Synthesis and SAR of a New Series of COX-2-Selective Inhibitors: Pyrazolo[1,5-*a*]pyrimidines

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Received August 7, 2000

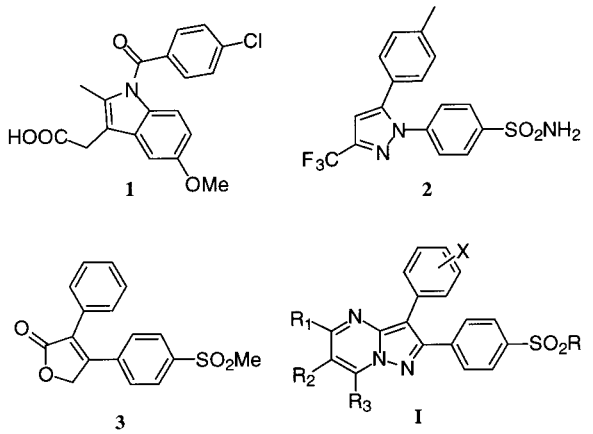
The synthesis and pharmacological activity of a series of bicyclic pyrazolo[1,5-*a*]pyrimidines as potent and selective cyclooxygenase-2 (COX-2) inhibitors are described. The new compounds were evaluated both in vitro (COX-1 and COX-2 inhibition in human whole blood) and in vivo (carrageenan-induced paw edema and air-pouch model). Modification of the pyrimidine substituents showed that 6,7-disubstitution provided the best activity and led to the identification of 3-(4-fluorophenyl)-6,7-dimethyl-2-(4-methylsulfonylphenyl)pyrazolo[1,5-*a*]pyrimidine (**10f**) as one of the most potent and selective COX-2 inhibitor in this series.

Introduction

Nonsteroidal antiinflammatory drugs (NSAIDs) exert their antiinflammatory action mainly through inhibition of cyclooxygenase (COX), one of the key enzymes in the arachidonic acid (AA) cascade.[1] This enzyme bis-oxygenates AA to PGG_2, which is subsequently degraded to vasoactive and inflammatory mediators such as prostaglandins (PGs), prostacyclin (PGI_2), and tromboxane A_2. The therapeutic use of NSAIDs has been associated with well-known side effects at the gastrointestinal level (mucosal damage, bleeding)[2] and, less frequently, at the renal level[3], and thus their use in chronic diseases such as rheumatoid arthritis has caused complications.

At the beginning of the 1990s two COX isoforms were discovered:[4] 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[5] that selective COX-2 inhibitors could provide antiinflammatory agents devoid of the undesirable effects associated with classical, nonselective NSAIDs.

Several approaches based on modification of established nonselective agents have been studied for the design of COX-2-selective inhibitors,[6] in a line of research in which the crystal resolution of the structures of both enzymes has also been somewhat helpful.[7] Lengthening the carboxyl side chain of indomethacin[8] (**1**) or substituting it with other groups[9] and modifying the preferential inhibitor nimesulide to flosulide,[10] L-745337,[11] and NS-398[12] has led to selective compounds, although none of them is being pursued for a variety of reasons. Based on the structure of early known antiinflammatory agents such as flumizole, a wide range of diarylheterocycle COX-2-selective inhibitors have been described.[13] To obtain good activity and selectivity such compounds require the presence of a 4-methylsulfonylphenyl group attached to an unsaturated (generally five-membered) ring in which an additional vicinal phenyl ring is present. The methylsulfonyl group can only be replaced by an SO_2NH_2,whereas the second phenyl group accepts lower alkyl or halogen substituents, the most common being 4-fluoro and 4-methyl. Two compounds in this class, celecoxib[14] (**2**) and rofecoxib[15] (**3**), are already in the market for the treatment of acute pain, osteoarthritis, and rheumatoid arthritis and seem to be as effective as other established NSAIDs and to have much better gastric tolerance.[16] In addition they are undergoing clinical trials for the treatment of certain forms of cancer and Alzheimer's disease.



In a new approach to diarylheterocycle COX-2-selective inhibitors, we decided to investigate the attachment of an additional six-membered ring to the usual five-membered central ring. The pyrazolo[1,5-*a*]pyrimidine nucleus **I** was selected for the SAR study in view of the easier access to substituent variation in the extra ring in comparison to other possible frameworks, which in turn could be prepared once the key features of **I** were identified. Two similar approaches taken with imidazo[1,2-*a*]pyridines[17] and isoindoles[18] have appeared in the patent literature.

Chemistry

The basic SAR around the pyrazolopyrimidines **I** was effected with the 3-(4-fluorophenyl)-2-(methylsulfo-

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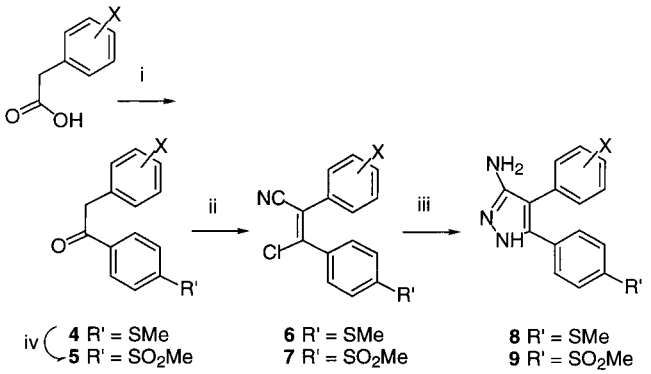
10.1021/jm0009383 CCC: $20.00
Published on Web 01/04/2001

Scheme 1[a]



a X = F; **b** X = Me; **c** X = 3,4-diF; **d** X = 2,4-diF

[a] (i) Thioanisole, $AlCl_3$, CH_2Cl_2, −10 °C, 1 h; (ii) 1. $POCl_3$/DMF, $CHCl_3$, 80 °C, 18 h, 2. NH_2OH·HCl, DMF, 20 °C, 4 h; (iii) NH_2NH_2·H_2O, EtOH, 78 °C, 18 h; (iv) Oxone, MeOH/THF, 20 °C, 1 h.

nylphenyl) derivatives **10**, which could be prepared from amine **9a**. Amines **8** and **9** were obtained from ketones **4** and **5**[19] in a process involving reaction with $POCl_3$/DMF followed by treatment with hydroxylamine in DMF to give a 3-chloropropenenitrile intermediate (**6**, **7**), which was cyclized with hydrazine in ethanol (Scheme 1).

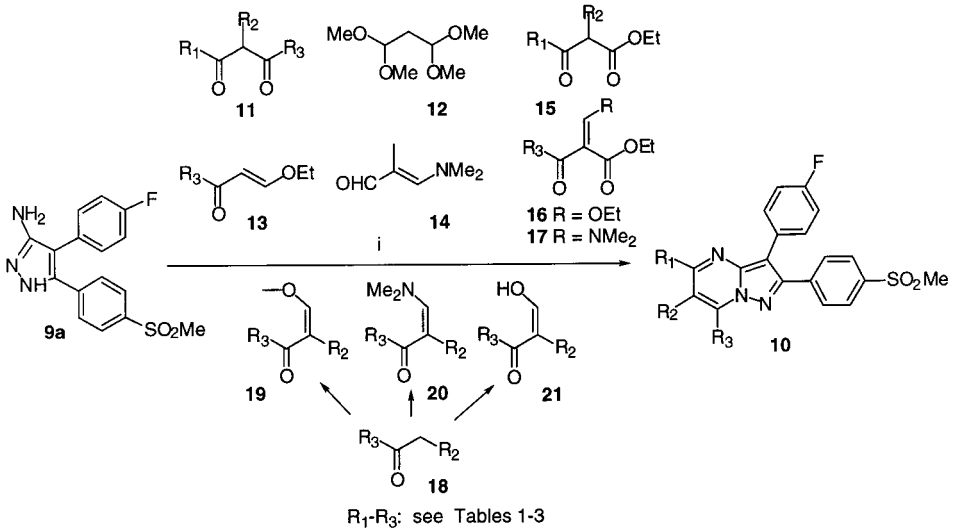
The cyclization of 3-aminopyrazoles with a 1,3-dicarbonyl compound is the usual method for preparing pyrazolopyrimidines.[20] When a nonsymmetrical reagent is used, usually isomeric mixtures are obtained. The proportion of isomers is quite erratic and depends on the substrate, the nature of the reagent, and the type of groups used to mask the carbonyl compounds.[20a,b]

As outlined in Scheme 2, compounds **10** were obtained by cyclization of **9a** with an appropriately substituted free or masked 1,3-dicarbonyl derivative (**11**−**17**, **19**−**21**), under conditions A−G indicated for each compound in Tables 1−3. When a free dicarbonyl derivative (**11** or **15**) was used, or in the case of the ethoxymethylene derivatives **13**, the reactions were run either in ethanol or in toluene (methods B, D). In the remaining cases, the presence of masked carbonyl groups in the reactants required acidic conditions and one of methods A (HCl/$ZnCl_2$/EtOH), C (HCl/EtOH), E (AcOH), or G (*p*-TsOH/toluene) was used. The 6-bromo derivative **10ab** (Table 2) was obtained from 3-bromo-4-diethylamino-3-buten-2-one (**20**, R_2 = Br, R_3 = Me) using HBr (method F), since the standard HCl gave its 6-chloro analogue, product of a halogen exchange.

The parent compound **10a** (Table 1) was obtained using the diprotected malonaldehyde **12** in the presence of $ZnCl_2$ (method A), while the 6-methyl **10d** was obtained from **14** under the conditions of method C. The isomeric 5-methyl **10b** and 7-methyl **10c** were obtained as a 1:4 mixture by reaction of **9a** with **13** (R_3 = Me) under the conditions of method B. The structural assignment was made on the basis of their ^1H and ^{13}C NMR spectra. The methyl groups of **10b** and **10c** showed proton shifts of 2.63 and 2.88 ppm, respectively, and carbon shifts of 26.5 and 18.5 ppm, respectively, in accordance with results previously reported for related compounds.[20a,b] The structural identity of **10b,c** was further confirmed by additional chemical transformations.[20c,d] Hence, **10s** was converted to **10b** upon hydrogenolysis with H_2 over Pd/C/NaOAc,[20c] whereas **10x** was converted to **10c** upon decarboxylation with H_2SO_4, using the same conditions described below for the preparation of **10j**.

