.	>300.	20.00	>15.0
				Standards			
Ind				0.84	0.31	180.0	<0.1
Pbz				32.4	384.0	284.0	1.4
Ibp				484.8	9.0	8.0	1.1
Nap					2.7	9.7	0.3
Pir				1.3	21.0	218.0	0.1
Asp				1942.7	1280.0	18000.0	0.1

[a] Standard error of the mean <±20%. [b] Standard deviation of the mean <±20%. Ind = indomethacin; Pbz = phenylbutazone; Ibp = ibuprofen; Nap = naproxen; Pir = piroxicam; Asp = aspirin.

Results and Discussion

Oral Antiinflammatory Activity. A structure–activity relationship analysis on the compounds in Table 1 show that the 4-(4-fluorophenyl)-5-[4-(methylsulfonyl)-phenyl]pyrrole (**1**) core was varied at position R1, R2, and R3 (Figure 1). Immediately it was observed that **1** and **20** were inactive in the AA assay and that they were the only members of the set where R2 = H, suggesting that there was something special about the 2-substituted pyrroles. Our ability to draw correlations between *in vivo* and *in vitro* effects within a class of compounds depends on the assumption of similar pharmacokinetics within the class. These compounds tend to display lower bioavailability than analogs with substitutions at R2 (see Table 3). Thus, the lack of oral antiinflammatory activity of **1** and **20** may reflect their very different pharmacokinetics and pharmacodynamics when compared to the remaining 18 compounds. Working on the assumption that oral activity was a function of pharmacology and pharmacokinetics, we subjected **2–19** to

Table 2. Parameters Used To Generate the Regression Eqs 4a–8[a]

	log		R1		R2					
compd	COX1	COX2	π	MR	MR	$\mathcal{F}$	R3 π	ClogP	CMR	NMR NH
1	2.57	1.10	0.00	1.03	1.03	0.00	0.00	3.372	8.492	11.51
2	2.34	1.86	0.00	1.03	13.40	0.49	0.00	3.785	9.776	12.67
3	1.56	1.54	0.00	1.03	13.82	0.23	0.00	3.955	9.762	11.73
4	2.12	1.60	0.00	1.03	8.88	0.45	0.00	4.396	9.269	12.15
5	2.76	2.47	0.00	1.03	11.17	0.54	0.00	3.737	9.502	13.10
6		1.97	0.00	1.03	13.94	0.42	0.00	4.656	9.799	12.30
7		2.43	0.00	1.03	13.49	0.53	0.00	2.188	9.829	12.63
8	1.97	1.62	0.00	1.03	6.03	0.42	0.00	4.246	8.984	12.20
9	2.71	1.55	0.56	5.65	8.88	0.45	0.00	4.743	9.733	na
10		1.81	0.00	1.03	6.33	0.51	0.00	3.262	8.97	11.20
11		1.80	0.00	1.03	7.36	0.65	0.00	3.532	9.218	12.10
12			1.62	26.77	8.88	0.45	0.00	nc	11.777	na
13	2.10		0.00	1.03	13.94	0.42	1.12	5.86	11.105	12.38
14			0.00	1.03	8.88	0.45	0.71	4.91	9.761	12.70
15	1.30	1.84	0.00	1.03	6.03	0.42	0.71	5.04	9.475	12.67
16	1.64	1.94	0.00	1.03	6.03	0.42	0.86	4.91	9.761	12.75
17	2.48	2.48	−0.55	11.18	8.88	0.45	0.00	nc	10.233	na
18	2.88	1.75	0.56	5.65	8.88	0.45	0.86	5.356	10.51	na
19	2.81	1.40	0.56	5.65	6.03	0.42	0.71	5.336	9.939	na
20		1.30	0.56	5.65	1.03	0.00	0.00	3.819	8.956	na

[a] nc = not calculated; na = not applicable.