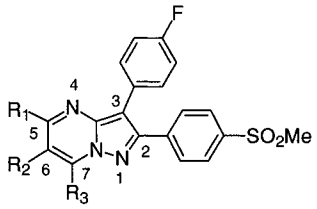
The 5,7-dimethyl derivative **10e** was prepared from **9a** and acetylacetone (**11**, R_2 = H, R_1 = R_3 = Me), whereas the isomeric **10f**,**g** were obtained in different proportions depending on the reactant employed. Thus, the α-hydroxymethylene derivative **21** (R_2 = R_3 = Me) led to **10f**,**g** in a 1:9 ratio, while the α-methoxymethylene derivative **19** (R_2 = R_3 = Me)[21] led to **10f**,**g** in a 9:1 ratio. In both cases the most abundant isomer was isolated by silica gel chromatography in 62% (**10g**) and 70% (**10f**) yield. The assignment of the structure to **10g** was based on its unambiguous synthesis by hydrogenolysis of the 7-chloropyrazolopyrimidine **10t** over Pd/

Scheme 2[a]



[a] (i) Method A: HCl, $ZnCl_2$, EtOH, 78 °C, 1 h; Method B: piperidine, EtOH, 78 °C, 18 h; Method C: HCl, EtOH, 78 °C, 1−18 h; Method D: toluene, 111 °C, 8 h; Method E: AcOH, 117 °C, 1 h; Method F: HBr, EtOH, 78 °C, 1 h; Method G: *p*-TsOH, toluene, 111 °C, 8 h. Specific methods and reagents used are indicated in Tables 1−3.

Table 1. Methylpyrazolopyrimidines of Formula **10**



							% Inh HWB[c]			IC_{50} Whole Cell[d]	
				reactant,	yield,	isomeric	COX-1	COX-2		COX-1	COX-2
compd	R_1	R_2	R_3	method[a]	%	ratio[b]	10 μM	10 μM	1 μM	(μM)	(μM)
10a	H	H	H	**12**, A	53	-	35.0	36.2	-	> 10	4.201
10b	Me	H	H	**13**, B	11	8:2	19.0	28.7	-	> 10	0.504
10c	H	H	Me	**13**, B	44	8:2	50.5	97.2	55.2	> 10	0.012
10d	H	Me	H	**14**, C	63	-	8.2	78.3	25.6	> 10	0.267
10e	Me	H	Me	**11**, B	54	-	8.3	93.1	17.1	> 10	0.154
10f	H	Me	Me	**19**, C	70	9:1	47.5	96.4	72.8	> 10	0.012
10g	Me	Me	H	**21**, D	62	1:9	32.6	57.2	-	> 10	2.133
10h	Me	Me	Me	**11**, D	53	-	61.3	100	56.1	> 10	0.192
2							34.5	94.6	67.0	5.1	0.080
1							100[e]	100	67.9	0.003	0.009

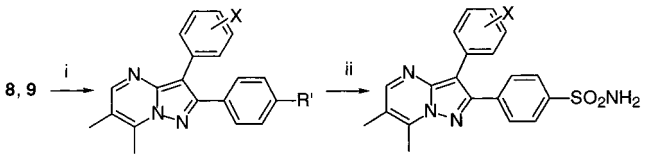
[a] Numbers indicate the type of reagent used as indicated in Scheme 2 and letters the cyclization method used for the preparation of compounds, as described in the Experimental Section. [b] Isomeric ratio of 7- vs 5-monosubstituted, 6,7- vs 5,6-disubstituted, or 5,7- vs 7,5-disubstituted compounds. [c] Percentage of inhibition of COX-1 (10 μM) or COX-2 (10 and 1 μM) activity in human whole blood. Assays were performed in duplicate or repeated, so that standard errors were inferior to 10%. [d] IC_{50} for whole cell inhibition in U-937 cells (COX-1) and 143982 cells (COX-2). Assays were performed in duplicate. [e] 82.3% inhibition at 1 μM.

C/NaOAc.[20c] It is noteworthy that the ^1H NMR spectra of the two isomers were characterized by the chemical shift of the 7-methyl (**10f**) at 2.85 ppm and the 5-methyl (**10g**) at 2.60 ppm. The downfield shift generally observed for the 7- over the 5-substituted derivatives allowed the identification of the remaining compounds of Tables 2 and 3.

As can be seen from the results outlined in Tables 2 and 3, where the observed isomeric ratios are indicated for every compound, the 7-substituted and 6,7-disubstituted isomers are favored over their 5-substituted and 5,6-disubstituted counterparts. This result is consistent with the proposed mechanism for this reaction, in which the most reactive carbonyl group (in this case the masked aldehyde function) first interacts with the amino group of the aminopyrazole, after which cyclization takes place.

Some compounds were obtained by further transformations of the pyrimidine substituents. Hence, the 7-hydroxy derivative **10q** was converted to the methoxy **10r** with methyl sulfate and to the chloro **10s** on treatment with $POCl_3$. Compound **10t** was prepared from its hydroxyl counterpart in an analogous way. Standard ester hydrolysis provided acids **10n**,**x**,**af**. Decarboxylation at position 6 by refluxing in H_2SO_4 for 18 h provided access to 7-monosubstituted derivatives such as **10j**. The preparation of the monohydroxymethyl derivatives **10y**,**ad** was more difficult. Attempts to reduce **10w** with $LiAlH_4$ or $NaBH_4$ were unsuccessful,

Scheme 3[a]



22 R' = SMe **24** R' = SCH_2OAc
23 R' = SO_2Me **25** R' = SO_2Na

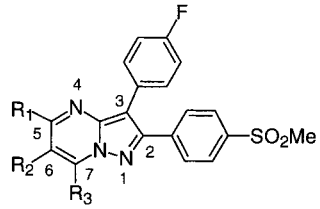
a X = F; **b** X = Me; **c** X = 3,4-diF; **d** X = 2,4-diF

[a] (i) **19** ($R_2 = R_3 =$ Me), HCl, EtOH, 78 °C, 4 h; (ii) 1. MCPBA, CH_2Cl_2, 20 °C, 2 h, 2. Ac_2O, AcONa, 140 °C, 8 h, 3. MMPP, CH_2Cl_2/MeOH, 20 °C, 18 h, 4. 1 N NaOH, THF/MeOH, 20 °C, 1 h, 5. NH_2OSO_3H, AcONa, H_2O, 20 C, 18 h.

since partial saturation of the pyrimidine ring was observed while the ethoxycarbonyl group was unaffected. However, using diisobutylaluminum hydride (DIBAL) in THF, **10y** could be prepared in good yield. Various approaches were studied for the preparation of **10ad**, but only the oxidation of **10f** with $KMnO_4/NEt_3/H_2SO_4$[22] allowed its isolation in moderate yields. The fluoromethyl derivative **10aa** was obtained upon treatment of **10y** with diethylaminosulfur trifluoride (DAST).

Other 6,7-dimethyl sulfones (**23**) were prepared from the corresponding amines **9** as described for **10f**. The sulfonamides **26** (Table 4, Scheme 3) were prepared from the methylthio derivatives **22** using a procedure[23] which involves monooxidation to the sulfoxide, Pummerer rearrangement to the acetoxymethylthio compound **24**, and oxidation with magnesium monoperoxy-

Table 2. Pyrazolopyrimidines of Formula **10**



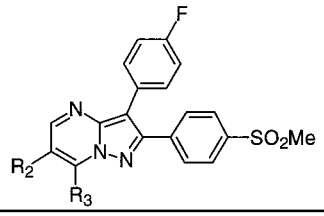
									% Inh HWB[e]		
				reactant, yield,		isomeric	mp[c],		COX-1	COX-2	
compd	R_1	R_2	R_3	method[a]	%	ratio[b]	°C	formula[d]	10 μM	10 μM	1 μM
10i	H	H	CF_3	**13**, B	46	10:0	213	$C_{20}H_{13}F_4N_3O_2S$	0.0	90.6	3.5
10j	H	H	Pr	-[g]	27	-	194	$C_{22}H_{20}FN_3O_2S.0.5H_2O$	37.3	56.1	32.2
10k	H	H	Ph	**20**, C	35	10:0	231	$C_{25}H_{18}FN_3O_2S.0.5H_2O$	5.7	90.4	0
10l	CF_3	H	Me	**11**, B	70	10:0	223	$C_{21}H_{15}F_4N_3O_2S$	2.5	57.2	-
10m[f]	COOEt	H	Me	**11**, B	68	5:5	98-99	$C_{23}H_{20}FN_3O_4S.0.5H_2O$	8.0	41.0	-
10n[f]	COOH	H	Me	-[g]	78	-	-	$C_{21}H_{16}FN_3O_4S.2H_2O$	33.5	24.6	-
10o	Bu	H	Me	**11**, B	31	5:5	57-61	$C_{24}H_{24}FN_3O_2S.0.5H_2O$	86.2[h]	100	51.5
10p	Me	H	Bu	**11**, B	33	5:5	183	$C_{24}H_{24}FN_3O_2S$	0.0	58.3	11
10q	Me	H	OH	**15**, D	54	10:0	198-200	$C_{20}H_{16}FN_3O_3S.H_2O$	8.1	21.9	-
10r	Me	H	OMe	-[g]	25	-	213	$C_{21}H_{18}FN_3O_3S.0.75H_2O$	0.0	0.0	-
10s	Me	H	Cl	-[g]	11	-	230-232	$C_{20}H_{15}ClFN_3O_2S.0.25H_2O$	32.5	14.3	-
10t	Me	Me	Cl	-[g]	88	-	253	$C_{21}H_{17}ClFN_3O_2S.H_2O$	1.2	45.8	-
10u	H	Et	Me	**19**, C	45	9:1	166-170	$C_{22}H_{20}FN_3O_2S.0.5H_2O$	91.8[i]	100	50.3
10v	H	COMe	Me	**20**, E	80	10:0	238	$C_{22}H_{18}FN_3O_3S$	71.1	100	60.8
10w	H	CO_2Et	Me	**17**, C	50	9.5:0.5	201	$C_{23}H_{20}FN_3O_4S$	0	100	40.5
10x	H	COOH	Me	-[g]	78	-	287-292	$C_{21}H_{16}FN_3O_4S.1.25CHCl_3$	8.3	56.3	-
10y	H	CH_2OH	Me	-[g]	28	-	268-270	$C_{21}H_{18}FN_3O_3S.0.75H_2O$	54.4	77.7	26.1
10z	H	CF_3	Me	**20**, E	36	8:2	208-215	$C_{21}H_{15}F_4N_3O_2S$	100[j]	100	69.0
10aa	H	CH_2F	Me	-[g]	75	-	218-219	$C_{21}H_{17}F_2N_3O_2S.0.5H_2O$	7.3	84.6	4.4
10ab	H	Br	Me	**20**, F	31	7:3	255-256	$C_{25}H_{15}BrFN_3O_2S.0.5H_2O$	83.8[k]	100	84.3
10ac	H	Me	Et	**19**, C	36	9:1	237	$C_{22}H_{20}FN_3O_2S.0.5H_2O$	82.8[l]	100	62.3
10ad	H	Me	CH_2OH	-[g]	33	-	226-227	$C_{21}H_{18}FN_3O_3S.0.5CH_2Cl_2$	42.7	100	56.5
10ae	H	Me	Ph	**20**, C	51	10:0	219	$C_{26}H_{20}FN_3O_2S.0.5H_2O$	0	12.3	-
10af	H	CO_2H	Pr	-[g]	70	-	257	$C_{23}H_{20}FN_3O_4S.0.5H_2O$	7.4	0.0	31.9

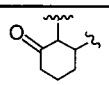
[a,b,e] See footnotes a–c of Table 1. [c] Melting points correspond generally to chromatographed products. [d] Elemental analyses for C, H, N, and S were within 0.4% of the theoretical values indicated. [f] Mixture of regioisomers. [g] See text and Experimental Section for method of synthesis. [h] 6.8% inhibition at 1 μM. [i] 0% inhibition at 1 μM. [j] 53.9% inhibition at 1 μM. [k] 18.9% inhibition at 1 μM. [l] 44.6% inhibition at 1 μM.

phthalate (MMPP), followed by treatment with NaOH to give the sodium sulfinate **25**. The reported process involved subsequent treatment with sulfuryl chloride and ammonia, but we found that on treatment of **25** with hydroxylamine *O*-sulfonic acid and NaOAc in H_2O, the sulfonamides **26** were produced in one step and in somewhat better yields.

Triazines **29** and **33** (Table 5) were prepared as described in Scheme 4. Formation of the diazonium salt of amine **9a** with $NaNO_2$ followed by treatment[24] with the in situ generated β-keto acids from **28** afforded the [1,2,4]triazines **29**. The [1,3,5]triazines **33** were obtained from **9a** upon treatment[25] with a suitable ethyl imidate **30**, followed by boiling in a triethyl orthoacylate **32**.

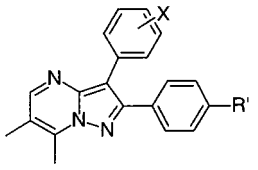
Table 3. Cyclopyrazolopyrimidines of Formula **10**



								% Inh HWB[e]	
			reactant, method[a]	yield, %	isomeric ratio[b]	mp[c], °C	formula[d]	COX-1 10 μM	COX-2 10 μM	COX-2 1 μM
compd	R_2	R_3								
10ag			**20**, E	30	10:0	145-149	$C_{23}H_{18}FN_3O_3S.0.9CHCl_3$	0	95.3	30.4
10ah			**21**, G	42	5:5	193-194	$C_{23}H_{20}FN_3O_2S.0.75H_2O$	40.4	100	57.7
10ai			**20**, E	30	10:0	232-233	$C_{22}H_{16}FN_3O_3S.0.3CHCl_3$	16.6	75.0	0
10aj			**21**, G	67	9.5:0.5	223-224	$C_{22}H_{18}FN_3O_2S.0.75H_2O$	0	90.4	48.3
10ak			**20**, E	18	10:0	217-219	$C_{21}H_{16}FN_3O_2S$	7.0	90.7	14.8
10al			**20**, E	44	10:0	229-232	$C_{21}H_{16}FN_3O_2S$	0	93.6	25.0

[a–e] See footnotes a–e of Table 2.

Table 4. 6,7-Dimethylpyrazolopyrimidines of Formulas **23** and **26**



			%Inh. HWB[b]		
			COX-1	COX-2	
compd[a]	R	R'	10 μM	10 μM	1 μM
26a	4-F	SO_2NH_2	22.0	99.1	22.5
23b	4-Me	SO_2Me	54.2	100	87.6
26b	4-Me	SO_2NH_2	33.5	100	65.7
23c	3,4-diF	SO_2Me	36.3	100	100
26c	3,4-diF	SO_2NH_2	68.2	100	97.2
23d	2,4-diF	SO_2Me	80.8	100	61.1

[a] Physicochemical properties described in the Experimental Section. [b] See footnote c of Table 1.

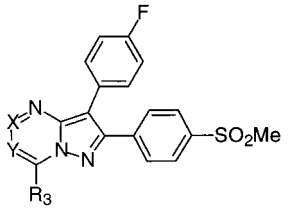
Other bicycles (Table 6) were prepared as described in Schemes 5 and 6. Imidazo[4,5-*c*]pyridines **38** were obtained from the corresponding 4-hydroxy-3-nitropyridines **34** upon treatment with $POCl_3$, followed by nucleophilic displacement with 4-fluoroaniline, reduction to the amine **37** with $SnCl_2$ in EtOH, and final cyclization with 4-methylsulfonylbenzoyl chloride in pyridine. On the other hand, the α-bromo ketone **39**, obtained from ketone **5a** upon treatment with bromine in CH_2Cl_2/AcOH, was reacted with the corresponding arylamines **40**, **42**, and **44** in DMF at 60 °C[26] to give imidazo[1,2-*b*]pyridazines **41**, imidazo[1,2-*a*]pyrazines **43,** and imidazo[1,2-*a*]pyrimidines **45**. Amines **40**, **42**, and **44** were commercially available or were prepared according to reported procedures (see Experimental Section). The Glaxo-reported bicycle **46** was also prepared from **39** as described.[17]

Results and Discussion

The new compounds were tested in vitro for their ability to inhibit COX-1 and COX-2 activity in a human whole blood (HWB) assay.[27] Percent inhibition at a 10 μM concentration was initially determined, and active compounds were also tested at 1 μM (Tables 1–5). All compounds showing more than 60% inhibition at 10 μM were tested in vivo in the rat carrageenan-induced paw edema assay (CPE)[28] at a 30 mg/kg dose; some of them were also tested in the carrageenan-induced air-pouch model[29] to estimate PG production at a 1 mg/kg dose.

The main SAR was carried out with the 3-(4-fluorophenyl)-2-(4-methylsulfonylphenyl) derivatives **10**, which are characterized by a 4-methylsulfonylphenyl group vicinal to a phenyl ring similarly to other COX-2-selective inhibitors. The gradual introduction of methyl groups into the poorly active parent unsubstituted compound (**10a**) was initially explored. In this initial series the activity in human cell lines expressing COX-1

Table 5. Pyrazolotriazines of Formulas **29** and **33**

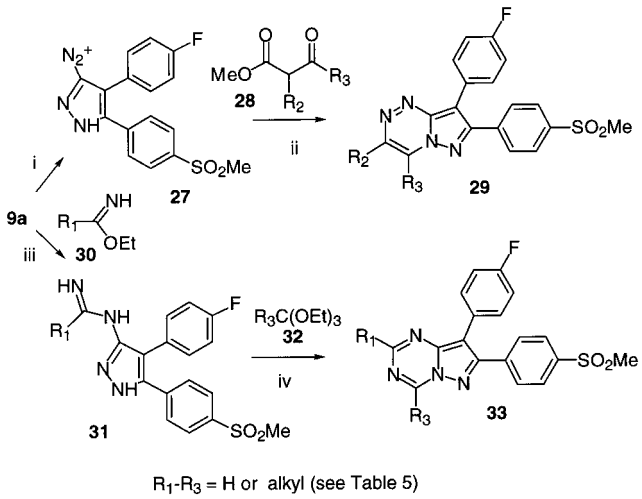


29 X = N; Y = CR_2;
33 X = CR_1; Y = N

			%Inh. HWB[b]		
			COX-1	COX-2	
Compd[a]	$R_{1,2}$	R_3	10 μM	10 μM	1 μM
29a	Me	Me	1.2	100	40.5
29b	H	Pr	29.3	100	43.9
29c	Et	Me	61.9	100	74.9
29d	Me	Pr	18.7	95.0	17.9
29e	H	Bu	69.8	89.7	0.9
29f	Et	Et	88.7	100	50.9
33a	Me	Me	4.75	90.3	18.1
33b	Me	Bu	7.7	54.6	-

[a,b] See footnotes a,b of Table 4.

Scheme 4[a]

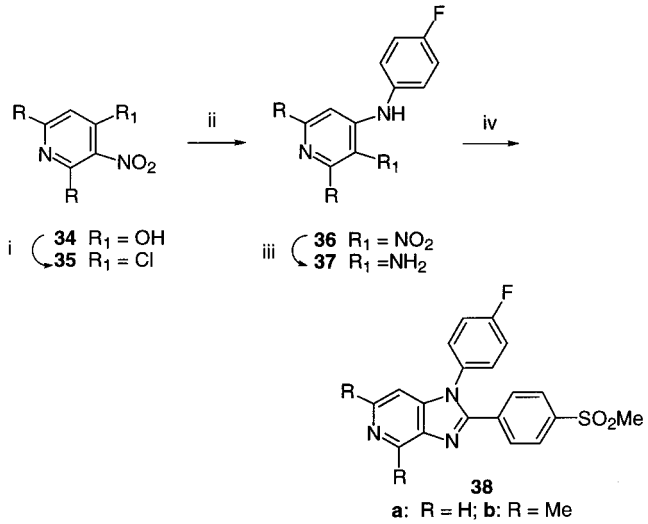


R_1-R_3 = H or alkyl (see Table 5)

[a] (i) $NaNO_2$, H_2O, 0 °C, 1 h; (ii) 1. KOH, H_2O, 0 °C, 24 h, 2. NaOAc, HCl, H_2O, 0−20 °C, 1 h; (iii) AcOH, 20 °C, 18 h; (iv) reflux, 18 h.