Table 3. Pharmacokinetic Studies in the Rat for Selected Antiinflammatory 4-(4-Fluorophenyl)-5-[4-(methylsulfonyl)phenyl]pyrroles[a]

compd	R_1	R_2	R_3	dose, μM/kg	$t_{1/2}$, h	V_d, L/kg	F	Cm_{ax}, μmol/L
1	H	H	H	iv, 15.9	9.1	7.6		
				po, 15.9	7.7		<0.1	<0.1
4	H	Br	H	iv, 6.3	10.2	3.5		2.0
				po, 12.7	9.9		1.06	3.0
8	H	Cl	H	iv, 7.1	6.1	9.0		1.4
				po, 14.3	5.8		1.00	2.3
9	CH_3	Br	H	iv, 12.2	13.7	10.0		2.9
				po, 12.2	4.3		0.64	1.5
10	H	CN	H	iv, 14.7	13.7	5.7		4.1
				po, 14.7	17.3		1.34	2.9

[a] $t_{1/2}$ = elimination half-life; V_d = volume of distribution; F = observed bioavailability; C_{max} = maximum blood level reached after an oral dose of drug.

Table 4. Correlation Matrix for Eq 4a

	−log AA	log(COX1)	log(COX2)	CMR
−log AA	1.000	−0.470	−0.791	−0.571
log(COX1)		1.000	0.135	0.460
log(COX2)			1.000	0.186
CMR				1.000

stepwise MLR analysis using log(COX1 IC_{50}) and log(COX2 IC_{50}) to represent pharmacology. Assuming similar drug metabolism (see Table 3), we used ClogP (molecular lipophilicity) and CMR (molecular size or dispersion) to represent pharmacokinetic contributors. Regression analysis produced eq 4a that suggests that the AA activity was the result of the inhibition of both COX1 and COX2 and the size of the molecule. Under these circumstances, hypothetically a pure COX1 inhibitor (COX1 IC_{50} = 1.0 μM, COX2 IC_{50} = 1000.0 μM) with a CMR = 9.74 would produce an AA ED_{50} = 43.2 μM. Conversely, a pure COX2 inhibitor (COX1 IC_{50} = 1000.0 μM, COX2 IC_{50} = 1.0 μM) with a CMR = 9.74 would produce an AA ED_{50} = 0.2 μM. It was interesting to observe that eq 4a did not contain the lipophilic parameter. The results in Table 4 also showed that the AA activity was moderately correlated with COX2 inhibition (see eq 5). The found vs predicted AA activity from eqs 4a and 5 are shown in Table 5.

Table 5. Found versus Predicted AA Activity from Eqs 4a, 5, 6a, and 7c[a]

	ED_{50}, μM/kg			COX2 IC_{50}, μM		COX1 IC_{50}, μM	
compd	found	predicted eq 4a	eq 5	found	predicted eq 6a	found	predicted eq 7c
1	>317.0			12.7	1.9	373.0	216.2
2	10.47	9.97	10.75	72.0	130.8	219.0	163.4
3	4.89	3.24	4.15	34.5	36.5	36.0	145.6
4	2.66	2.98	4.96	40.0	57.1	132.5	107.9
5	47.16	38.04	65.88	298.2	124.3	580.0	168.8
6	49.86		14.90	92.3	98.4	>300	(90.5)
7	93.02		58.50	267.0	162.7	>300	(482.8)
8	1.43	2.16	5.27	42.0	33.0	94.0	119.5
9	4.16	5.57	4.28	35.5	10.3	515.7	835.1
10	3.82		9.26	64.4	54.6	nd	(233.0)
11	24.97		8.99	63.0	129.2	nd	(194.0)
12	32.00			>300	0.4	>300	nc
13	72.82			>300	499.6	127.0	40.0
14	9.98			nd	159.8	nd	(76.2)
15	3.9	4.32	10.13	69.0	92.4	20.0	69.7
16	7.93	8.48	13.63	88.0	114.9	44.0	76.2
17	82.95	68.69	67.87	300.0	307.5	300.0	nc
18	8.54	19.80	7.75	55.7	35.8	766.7	551.0
19	12.55	5.03	2.74	25.0	16.6	650.0	558.5
20	>303			20.0	0.3	>300	(1563.3)

[a] Data in parentheses = not in the regression, calculated from regression equation; nc = not calculated, necessary data not available; nd = not determined.

$$-\log \mathrm{AA} = -0.20(\pm 0.15)\log(\mathrm{COX1}) - 1.00(\pm 0.21)\log(\mathrm{COX2}) - 9.45(\pm 4.47)\log\mathrm{CMR} + 10.71(\pm 4.23) \quad (4a)$$

$$n = 11 \quad r = 0.924 \quad \mathrm{SE} = 0.237 \quad F = 13.599 \quad \mathrm{prob} > F = 0.003$$