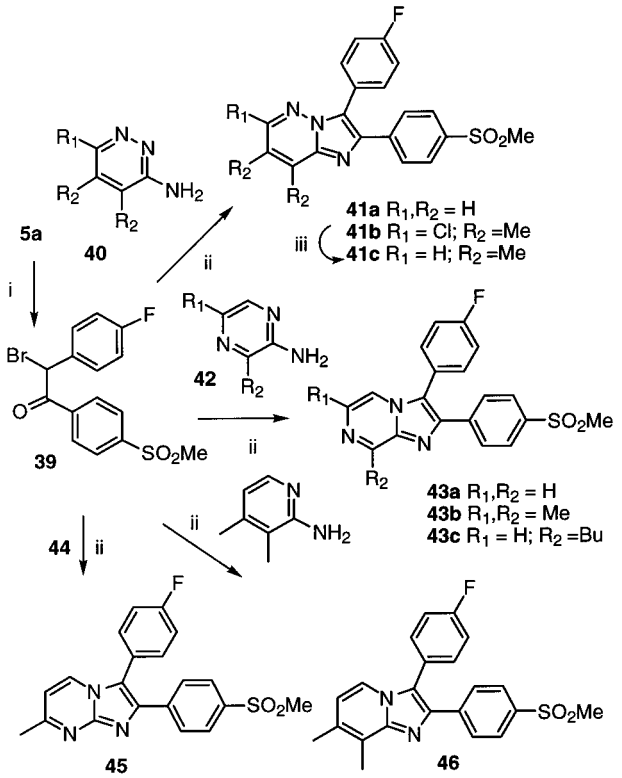
and COX-2[30] was also evaluated (Table 1). While substitution at the 5- or 6-position provided some increase in whole cell activity, a substantial gain was observed for the 7-methyl derivative **10c**. Its activity was maintained with a second methyl group in position 6 (**10f**) and impaired by introduction of additional methyl groups at the 5 (**10e**) or 5,6 (**10h**) positions. These results, together with the poor activity of the 5,6-disubstituted compound **10g**, indicated the need for substitution at position 7, which then tolerated a second substituent at position 6. The whole cell results cor-

Scheme 5[a]



[a] (i) $POCl_3$, DMF, toluene, 110 °C, 7 h; (ii) 4-fluoroaniline, NEt_3, $CHCl_3$, 67 °C, 48 h; (iii) $SnCl_2$, EtOH, 78 °C, 45 min; (iv) 4-methylsulfonylbenzoyl chloride, pyridine, 115 °C, 18 h.

Scheme 6[a]



[a] (i) Br_2, CH_2Cl_2, AcOH, 20 °C, 18 h; (ii) DMF, 60 °C, 18−36 h; (iii) H_2, Pd/C, TEA, 200 psi, 18 h.

related quite well with those obtained in the whole blood assay. The most potent compounds in the whole cell assay, **10c**,**f**, gave also the highest inhibitions in HWB. When their IC_{50} in HWB were calculated (**10c**: COX-1 = 10 μM, COX-2 = 0.6 μM; **10f**: COX-1 > 10 μM, COX-2 = 0.08 μM) the superior activity shown at 1 μM by **10f** was confirmed. This compound had excellent in vitro activity, which compared well with celecoxib (**2**), whether in whole cells (Table 1) or in HWB (**2** IC_{50}: COX-1 = 13 μM, COX-2 = 0.6 μM).

Among the methylated derivatives only compound **10f** showed some in vivo activity. In the air-pouch model it

showed 74% inhibition of PGE_2 production at 1 mg/kg, approximately one-third of that shown by **2** (93.4% and 71.3% inhibition at 1 and 0.3 mg/kg, respectively). In the CPE model **10f** provided around 20% of edema inhibition at 30 as well as at 10 mg/kg, in comparison to 43% shown by **2** at 10 mg/kg. In initial pharmacokinetic evaluation in the rat, compound **10f** had a short half-life (1 h) and poor bioavailability.

With the aim of improving these results and keeping the 7-position substituted, we introduced further variation. Changing the methyl of **10c** for CF_3 (**10i**) or lengthening to propyl (**10j**) or phenyl (**10k**) clearly diminished activity. Replacing the 5-methyl of **10e** by CF_3, COOEt, or COOH (**10l**−**n**) was clearly detrimental, while lengthening it to butyl provided the more active albeit less selective compound **10o**. Substitution of the 7-methyl of **10e** by butyl, hydroxy, methoxy, or chloro (**10p**−**t**) produced a complete loss in activity. Next, some 6,7-disubstituted analogues of **10f** were prepared and evaluated. Replacing the 6-methyl group by ethyl (**10u**), acetyl (**10v**), CF_3 (**10z**), or Br (**10ab**) maintained activity but provided some decrease in selectivity, whereas replacement by COOH, COOEt, CH_2OH, or CH_2F (**10w**−**y**,**aa**) was more detrimental. Elongation of the 7-methyl group to ethyl provided a loss in selectivity (**10ac**), while the 7-phenyl (**10ae**) completely lost potency, as in the case of **10k**. The 7-hydroxymethyl derivative, which was prepared together with **10y** as a possible metabolite of **10f**, retained its potency. Among these derivatives only the ethyl ester **10w** and the monohydroxylated **10y** showed some in vivo activity in the CPE test (20.2 and 17.3% inhibition at 30 mg/kg, respectively), somewhat inferior to that of **10f**.

Since 6,7-disubstitution seemed to provide the best activity, several 6,7-cyclo derivatives (Table 3) were prepared. The naked cyclohexyl (**10ah**) and cyclopentyl (**10aj**) derivatives had a profile similar to that of **10f**, while substitution by heteroatoms was detrimental. Despite this low in vitro potency, the cyclohexanone **10ag** and cyclopentanone **10ai** were the only ones displaying activity in the CPE test (22.4 and 26.3% inhibition at 30 mg/kg, respectively).

These results showed a restricted SAR around the pyrimidine substituents, which precluded their further optimization. Maintaining the 6,7-dimethyl groups, we decided to introduce some changes in the nature of the aromatic ring and explore changing the SO_2Me for an SO_2NH_2 group, a switch that in some instances has been shown to improve in vivo activity through an improvement of bioavailability.[14] In this case, the sulfonamide **26a** was equipotent to its sulfone counterpart **10f** in the whole cell assay (IC_{50}: COX-1 > 10 μM, COX-2 = 0.019 μM), although in the HWB assay **26a** showed less potency than expected. This result contrasted with that generally observed in the diaryl C5-heterocycle class, where this group usually provides more potency albeit less selectivity.[14] Although the 4-methyl and difluoro derivatives **23b**−**d** and **26b,c** were highly potent in the HWB assay, none of them showed improved in vivo results and only compound **26b** showed some activity in the CPE test (22.8% inhibition at 10 mg/kg).

To improve in vivo results, the introduction of a second nitrogen atom in the pyrimidine ring was envisaged and triazines **29** and **33** were prepared (Table 5).

Table 6. Heterocycle Variations: Compounds of Schemes 5 and 6

	% Inh. HWB[b]		
	COX-1	COX-2	
Compd[a]	10 μM	10 μM	1 μM
38a	34.3	21.3	-
38b	0	4.3	-
41a	47.0	61.2	-
41b	0	100	35.4
41c	0	100	37.3
43a	6.0	42.5	-
43b	0	82.4	0
43c	9.8	0	-
45	13.5	50.7	-
46	0	66.7	59.3

[a,b] See footnotes a,b of Table 4.

The HWB activity of the 6,7-dimethyl-1,3,5-triazine **29a** was inferior to that of **10f**, although in this case it was improved upon lengthening to the 6-ethyl derivative **29c**. However, a second ethyl group (**29f**) diminished selectivity. The 7-propyl **29b** was more active than its pyrimidine counterpart **10j**, but further lengthening (**29e**) or introduction of a methyl group (**29d**) failed to improve this result. On the other hand, the 1,3,4-triazines **33** lost activity in relation to their pyrimidine counterparts. Among these compounds only **29a**,**b** showed in vivo activity, but again neither of them improved the potency of **10f**.

Finally, other bicyclic frameworks, which eventually could provide the desired in vivo properties, were evaluated (Table 6). As in the pyrazolopyrimidine series, the unsubstituted imidazopyridine compound **38a** was inactive, but in this case the introduction of two methyl groups at the 6- and 7-positions (**38b**) impaired activity even further. As far as the imidazopyrazines were concerned, the poor activity of the parent **43a** could not be improved upon introduction of alkyl groups, and compounds **43b**,**c** were much less potent than their pyrazolopyrimidine counterparts **10c**,**e**. Moreover, around 10 different substituted imidazopyrimidines were prepared (results not shown), and as in the case of **45**, none of them showed significant COX-2 inhibitory activity.

Among these modifications only the imidazopyridazine framework **41** showed any activity. The presence of the two methyl groups was also important in this case, since **41c** was much more active than the parent **41a**. However, none of these derivatives showed any in vivo activity. For purposes of comparison, the imidazopyridine **46** described in the patent literature[17] was prepared but exhibited less potency both in vitro and in vivo than its counterpart **10f**.

Altogether these results indicate that a nitrogen atom at position 4 (**10f** vs **46**) increases activity, whereas it is merely tolerated at position 5 (**29**). On the contrary,

its presence in position 6 (compounds **33**, **38**, and **43**) or 7 (**45**) is clearly detrimental.

Conclusion

In summary, the tight SAR around the pyrazolo[1,5-*a*]pyrimidine nucleus indicated that 6,7-disubstitution provided the best activity and led to identification of the 6,7-dimethylated derivative **10f** as one of the most potent and selective COX-2 inhibitor in this series. While **10f** exhibited a remarkable in vitro potency and selectivity profile in relation to reference compounds, it was less active in vivo due to low oral bioavailability. In vivo activity could not be improved by introducing more polar groups in the side chains (Tables 1, 2), changing the methylsulfonyl to sulfonamide groups (Table 4), or introducing more nitrogen atoms to the nucleus (Table 6). The pyrazolo[1,5-*a*]pyrimidine and imidazo[1,2-*b*]pyridazine were the best bicyclic frameworks among those studied, indicating that the presence of a nitrogen atom at position 4 of the central core was positive, at position 5 was tolerated, and at position 6 or 7 was clearly detrimental. Further efforts to discover novel orally active COX-2-selective inhibitors related to these structures are underway and will be reported in due course.

Experimental Section

Melting points were determined with a Mettler FP 80 central processor melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 983 spectrophotometer. ^{1}H (80 MHz) and ^{13}C (20.1 MHz) NMR spectra were recorded on a Brücker AC 80 spectrometer and ^{1}H (300 MHz) NMR spectra were recorded on a Brucker Avance DPX-300 spectrometer. They are reported in ppm on the δ scale, from the indicated reference. Combustion analyses were performed with a Carlo Erba 1106 analyzer. Liquid chromatography was performed with a forced flow (flash chromatography) of the indicated solvent system on SDS silica gel Chromagel 60 ACC (230−400 mesh). Analytical thin-layer chromatography (TLC) was performed with Macherey-Nagel 0.25-mm silica gel SIL G-25 plates.

3-Chloro-2-(4-fluorophenyl)-3-(4-methylsulfonylphenyl)-propenenitrile (7a). A mixture of DMF (33.5 mL, 40 mmol) and $POCl_3$ (14.25 mL, 155 mmol) was stirred at 0 °C under argon for 15 min. To this solution, **5a**[19] (15.2 g, 52 mmol) in $CHCl_3$ (310 mL) was added dropwise and the mixture was heated at 80 °C overnight. The mixture was cooled at 0 °C and $NH_2OH{\cdot}HCl$ (10.5 g, 154 mmol) in DMF (29.25 mL) was added and stirred for 4 h at room temperature. The suspension was poured over saturated $NaHCO_3$ solution and extracted with $CHCl_3$. The organic phase was dried and concentrated to a crude product which was used directly in the next step (20 g): ^{1}H NMR ($CDCl_3$ δ TMS) 3.10 (s, 3 H), 7.06 (m, 2 H), 7.23 (m, 2 H), 7.50 (d, J = 7 Hz, 2 H), 7.90 (d, J = 7 Hz, 2 H).

3-Amino-4-(4-fluorophenyl)-5-(4-methylsulfonylphenyl)-pyrazole (9a). To a solution of crude **7a** (20 g, 52 mmol) in EtOH (546 mL), $NH_2NH_2{\cdot}H_2O$ (5.4 mL, 104 mmol) was added and the mixture was stirred at reflux overnight. The solvent was concentrated and the residue poured over a mixture of H_2O and AcOEt. The organic phase was extracted with AcOEt and the combined organic extracts were dried and concentrated to a crude product. This was chromatographed on silica gel (hexane−AcOEt mixtures) to afford **9a** as a white solid (9.0 g, 52%): mp 192−193 °C; ^{1}H NMR ($CDCl_3$ δ TMS) 3.06 (s, 3 H), 3.92 (s, 3 H), 7.05 (m, 2 H), 7.20 (m, 2 H), 7.52 (d, J = 7 Hz, 2 H), 7.80 (d, J = 7 Hz, 2 H).

3-(4-Fluorophenyl)-2-(4-methylsulfonylphenyl)pyrazolo-[1,5-*a*]pyrimidine (10a). Method A. A mixture of **9a** (0.41 g, 1.33 mmol), EtOH (1 mL), concentrated HCl (2.6 mL) and $ZnCl_2$ (0.08 g, 0.57 mmol) was heated to reflux and 1,1,3,3-tetramethoxypropane (**12**; 0.164 mL, 1 mmol) in EtOH (0.4 mL) was added. After 1 h at reflux the solution thus obtained was poured over ice and basified with NH_4OH. The mixture was extracted with AcOEt and the organic phase dried and concentrated to a crude product. This was chromatographed on silica gel (hexane−AcOEt mixtures) to afford **10a** as a white solid (0.34 g, 76%): mp 184−185 °C; ^{1}H NMR ($CDCl_3$ δ TMS) 3.06 (s, 3 H), 6.93 (dd, J = 4 Hz, J = 7 Hz, 1 H), 7.13 (m, 2 H), 7.48 (m, 2 H), 7.86 (d, J = 7 Hz, 2 H), 7.95 (d, J = 7 Hz, 2 H), 8.49 (dd, J = 1.8 Hz, J = 4 Hz, 1 H), 8.69 (dd, J = 1.8 Hz, J = 7 Hz, 1 H). Anal. ($C_{19}H_{14}FN_3O_2S{\cdot}0.25H_2O$) C, H, N, S.

3-(4-Fluorophenyl)-5,7-dimethyl-2-(4-methylsulfonylphenyl)pyrazolo[1,5-*a*]pyrimidine (10e). Method B. A mixture of **9a** (0.51 g, 1.7 mmol), acetylacetone (**11**, R_1, R_3 = Me, R_2 = H; 0.176 g, 1.7 mmol), 1 drop of piperidine and EtOH (8 mL) was refluxed for 18 h. The solvent was concentrated and the residue was chromatographed on silica gel (hexane−AcOEt mixtures) to afford **10e** as a white solid (0.48 g, 80%): mp 220 °C; ^{1}H NMR ($CDCl_3$ δ TMS) 2.59 (s, 3 H), 2.81 (s, 3 H), 3.07 (s, 3 H), 6.67 (s, 1 H), 7.07 (m, 2 H), 7.47 (m, 2 H), 7.87 (d, J = 7 Hz, 2 H), 7.93 (d, J = 7 Hz, 2 H). Anal. ($C_{21}H_{18}FN_3O_2S{\cdot}0.25H_2O$) C, H, N, S.

Using the same procedure but starting from 4-ethoxy-3-buten-2-one (**13**, R_3 = Me), a 1:4 mixture of **10b**,**c** was obtained.

10b: 11% yield; mp 209−216 °C; ^{1}H NMR ($CDCl_3$ δ TMS) 2.63 (s, 3 H, 5-Me), 3.09 (s, 3 H, SO_2Me), 6.78 (d, J = 7.2 Hz, 1 H, H_6), 7.09 (m, 2 H, C_6H_4F), 7.48 (m, 2 H, C_6H_4F), 7.82 (d, J = 7 Hz, 2 H, $C_6H_4SO_2Me$), 7.94 (d, J = 7 Hz, 2 H, $C_6H_4SO_2Me$), 8.56 (d, J = 7.2 Hz, 1 H, H_7); ^{13}C NMR ($CDCl_3$ δ TMS) 26.51 (CH_3, 5-Me), 45.90 (CH_3, SO_2Me), 109.44 (C), 110.64 (CH), 117.11 (CH, d, J_{F-C} = 20 Hz), 128.54 (C), 129.73 (CH), 131.03 (CH), 133.19 (CH, d, J_{F-C} = 8 Hz), 135.52 (CH, C_7), 140.14 (C), 141.59 (C), 147.88 (C), 152.97 (C), 161.43 (C), 163.45 (C, d, J_{F-C} = 245 Hz). Anal. ($C_{20}H_{16}FN_3O_2S{\cdot}0.25H_2O$) C, H, N, S.

10c: 44% yield; mp 192 °C; ^{1}H NMR ($CDCl_3$ δ TMS) 2.88 (s, 3 H, 7-Me), 3.09 (s, 3 H, SO_2Me), 6.80 (d, J = 4 Hz, 1 H, H_6), 7.10 (m, 2 H, C_6H_4F), 7.48 (m, 2 H, C_6H_4F), 7.89 (d, J = 7 Hz, 2 H, $C_6H_4SO_2Me$), 7.96 (d, J = 7 Hz, 2 H, $C_6H_4SO_2Me$), 8.46 (d, J = 4 Hz, 1 H, H_5); ^{13}C NMR ($CDCl_3$ δ TMS) 18.53 (CH_3, 7-Me), 45.90 (CH_3, SO_2Me), 110.16 (CH), 110.49 (C), 117.22 (CH, d, J_{F-C} = 20 Hz), 128.75 (C), 128.96 (CH), 131.14 (CH), 133.05 (CH, d, J_{F-C} = 8 Hz), 140.24 (C), 141.61 (C), 147.58 (C), 148.59 (C), 150.85 (CH, C_5), 152.35 (C), 163.65 (C, d, J_{F-C} = 245 Hz). Anal. ($C_{20}H_{16}FN_3O_2S{\cdot}H_2O$) C, H, N, S.

Compound **10b** was also obtained by the following procedure: A mixture of **10s** (0.13 g, 0.35 mmol), EtOH (10 mL), Pd/C (5%) (0.075 g) and NaOAc (0.070 g, 0.85 mmol) was hydrogenated at atmospheric pressure for 1 h. The suspension thus obtained was filtered over Celite, concentrated, and recrystallized twice from AcOEt to give **10b** as a white solid (0.098 g, 71%).

Compound **10c** was also obtained on decarboxylation of **10x** using the conditions described below for **10j**.

3-(4-Fluorophenyl)-6,7-dimethyl-2-(4-methylsulfonylphenyl)pyrazolo[1,5-*a*]pyrimidine (10f). Method C. A mixture of **9a** (0.4 g, 1.2 mmol), 3-methyl-4-methoxy-3-buten-2-one[21] (**19**, R_2 = R_3 = Me; 0.176 g, 1.2 mmol), HCl (0.1 mL) and EtOH (8 mL) was refluxed for 4 h. The solvent was concentrated and the residue was chromatographed on silica gel (hexane−AcOEt mixtures) to afford **10f** as a white solid (0.330 g, 70%): mp 202 °C; ^{1}H NMR ($CDCl_3$ δ TMS) 2.42 (s, 3 H, 6-Me), 2.85 (s, 3 H, 7-Me), 3.08 (s, 3 H, SO_2Me), 7.12 (m, 2 H, C_6H_4F), 7.48 (m, 2 H, C_6H_4F), 7.88 (d, J = 7 Hz, 2 H, $C_6H_4SO_2Me$), 7.94 (d, J = 7 Hz, 2 H, $C_6H_4SO_2Me$), 8.36 (s, 1 H, H_5). Anal. ($C_{21}H_{18}FN_3O_2S{\cdot}0.25H_2O$) C, H, N, S. Additionally, 0.039 g of **10f** was obtained (8%).

The same procedure was used for the preparation of **10d** and **23b**−**d** starting from **9b**−**d** respectively. **10d**: mp 234 °C; Anal. ($C_{20}H_{16}FN_3O_2S{\cdot}0.25H_2O$) C, H, N, S. **23b**: mp 244 °C; Anal. ($C_{22}H_{21}N_3O_2S{\cdot}0.25H_2O$) C, H, N, S. **23c**: mp 230−232 °C; Anal. ($C_{21}H_{17}F_2N_3O_2S{\cdot}0.5H_2O$) C, H, N, S. **23d**: mp 189−194 °C; Anal. ($C_{21}H_{17}F_2N_3O_2S$) C, H, N, S.