When −log AA was subjected to stepwise MLR against log(COX1), log(COX2), ClogP, log(CMR), and log(NH ppm), eq 4b resulted which contained the lipophilic parameter and did not contain log(COX1). A comparison of eqs 4a,b would suggest that for this set of compounds in this antiinflammatory model, the inhibition of COX2 was more important to antiinflammatory activity than that of COX1 and that the missing lipophilic component in eq 4a might be nested in the requirements for COX1 inhibition. Since a large number of marketed NSAIDs are acidic drugs, and the pyrroles are generally weakly acidic, we had hoped that pK_a of the pyrrole nitrogen, as represented by log(NH

$$-\log AA = -0.97(\pm 0.40)\log(COX2) + 0.15(\pm 0.15)ClogP - 12.77(\pm 5.90)\log CMR + 12.70(5.36) \quad (4b)$$

$n = 14$ $r = 0.823$ SE $= 0.344$ $F = 7.012$ prob > $F = 0.008$

$$-\log AA = -1.29(\pm 0.28)\log(COX2) + 1.37(\pm 0.54) \quad (5)$$

$n = 15$ $r = 0.781$ SE $= 0.367$ $F = 20.380$ prob > $F = 0.001$

ppm), might be important for systemic activity and appear in the regression equation. It did not, but its antiinflammatory influence might be embedded in the inhibition of one or both of the isozymes of COX. Note: using the compounds in Table 1 where R1 = H, the NMR resonance for the pyrrole N-H, and the equation reported by Perrin et al.[27] ($pK_a = -4.28\Sigma\sigma + 17$), we found that the regression coefficient (r) was 0.316. The outlier to the relationship was **10** (R2 = CN). When **10** was removed from the regression, a modest relationship resulted with $r = 0.606$. Though an improvement, the correlation was not strong enough for QSAR predictive purposes.

These finding would appear to be in conflict with the previously reported absence of activity against BSV PGS and the lack of activity in the PQW and CAR assays. Our earlier assays for BSV PGS activity did not involve preincubation and thereby may have masked potency against the bovine enzyme. Some of the diarylpyrroles were later found to be time dependent inhibitors of COX1 (data not included). The PQW test is a general analgesia test and not well correlated with either COX1 or COX2 inhibition, yet Tylenol works in this model while not inhibiting either COX isozyme. We consider the AA model of inflammation to be a more stringent model than the CAR assay. In general our compounds were active in the Randall–Selito model,[28] which is similar to the CAR, but as with human rCOX1, not enough compounds were tested as to produce a statistically suitable data set. There has also been some concern about deriving *in vivo–in vitro* relationships when the biology involved different species (AA in rat, COX1 from ovine enzyme, and COX2 from human rCOX2).[21] We have assayed some of the compounds in Table 1 against the human rCOX1 and found no difference in K_i from that determined for the ovine enzyme. Because of availability and no demonstrated difference between the ovine and human enzyme, we routinely use the former. Presently there is no alternative to the use of animal models for the evaluation of antiinflammatory activity of experimental chemical entities.

We explored the QSAR for COX inhibition by these diarylpyrroles using stepwise MLR. Since the set of compounds varied at R1, R2, and R3, we initiated the regressions with substituent parameters for lipophilicity (π), size (MR and Sterimol), and electronic effects (σ_p, σ_m, σ_I, $\mathcal{F}$, and $\mathcal{R}$) for R1, R2, and R3. We also considered the NMR chemical shift for the pyrrole N-H where present since this parameter may reflect the pK_a for the proton and/or the ability of the N-H to form hydrogen bonds in the active site of the enzyme(s). The results of this study were eq 6a and its correlation matrix (Table 6). The found vs predicted values are shown in Table 5. Of the parameters in eq 6a, the least confidence was in the intercept ($t = 0.116$), and the most interesting observation was the failure to predict the activity of **12** (R1 = ^{t}Boc, R2 = Br, R3 = H).