3-(4-Fluorophenyl)-5,6-dimethyl-2-(4-methylsulfonylphenyl)pyrazolo[1,5-*a*]pyrimidine (10g). Method D. A mixture of **9a** (0.30 g, 0.9 mmol), 4-hydroxy-3-methyl-3-buten-2-one (**21**, $R_2 = R_3 =$ Me; 0.11 g, 0.9 mmol) and toluene (2 mL) was refluxed for 2 h. The solvent was concentrated and the residue was chromatographed on silica gel (hexane−AcOEt mixtures) to afford **10g** as a white solid (0.22 g, 62%): mp 242−243 °C; ^{1}H NMR ($CDCl_3$ δ TMS) 2.37 (s, 3 H, 6-Me), 2.60 (s, 3 H, 5-Me), 3.10 (s, 3 H, SO_2Me), 7.12 (m, 2 H, C_6H_4F), 7.48 (m, 2 H, C_6H_4F), 7.84 (d, $J = 7$ Hz, 2 H, $C_6H_4SO_2Me$), 7.95 (d, $J = 7$ Hz, 2 H, $C_6H_4SO_2Me$), 8.42 (s, 1 H, H_7). Anal. ($C_{21}H_{18}FN_3O_2S\cdot CHCl_3$) C, H, N, S. Additionally, 0.024 g of **10f** was obtained (6%).

The same procedure was used for the preparation of **10h**: mp 202 °C; Anal. ($C_{22}H_{20}FN_3O_2S\cdot 0.5H_2O$) C, H, N, S.

Compound **10g** was also obtained on hydrogenation of **10t** over Pd/C/NaOAc as described above for the preparation of **10b**.

6-Acetyl-3-(4-fluorophenyl)-7-methyl-2-(4-methylsulfonylphenyl)pyrazolo[1,5-*a*]pyrimidine (10v). Method E. A mixture of **9a** (0.30 g, 0.9 mmol), 3-dimethylaminomethylene-2,4-pentanedione (0.13 g, 0.9 mmol; **20**, $R_3 =$ Me, $R_2 =$ COMe, obtained by reaction of 2,4-pentanedione and 1.6 equiv of dimethylformamide dimethylacetal in THF at room temperature overnight) and AcOH (1.5 mL) was refluxed for 1 h. The solvent was eliminated and the residue was dissolved in $CHCl_3$ and washed with saturated $NaHCO_3$ and NaCl solution. The organic phase was dried and concentrated to a crude product. This was chromatographed on silica gel (hexane−AcOEt mixtures) to afford **10v** as a white solid (0.14 g, 37%): mp 238 °C; ^{1}H NMR ($CDCl_3$ δ TMS) 2.73 (s, 3 H), 3.09 (s, 3 H), 3.24 (s, 3 H), 7.14 (m, 2 H), 7.48 (m, 2 H), 7.90 (d, $J = 7$ Hz, 2 H), 7.97 (d, $J = 7$ Hz, 2 H), 8.90 (s, 1 H). Anal. ($C_{22}H_{18}FN_3O_3S$) C, H, N, S.

6-Bromo-3-(4-fluorophenyl)-7-methyl-2-(4-methylsulfonylphenyl)pyrazolo[1,5-*a*]pyrimidine (10ab). Method F. A mixture of **9a** (0.4 g, 1.2 mmol), 3-bromo-4-diethylamino-3-buten-2-one (**20**, $R_2 =$ Br, $R_3 =$ Me, obtained by bromination with Br_2 in $CHCl_3$ of 4-diethylamino-3-buten-2-one; 0.340 g, 1.6 mmol), HBr (0.1 mL) and EtOH (4 mL) was refluxed for 1 h. The solvent was concentrated and the residue was chromatographed on silica gel (hexane−AcOEt mixtures) to afford **10ab** as a white solid (0.170 g, 31%): mp 255−256 °C; ^{1}H NMR ($CDCl_3$ δ TMS) 3.01 (s, 3 H), 3.08 (s, 3 H), 7.13 (m, 2 H), 7.47 (m, 2 H), 7.89 (d, $J = 7$ Hz, 2 H), 7.94 (d, $J = 7$ Hz, 2 H), 8.52 (s, 1 H). Anal. ($C_{25}H_{15}BrFN_3O_2S\cdot 0.5H_2O$) C, H, N, S.

3-(4-Fluorophenyl)-6,7,8,9-tetrahydro-2-(4-methylsulfonylphenyl)-6-oxopyrazolo[1,5-*a*]quinazoline (10ag). Method E. Following the same procedure described for the preparation of **10v** but starting from 1,3-cyclohexanedione, compound **10ag** was obtained as a sole isomer in 30% yield: mp 145−149 °C; ^{1}H NMR ($CDCl_3$ δ TMS) 2.41 (m, 2 H), 2.78 (m, 2 H), 3.10 (s, 3 H), 3.58 (m, 2 H), 7.14 (m, 2 H), 7.47 (m, 2 H), 7.89 (d, $J = 7$ Hz, 2 H), 7.97 (d, $J = 7$ Hz, 2 H), 9.05 (s, 1 H).

3-(4-Fluorophenyl)-6,7,8,9-tetrahydro-2-(4-methylsulfonylphenyl)pyrazolo[1,5-*a*]quinazoline (10ah). Method G. A mixture of **9a** (0.5 g, 1.5 mmol), 2-hydroxymethylenecyclohexanone[31] (**21**; 0.189 g, 1.5 mmol), *p*-toluensulfonic acid (0.011 g) and toluene (19 mL) was refluxed for 1 h. The solvent was concentrated and the residue chromatographed on silica gel (hexane−AcOEt mixtures) to afford **10ah** as a white solid (0.27 g, 43%), together with its regioisomer 3-(4-fluorophenyl)-6,7,8,9-tetrahydro-2-(4-methylsulfonylphenyl)pyrazolo[5,1-*b*]-quinazoline (0.26 g, 42%): mp 193−194 °C; ^{1}H NMR ($CDCl_3$ δ TMS) 2.0 (m, 4 H), 2.85 (m, 2 H), 3.07 (s, 3 H), 3.21 (m, 2 H), 7.10 (m, 2 H), 7.47 (m, 2 H), 7.87 (d, $J = 7$ Hz, 2 H), 7.93 (d, $J = 7$ Hz, 2 H), 8.32 (s, 1 H). Anal. ($C_{23}H_{20}FN_3O_2S\cdot 0.75H_2O$) C, H, N, S.

3-(4-Fluorophenyl)-2-(4-methylsulfonylphenyl)-7-propylpyrazolo[1,5-*a*]pyrimidine (10j). A mixture of **10af** (0.124 g, 0.27 mmol) and 40% H_2SO_4 (1.2 mL) was refluxed for 18 h. After cooling, the mixture was poured over H_2O and basified with 2 N NaOH. The aqueous phase was extracted with $CHCl_3$ and the organic phase was dried and concentrated to a crude product, which was chromatographed on silica gel (hexane−AcOEt mixtures) to afford **10j** as a white solid (0.03 g, 27%): mp 194 °C; ^{1}H NMR ($CDCl_3$ δ TMS) 1.15 (t, $J = 7$ Hz, 3 H), 2.02 (m, 2 H), 3.10 (s, 3 H), 3.26 (t, $J = 7$ Hz, 2 H), 6.80 (d, $J = 4.5$ Hz, 1 H), 7.14 (m, 2 H), 7.49 (m, 2 H), 7.90 (d, $J = 7$ Hz, 2 H), 7.96 (d, $J = 7$ Hz, 2 H), 8.49 (d, $J = 4.5$ Hz, 1 H). Anal. ($C_{22}H_{20}FN_3O_2S\cdot 0.5H_2O$) C, H, N, S.

3-(4-Fluorophenyl)-5-methyl-2-(4-methylsulfonylphenyl)-7-methoxypyrazolo[1,5-*a*]pyrimidine (10r). A mixture of **10q** (0.51 g, 1.36 mmol), acetone (4.2 mL), Me_2SO_4 (0.13 mL, 1.36 mmol), K_2CO_3 (0.186 g, 1.36 mmol) and KI (0.042 g, 0.2 mmol) was refluxed for 2 h. The solvent was concentrated and the residue dissolved in a mixture $CHCl_3$−H_2O. The organic phase was extracted with $CHCl_3$ and the combined organic extracts were dried and concentrated to a crude product. This was chromatographed on silica gel (hexane−AcOEt mixtures) to afford **10r** as a white solid (0.18 g, 25%): mp 213 °C; ^{1}H NMR ($CDCl_3$ δ TMS) 2.40 (s, 3 H), 3.14 (s, 3 H), 3.88 (s, 3 H), 6.10 (s, 1 H), 7.03 (m, 2 H), 7.37 (m, 2 H), 7.61 (d, $J = 7$ Hz, 2 H), 8.04 (d, $J = 7$ Hz, 2 H). Anal. ($C_{21}H_{18}FN_3O_3S\cdot 0.75H_2O$) C, H, N, S.

7-Chloro-3-(4-fluorophenyl)-5-methyl-2-(4-methylsulfonylphenyl)pyrazolo[1,5-*a*]pyrimidine (10s). A mixture of **10q** (0.33 g, 0.9 mmol) and $POCl_3$ (1 mL) was refluxed for 1 h. After concentration the residue was dissolved in ice−H_2O and basified with NH_4OH. The mixture was extracted with AcOEt and the organic phase was dried and concentrated to a crude product. This was chromatographed on silica gel (hexane−AcOEt mixtures) to afford **10s** as a white solid (0.04 g, 11%): mp 230−232 °C; ^{1}H NMR ($CDCl_3$ δ TMS) 2.65 (s, 3 H), 3.09 (s, 3 H), 6.96 (s, 1 H), 7.13 (m, 2 H), 7.48 (m, 2 H), 7.87 (d, $J = 7$ Hz, 2 H), 7.96 (d, $J = 7$ Hz, 2 H). Anal. ($C_{20}H_{15}ClFN_3O_2S\cdot 0.25H_2O$) C, H, N, S.

Using the same procedure from the corresponding hydroxy derivative [3-(4-fluorophenyl)-5,6-dimethyl-2-(4-methylsulfonylphenyl)pyrazolo[1,5-*a*]pyrimidin-7-ol, obtained from **9a** and **15** (R_1, $R_2 =$ Me) under conditions of method D: mp 293−298 °C; Anal. ($C_{21}H_{18}FN_3O_3S\cdot H_2O$) C, H, N, S] **10t** was obtained in 88% yield.