Table 6. Correlation Matrix of Eq 6a

	−log(COX2)	R1 π	R2 MR	R2 $\mathcal{F}$	R3 π
−log(COX2)	1.000	0.561	−0.622	−0.752	−0.070
R1 π		1.000	−0.266	−0.232	0.348
R2 MR			1.000	0.542	−0.213
R2 $\mathcal{F}$				1.000	0.089
R3 π					1.000

$$-\log(COX2) = 1.33(\pm 0.40)R1\pi - 0.06(\pm 0.03)R2MR - 2.23(\pm 0.76)R2\mathcal{F} - 0.63(\pm 0.34)R3\pi - 0.22(\pm 0.31) \quad (6a)$$

$n = 17$ $r = 0.901$ SE $= 0.422$ $F = 13.012$ prob > F 0.000

A similar approach was applied to COX1 to give eqs 7a and 7c (see Table 5), both of which were statistically disappointing. Both equations contain the Sterimol parameter B1, and instead of the electronic component being $\mathcal{F}$ as in −log(COX2), the equations for −log(COX1) contain the resonance parameter $\mathcal{R}$. Note in eqs 6a and 7a,b that the best enzyme inhibitors occur when $\mathcal{F}$ or $\mathcal{R}$ is large and positive. Both equations contain a lipophilic parameter (π in eq 7a and ClogP in eq 7b); however, the standard error for ClogP and the intercept were quite large.

$$-\log(COX1) = -0.67(\pm 0.29)R1\pi - 1.15(\pm 0.30)R1B1 - 1.98(\pm 0.61)R2\mathcal{R} - 1.00(\pm 0.36) \quad (7a)$$

$n = 13$ $r = 0.858$ SE $= 0.307$ $F = 8.384$ prob > $F = 0.006$

$$-\log(COX1) = 0.06(\pm 0.18)ClogP - 1.92(\pm 0.47)R1B1 - 1.84(\pm 0.78)R1\mathcal{R} - 0.47(\pm 0.75) \quad (7b)$$

$n = 12$ $r = 0.857$ SE $= 0.323$ $F = 7.400$ prob > $F = 0.011$

A comparison of eqs 6a and 7a would suggest that there are different requirements for the two enzymes at R1 since the sign for the coefficient is different. Increasing the lipophilicity of R1 would result in decreased potency for COX2 while increasing COX1 potency as seen for all comparable cases in Table 1 where R1 ≠ H. On the basis of the data in Table **1** and eq 4a, the better antiinflammatory compounds occur when R1 = R3 = H, where $H_\pi = 0.00$, and $H_{B1} = 1.00$. Thus, eq 6a becomes eq 6b, eq 7a become eq 7c, and eq 7b becomes 7d.

$$-\log(COX2)_{(R1=R3=H)} = -0.06R2MR - 2.23R2\mathcal{F} - 0.22 \quad (6b)$$

$$-\log(COX1)_{(R1=R3=H)} = -1.98R2\mathcal{R} - 2.15 \quad (7c)$$

$$-\log(\mathrm{COX1})_{(R1=R3=H)} = 0.06\mathrm{ClogP} - 1.84\mathrm{R1}\mathscr{R} - 2.39 \quad (7d)$$

In an attempt to understand the importance of the pyrrole N-H to enzyme inhibition, we conducted a stepwise MLR for COX2 using ClogP, CMR, R1π, R1MR, R2MR, R2$\mathscr{F}$, R3π, and (NH ppm) to produce eq 6c and its cross-correlation matrix (Table 7). The correlation table shows that CMR, R2$\mathscr{F}$, and (NH ppm) were all moderately correlated with COX2 inhibition and that (NH ppm) and R2$\mathscr{F}$ were not cross-correlated. However, CMR and (NH ppm) were moderately cross-correlated. Care was taken in the interpretation of this equation with four independent variables for 12 observations.[29,30] A similar approach for COX1 inhibition failed, but its cross-correlation matrix (Table 8) shows an extremely poor correlation between −log(COX1) and (NH ppm) (r = 0.046). Thus, the electronic environment of the pyrrole proton would appear to be more important to the inhibition of COX2 than of COX1.

$$-\log(\mathrm{COX2}) = 0.14(\pm0.07)\mathrm{ClogP} - 0.25(\pm0.16)\mathrm{CMR} - 0.91(\pm0.34)\mathrm{R2}\mathscr{F} - 0.29(\pm0.13)(\mathrm{NH\ ppm}) + 3.85(\pm1.33) \quad (6c)$$

$n = 12$ $r = 0.929$ SE $= 0.172$ $F = 11.097$ prob > $F = 0.004$

COX2 Selectivity. While most of the antiinflammatory efficacy appears to be related to COX2 inhibition, the ability of a compound to also inhibit COX1 will greatly affect its clinical utility, in that COX1 inhibition is presumed to be associated with the ulcerogenicity of NSAIDs. Indeed, the greatest motivation for exploring COX2 selective inhibitors as antiinflammatory agents is not to provide greater clinical efficacy but rather to provide greater gastrointestinal and renal safety while maintaining efficacy. Hence it is not only COX2 inhibitory potency but also COX2 *selectivity* that is important in designing molecules of clinical value. Therefore, an attempt to understand the molecular requirements for selective inhibition of COX2, as expressed by the ratio of isozyme IC_{50} values (COX1/COX2), was undertaken. Using all of the above-mentioned molecular parameters in a stepwise MLR, eq 8 resulted which showed that for this set of inhibitors, selectivity could be influenced by the size (MR) of R1 and R2 and the molecular lipophilicity (ClogP). This finding was in agreement with that demonstrated in eqs 4a−7.

$$\log\mathrm{COX}(1/2) = 0.38(\pm0.06)\mathrm{R1MR} - 0.06(\pm0.02)\mathrm{R2MR} - 0.84(\pm0.18)\mathrm{ClogP} + 3.92(\pm0.76) \quad (8)$$

$n = 11$ $r = 0.895$ SE $= 0.281$ $F = 17.137$ prob > $F = 0.001$

The results, as shown in eqs 4a and 8, described the molecular parameters needed to maximize *in vivo* antiinflammatory activity (AA ED_{50}) and maximize COX selectivity (COX1 IC_{50}/COX2 IC_{50}) for this set of 4,5-diarylpyrroles. Clearly the pyrroles where R1 = R3 = H and R2 is inductively electron withdrawing produced the best activity.

QSAR Utility. The QSAR studies showed that the best 1-, 2-, or 3-substituted-4-(4-fluorophenyl)-5-[4-(methylsulfonyl)phenyl]pyrroles were obtained when R1 = R3 = H. Using published data,[25] we selected substituents where the substituent constants for MR, $\mathscr{F}$, $\mathscr{R}$, and Sterimol were available. Substituents in Table 1 and those considered not to be compatible with biological systems were eliminated. The remaining substituents for the hypothetical compounds were subjected to eq 6b. A similar approach was applied to eq 7b. When the predicted values for log(COX2) from eq 6b, log(COX1) from eq 7b, and log(CMR) were substituted in eq 4a, the predicted −log(AA) was obtained. Taking the reciprocal antilog resulted in predicted AA ED_{50} values. The selected predicted values from eqs 6b, 7b, and 4a are shown in Table 9.

The data in Table 9 highlighted an interesting relationship between the hypothetical compounds where R2 = CO_2Me, CO_2H, and the carboxylate anion CO_2^-. The carboxylic acid is statistically less active and selective than its corresponding anion. The active species would depend on the degree of ionization that existed at physiological pH. The bioavailability and

Table 7. Correlation Matrix for Eq 6c

	−log(COX2)	ClogP	CMR	R2$\mathscr{F}$	NH ppm
−log(COX2)	1.000	0.213	−0.640	−0.724	−0.770
ClogP		1.000	0.157	−0.123	0.245
CMR			1.000	0.307	0.626
R2$\mathscr{F}$				1.000	0.394
NH ppm					1.000

Table 8. Correlation Matrix for −log(COX2)

	−log(COX2)	ClogP	CMR	R2$\mathscr{F}$	NH ppm
−log(COX2)	1.000	0.521	0.199	0.033	−0.032
ClogP		1.000	0.741	0.409	0.310
CMR			1.000	0.385	0.370
R2 $\mathscr{F}$				1.000	0.860
NH ppm					1.000

Table 9. Hypothetical 2-Substituted-4-(4-fluorophenyl)-5-[4-methylsulfonyl)phenyl]-1*H*-pyrroles (Predicted COX1 IC_{50}, COX2 IC_{50}, and AA ED_{50} using regression eqs 6b, 7b, and 4a, respectively)[a]