3-(4-Fluorophenyl)-7-methyl-2-(4-methylsulfonylphenyl)pyrazolo[1,5-*a*]pyrimidine-6-carboxylic Acid (10x). A mixture of **10w** (0.23 g, 0.54 mmol), KOH (0.13 g, 1.4 mmol), H_2O (1 mL) and EtOH (10 mL) is heated at reflux overnight. The solvent was concentrated and the residue poured over a mixture of H_2O and AcOEt. The aqueous phase was washed with AcOEt and acidified with 6 N HCl. This was extracted with $CHCl_3$, which was dried and concentrated to afford **10x** as a white solid (78%): mp 287−292 °C; ^{1}H NMR ($CDCl_3$ + CD_3OD δ TMS) 3.09 (s, 3 H), 3.25 (s, 3 H), 4.28 (s, 1 H + H_2O), 7.07 (m, 2 H), 7.42 (m, 2 H), 7.89 (d, $J = 7$ Hz, 2 H), 7.91 (d, $J = 7$ Hz, 2 H), 8.97 (s, 1 H). Anal. ($C_{21}H_{16}FN_3O_4S\cdot 1.25CHCl_3$) C, H, N, S.

The same procedure was used for the preparation of **10n** from **10m**.

3-(4-Fluorophenyl)-6-hydroxymethyl-7-methyl-2-(4-methylsulfonylphenyl)pyrazolo[1,5-*a*]pyrimidine (10y). To a solution of **10w** (0.2 g, 0.44 mmol) in THF (4 mL) at −78 °C, a solution of DIBAL (1.24 mL, 1.24 mmol) 1 M in THF was added and the mixture stirred at −78 °C for 2 h and at room temperature overnight. H_2O was added and the solution extracted with AcOEt. The organic phase was dried and concentrated to a crude product, which was chromatographed on silica gel (hexane−AcOEt mixtures) to afford **10y** as a yellow solid (0.05 g, 28%): mp 268−270 °C; ^{1}H NMR ($CDCl_3$ + CD_3OD δ TMS) 2.95 (s, 3 H), 3.11 (s, 3 H), 3.49 (s, 1 H + H_2O), 4.79 (s, 2 H), 7.13 (m, 2 H), 7.46 (m, 2 H), 7.89 (d, $J = 7$ Hz, 2 H), 7.94 (d, $J = 7$ Hz, 2 H), 8.51 (s, 1 H). Anal. ($C_{21}H_{18}FN_3O_3S\cdot 0.75H_2O$) C, H, N, S.

3-(4-Fluorophenyl)-6-fluoromethyl-7-methyl-2-(4-methylsulfonylphenyl)pyrazolo[1,5-*a*]pyrimidine (10aa). A mixture **10y** (0.2 g, 0.48 mmol), DAST (0.067 g, 0.52 mmol) and CH_2Cl_2 (8 mL) was stirred at room temperature for 2 h. The solution thus obtained was washed with H_2O and the

organic phase was dried and concentrated to a crude product. This was chromatographed on silica gel (hexane−AcOEt mixtures) to afford **10aa** as a white solid (0.15 g, 75%): mp 218−219 °C; ^{1}H NMR ($CDCl_3$ δ TMS) 2.99 (d, J = 3 Hz, 3 H), 3.09 (s, 3 H), 3.57 (d, J = 48 Hz, 2 H), 7.13 (m, 2 H), 7.47 (m, 2 H), 7.89 (d, J = 7 Hz, 2 H), 7.94 (d, J = 7 Hz, 2 H), 8.50 (s, 1 H). Anal. ($C_{21}H_{17}F_2N_3O_2S\cdot0.5H_2O$) C, H, N, S.

3-(4-Fluorophenyl)-7-hydroxymethyl-6-methyl-2-(4-methylsulfonylphenyl)pyrazolo[1,5-*a*]pyrimidine (10ad). A mixture of **10f** (0.2 g, 0.5 mmol), 6 N H_2SO_4 (0.6 mL) and $CHCl_3$ (0.5 mL) was added to a suspension of $KMnO_4$ (0.079 g, 0.5 mmol), NEt_3 (0.07 g, 0.5 mmol), H_2O (0.1 mL) and $CHCl_3$ (2 mL) and it was stirred at room temperature for 3 days. The mixture was filtered over a silica gel/$CaCl_2$ (2.5 g/0.5 g) mixture and washed thoroughly with $CHCl_3$. The organic phase was washed with a saturated $NaHCO_3$ solution, dried and concentrated to a crude product. This was chromatographed on silica gel (hexane−AcOEt mixtures) to afford **10ad** as a white solid, which was recrystallized from CH_2Cl_2 (0.07 g, 33%): mp 226−227 °C; ^{1}H NMR ($CDCl_3$ δ TMS) 1.55 (s, 1 H), 2.25 (s, 3 H), 3.09 (s, 3 H), 5.20 (s, 2 H), 7.13 (m, 2 H), 7.47 (m, 2 H), 7.89 (m, 4 H), 8.42 (s, 1 H). Anal. ($C_{21}H_{18}FN_3O_3S\cdot0.5CH_2Cl_2$) C, H, N, S.

3-(4-Fluorophenyl)-2-(4-methylsulfonylphenyl)-7-propylpyrazolo[1,5-*a*]pyrimidine-6-carboxylic Acid (10af). A mixture of ethyl 3-(4-fluorophenyl)-2-(4-methylsulfonylphenyl)-7-propylpyrazolo[1,5-*a*]pyrimidine-6-carboxylate [obtained from **9a** and **16** (R_3 = Pr) under conditions of method C; 0.26 g, 0.54 mmol], KOH (0.13 g, 1.4 mmol), H_2O (1 mL) and EtOH (10 mL) was heated at reflux overnight. The solvent was concentrated and the residue poured over a mixture of H_2O and AcOEt. The aqueous phase was washed with AcOEt and acidified with 6 N HCl. This was extracted with $CHCl_3$, which was dried and concentrated to afford **10af** as a white solid (70%): mp: 257 °C; ^{1}H NMR ($CDCl_3$ δ TMS) 1.20 (t, J = 7 Hz, 3 H), 1.98 (m, 2 H), 3.12 (s, 3 H), 3.85 (t, J = 7 Hz, 2 H), 7.17 (m, 2 H), 7.48 (m, 2 H), 7.91 (d, J = 7 Hz, 2 H), 8.00 (d, J = 7 Hz, 2 H), 9.09 (s, 1 H), 10.63 (s, 1 H). Anal. ($C_{23}H_{20}FN_3O_4S\cdot0.5H_2O$) C, H, N, S.

4-[3-(4-Fluorophenyl)-6,7-dimethylpyrazolo[1,5-*a*]pyrimidin-2-yl]benzenesulfonamide (26a). To a solution of **22a** (0.37 g, 1.0 mmol, obtained from **8a** as described for **10f**) in CH_2Cl_2 (2.5 mL) was added *m*-chloroperbenzoic acid (0.32 g, 1.0 mmol) at 0 °C and the mixture was stirred for 2 h at room temperature. The suspension was washed with 1 N NaOH and the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were dried and concentrated to a crude product. This was chromatographed on silica gel (hexane−AcOEt mixtures) to afford 3-(4-fluorophenyl)-6,7-dimethyl-2-(4-methylsulfinylphenyl)pyrazolo[1,5-*a*]pyrimidine as a white solid (0.32 g, 82%): ^{1}H NMR ($CDCl_3$ δ TMS) 2.41 (s, 3 H), 2.75 (s, 3 H), 2.84 (s, 3 H), 7.10 (m, 2 H), 7.46 (m, 2 H), 7.67 (d, J = 7 Hz, 2 H), 7.83 (d, J = 7 Hz, 2 H), 8.35 (s, 1 H).

The previous compound was treated with Ac_2O (2.6 mL) and AcONa (0.26 g, 3.2 mmol) at reflux for 8 h. The solvent was eliminated and the crude 2-[4-(acetoxymethylthio)phenyl]-3-(4-fluorophenyl)-5,7-dimethylpyrazolo[1,5-*a*]pyrimidine (**24a**) thus obtained was used directly in the next step.

A solution of the previous compound in CH_2Cl_2 (2.6 mL) and MeOH (1.7 mL) at 0 °C was treated with hexahydrated magnesium monoperoxyphthalate (0.58 g, 0.97 mmol) and the mixture stirred at room temperature overnight. Saturated $NaHCO_3$ solution was added and the mixture extracted with CH_2Cl_2. The solvent was eliminated and the residue dissolved in THF (2.6 mL) and MeOH (1.3 mL) and cooled to 0 °C. 1 N NaOH (0.84 mL) was added and the mixture stirred at room temperature for 1 h. The solvent was eliminated and the residue vacuum-dried to afford crude sodium 4-[3-(4-fluorophenyl)-5,7-dimethylpyrazolo[1,5-*a*]pyrimidin-2-yl]benzenesulfinate (**25a**; 0.28 g).

A mixture of the previous crude, H_2O (3.6 mL), AcONa (0.063 g, 0.76 mmol) and hydroxylamine *O*-sulfonic acid (0.086 g, 0.76 mmol) was stirred at room temperature overnight. The suspension thus obtained was filtered and washed with AcOEt and H_2O. The phases were separated and the aqueous phase was extracted with AcOEt. The combined organic phases were dried and concentrated to a crude product. This was chromatographed on silica gel (hexane−AcOEt mixtures) to afford **26a** as a white solid (0.083 g, 30%): mp 228−229 °C; ^{1}H NMR ($CDCl_3$ + CD_3OD δ TMS) 2.39 (s, 3 H), 2.81 (s, 3 H), 4.09 (s, 2 H + H_2O), 7.07 (m, 2 H), 7.40 (m, 2 H), 7.74 (d, J = 7 Hz, 2 H), 7.85 (d, J = 7 Hz, 2 H), 8.30 (s, 1 H). Anal. ($C_{20}H_{17}FN_4O_2S\cdot0.75H_2O$) C, H, N, S.

The same procedure was used to obtain **26b**: mp 281 °C; Anal. ($C_{21}H_{20}N_4O_2S\cdot0.25H_2O$) C, H, N, S. **26c**: mp 250−252 °C; Anal. ($C_{20}H_{16}F_2N_4O_2S\cdot0.5H_2O$) C, H, N, S.