	predicted			
R2	COX2 IC_{50} eq 6b	COX1 IC_{50} eq 7b	AA ED_{50} eq 4a	calcd COX selectivity
CO_2^-*	1.8	255.5	nc	144
$B(OH)_2$	5.3	320.9	0.4	60
CH_2OH	4.5	141.3	0.3	32
Me	2.9	78.1	0.1	26
$CH_2CH{=}CH_2$	7.0	117.7	0.9	17
CH_2OMe	9.2	154.7	0.9	17
CH_2NH_2	5.5	89.5	0.4	16
CF_3	23.4	335.9	1.5	14
$C({=}O)NH_2$	22.1	267.4	2	12
CHO	2.1	255.5	1.3	12
CO_2H*	23.5	279.9	1.7	12
$CH_2N(Me)_2$	17.1	177.4	3.3	10
$CH{=}CH_2$	10.9	98.1	0.9	9
C(=O)Me	40.2	351.6	4.2	9
CH_2OAc	19.9	154.7	3.2	8
C(=O)OMe*	53.5	279.9	6.2	5
SCF_3	67.5	320.9	9.8	5
SO_2NH_2	74.3	335.9	10.1	5
OCF_3	34.6	141.3	2.2	4
$C({=}O)CF_3$	137.7	529.9	16.3	4
NHAc	13.6	39.4	1.2	3
C(=O)NHMe	71.2	177.4	9.3	2
F	17.1	30.0	0.4	2
NH_2	3.9	6.4	0.1	2
4-Pyr	345.5	506.3	135.3	1
SO_2CF_3	416.4	462.2	69.3	1
$N(Me)_2$	23.8	2.1	1.2	0

[a] nc = CMR value not calculated.

efficacy of the two species may be different depending on the site of oral absorption, the environment of the inflamed tissues, and the $t_{1/2}$ of the three species. It is possible that the corresponding ester(s) might function as 'prodrugs' to GI absorption and tissue distribution and penetration. The metabolic fate of some of these compounds also presents an interesting problem for the drug designer. If the metabolism of the pyrroles is similar to that of DuP697 (2-Br → 2-SO_2Me) as reported by Pinto et al.,[22] then **4** (R2 = Br, COX2 IC_{50} = 40.0 μM, COX1 IC_{50} = 132.5 μM, AA ED_{50} = 2.7 μM) or **8** (R2 = Cl, COX2 IC_{50} = 42.0 μM, COX1 IC_{50} = 94.0 μM, AA ED_{50} = 1.4 μM) might be expected to produce **7** (R2 = SO_2Me, COX2 IC_{50} = 267.0 μM, COX1 IC_{50} > 300.0 μM, AA ED_{50} = 93.0 μM). For some of our diaryl heterocyclic antiinflammatory compounds, there is evidence that the SO_2Me species is deposited in fat tissue and may be responsible for some toxicity. Thus, the judicious use of these equations and relationships in conjunction with SAR–QSAR for drug metabolism and toxicology may reduce the amount of resources required to discover and develop a potent and safe oral antiinflammatory selective cyclooxygenase-2 inhibitor.

We used QSAR to facilitate the understanding of the interaction between the chemical entity and the enzyme, determine if we have failed to synthesize the best compound, identify new leads, and prioritize syntheses and pharmacological evaluation of new chemical entities. To be of maximum value, the QSAR equations must contain parameters that can be obtained from the literature or calculated prior to synthesis.

Conclusion

The results of this study have been used to better understand the relationship between oral antiinflammatory activity of a series of 2-substituted and 2,3-disubstituted-4-(4-fluorophenyl)-5-[4-(methylsulfonyl)-phenyl]-1*H*-pyrroles and their ability to inhibit COX1 and COX2. Because these compounds did not inhibit BSV PGS, we did not think that the antiinflammatory activity was due to the reduction in the inflammatory mediators associated with arachidonic acid metabolism. The compounds were inactive in the animal models considered to be sensitive to COX1 inhibition. The antiinflammatory activity was best demonstrated in the rat-established adjuvant arthritis assay that has been associated with delayed hypersensitivity response and may best represent autoimmune diseases.[10,31] These observations and information, along with the low GI toxicity, further mitigated against the involvement of arachidonic acid metabolism inhibition. This study did demonstrate that the series did selectivity inhibit COX2. Using MLR analysis techniques, QSAR equations have been generated which can be used to describe and predict the *in vivo* and *in vitro* activity of these diarylpyrroles. The data presented here provide new insight into the physicochemical parameters germane to selective inhibition of COX2; this information may lead to the discovery and development of more potent and selective inhibitors of COX2 that can be useful in treating inflammatory diseases.[17]

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