8-(4-Fluorophenyl)-3,4-dimethyl-7-(4-methylsulfonylphenyl)pyrazolo[5,1-*c*][1,2,4]triazine (29a). A mixture of ethyl 2-methylacetoacetate (0.21 mL, 1.5 mmol) and KOH (0.13 g, 1.9 mmol) in H_2O (1 mL) was stirred at 0 °C for 24 h. After adjusting the pH to 6 with concentrated HCl, a suspension of **27** [prepared from **9a** (0.5 g, 1.5 mmol) by reaction at 0 °C with $NaNO_2$ (0.104 g, 1.5 mmol) in H_2O (0.27 mL)] in HCl (0.37 mL) and H_2O (0.27 mL) was added under the surface followed by AcONa (0.38 g, 4.6 mmol). The mixture was stirred at 0 °C for 1 h and allowed to reach room temperature. $CHCl_3$ was added and the phases were separated. The aqueous phase was extracted with $CHCl_3$ and the combined organic phases were dried and concentrated to a crude product. This was chromatographed on silica gel (hexane−AcOEt mixtures) to afford **29a** as a white solid (0.100 g, 17%): mp 219 °C; ^{1}H NMR ($CDCl_3$ δ TMS) 2.91 (s, 3 H), 2.93 (s, 3 H), 3.11 (s, 3 H), 7.17 (m, 2 H), 7.63 (m, 2 H), 7.94 (d, J = 7 Hz, 2 H), 7.99 (d, J = 7 Hz, 2 H). Anal. ($C_{20}H_{17}FN_4O_2S\cdot2H_2O$) C, H, N, S.

The same procedure was used to obtain **29b**: mp 164 °C; Anal. ($C_{21}H_{19}FN_4O_2S$) C, H, N, S. **29c**: mp 213−214 °C; Anal. ($C_{21}H_{19}FN_4O_2S$) C, H, N, S. **29d**: mp 221−224 °C; Anal. ($C_{22}H_{21}FN_4O_2S$) C, H, N, S. **29e**: mp 170−176 °C; Anal. ($C_{22}H_{21}FN_4O_2S\cdot0.5H_2O$) C, H, N, S. **29f**: mp 186−190 °C; Anal. ($C_{22}H_{21}FN_4O_2S\cdot0.25H_2O$) C, H, N, S.

8-(4-Fluorophenyl)-2,4-dimethyl-7-(4-methylsulfonylphenyl)pyrazolo[1,5-*a*][1,3,5]triazine (33a). A mixture of ethyl acetimidate hydrochloride (**30**, R_1 = Me; 0.25 g, 2 mmol), K_2CO_3 (0.28 g, 2 mmol) and CH_3CN (5 mL) was stirred for 5 min and the suspension thus obtained was filtered. To the resulting solution a mixture of **9a** (0.3 g, 0.9 mmol) in AcOH (0.05 mL) was added and stirring continued overnight. The suspension thus obtained was filtered and the solvent was concentrated to give **31a** (R_1 = Me; 0.3 g). This compound was dissolved in ethyl orthoacetate (**32**, R_2 = Me; 1 mL) and heated at reflux overnight. The solvent was concentrated to a crude product, which was chromatographed on silica gel (hexane−AcOEt mixtures) to afford **33a** as a white solid (0.075 g, 21%): mp 190 °C; ^{1}H NMR ($CDCl_3$ δ TMS) 2.71 (s, 3 H), 3.02 (s, 3 H), 3.11 (s, 3 H), 7.13 (m, 2 H), 7.45 (m, 2 H), 7.86 (d, J = 7 Hz, 2 H), 7.99 (d, J = 7 Hz, 2 H). Anal. ($C_{20}H_{17}FN_4O_2S$) C, H, N, S.

The same procedure was used to obtain **33b**: mp 177−183 °C; Anal. ($C_{23}H_{23}FN_4O_2S\cdot1.25H_2O$) C, H, N, S. **33c**: mp 170−173 °C; Anal. ($C_{22}H_{21}FN_4O_2S\cdot0.25H_2O$) C, H, N, S.

4-Chloro-2,6-dimethyl-3-nitropyridine (35b). A mixture of 2,6-dimethyl-4-hydroxy-3-nitropyridine (**34b**;[32] 4.6 g, 27.3 mmol), $POCl_3$ (2.5 mL), DMF (2 mL) and toluene (20 mL) was heated at 110 °C for 7 h. After cooling to 0 °C, the mixture was basified with Na_2CO_3 and the solid thus obtained filtered and dried to afford **35b** as an oil (3.54 g, 69%): ^{1}H NMR ($CDCl_3$ δ TMS) 2.56 (s, 3 H), 2.57 (s, 3 H), 7.18 (s, 1 H).

2,6-Dimethyl-4-(4-fluorophenylamino)-3-nitropyridine (36b). To a solution of **35b** (3.54 g, 19 mmol) in $CHCl_3$ (3 mL) was added, at 0 °C under argon, a solution of 4-fluoroaniline (2.2 mL, 23 mmol) and NEt_3 (3.7 mL) in $CHCl_3$ (6.5 mL) and the mixture was refluxed for 48 h. The solvent was eliminated and the residue was dissolved with AcOEt and H_2O. The phases were separated and the aqueous phase was extracted with AcOEt. The combined organic phases were dried and concentrated to a crude product. This was chromatographed on silica gel (hexane−AcOEt mixtures) to afford **36b** as a yellow solid (4.0 g, 81%): mp 128 °C; ^{1}H NMR ($CDCl_3$

δ TMS) 2.35 (s, 3 H), 2.75 (s, 3 H), 6.52 (s, 1 H), 7.20 (m, 4 H), 8.60 (s, 1 H).

3-Amino-2,6-dimethyl-4-(4-fluorophenylamino)pyridine (37b). A mixture of **36b** (2.0 g, 7.6 mmol), $SnCl_2$ (7.2 g, 38 mmol) and EtOH (54 mL) was refluxed for 45 min. The solvent was eliminated and the residue was dissolved with H_2O and basified with 25% NaOH. The aqueous phase was extracted with $CHCl_3$ and the organic phase was dried and concentrated to afford **37b** as an oil (1.0 g, 56%): ^{1}H NMR ($CDCl_3$ δ TMS) 2.34 (s, 3 H), 2.44 (s, 3 H), 3.19 (s, 2 H), 5.91 (s, 1 H), 6.63 (s, 1 H), 7.04 (m, 4 H).

4,6-Dimethyl-1-(4-fluorophenyl)-2-(4-methylsulfonylphenyl)imidazo[4,5-*c*]pyridine (38b). A mixture of **37b** (1.0 g, 4.0 mmol), 4-methylsulfonylbenzoyl chloride (0.98 g, 4.5 mmol) and pyridine (16 mL) was refluxed overnight. The solvent was eliminated and the residue was dissolved with H_2O and extracted with $CHCl_3$. The organic phase was washed with 0.1 N NaOH, dried and concentrated to afford a crude product. This was chromatographed on silica gel (hexane−AcOEt mixtures) to afford **38b** as a white solid (0.46 g, 29%): mp 259 °C; ^{1}H NMR ($CDCl_3$ δ TMS) 2.62 (s, 3 H), 2.94 (s, 3 H), 3.06 (s, 3 H), 6.85 (s, 1 H), 7.28 (m, 4 H), 8.76 (d, J = 7 Hz, 2 H), 7.91 (d, J = 7 Hz, 2 H). Anal. ($C_{21}H_{18}FN_3O_2S\cdot0.25H_2O$) C, H, N, S.

The same procedure was used to obtain **38a**: mp 218−221 °C; Anal. ($C_{19}H_{14}FN_3O_2S\cdot0.25H_2O$) C, H, N, S.

2-Bromo-2-(4-fluorophenyl)-1-(4-methylsulfonylphenyl)-ethanone (39). To a solution of **5a** (5 g, 17.1 mmol) in CH_2Cl_2 (44 mL) was added a solution of Br_2 (0.98 mL, 19.2 mmol) in AcOH (107 mL) and the mixture was stirred at room temperature overnight. The solvent was eliminated and the residue was dried to afford **39** as a white solid (6.35 g, 100%): mp 113−116 °C; ^{1}H NMR ($CDCl_3$ δ TMS) 3.13 (s, 3 H), 6.34 (s, 1 H), 7.22 (m, 2 H), 7.58 (m, 2 H), 8.09 (d, J = 7 Hz, 2 H), 8.21 (d, J = 7 Hz, 2 H).

3-Amino-6-chloro-4,5-dimethylpyridazine (40b). A mixture of 3,6-dichloro-4,5-dimethylpyridazine[33] (0.5 g, 2.8 mmol), 30% aqueous NH_3 (20 mL) and THF (5 mL) was stirred in a pressure vessel at 150 °C for 36 h. The solvent was eliminated and the residue was chromatographed on silica gel (hexane−AcOEt mixtures) to afford **40b** as an oil (0.22 g, 50%): ^{1}H NMR ($CDCl_3$ δ TMS) 2.14 (s, 3 H), 2.33 (s, 3 H), 4.81 (s, 2 H).

6-Chloro-7,8-dimethyl-3-(4-fluorophenyl)-2-(4-methylsulfonylphenyl)imidazo[1,2-*b*]pyridazine (41b). A mixture of **40b** (0.22 g, 1.4 mmol), **39** (0.52 g, 1.4 mmol) and DMF (3 mL) was kept at 60 °C overnight. The solvent was eliminated and the residue was dissolved with H_2O and extracted with $CHCl_3$. The organic phase was washed with saturated $NaHCO_3$ solution, dried and concentrated to afford a crude product. This was chromatographed on silica gel (hexane−AcOEt mixtures) to afford **41b** as a white solid (0.46 g, 29%): mp 192−194 °C; H NMR ($CDCl_3$ δ TMS) 2.44 (s, 3 H), 2.74 (s, 3 H), 3.05 (s, 3 H), 7.22 (m, 2 H), 7.54 (m, 2 H), 7.94 (s, 4 H). Anal. ($C_{21}H_{17}ClFN_3O_2S\cdot0.25H_2O$) C, H, N, S.

The same procedure was used to obtain **41a**: mp 216 °C; Anal. ($C_{19}H_{14}FN_3O_2S\cdot0.25H_2O$) C, H, N, S.

7,8-Dimethyl-3-(4-fluorophenyl)-2-(4-methylsulfonylphenyl)imidazo[1,2-*b*]pyridazine (41c). A mixture of **41b** (0.15 g, 0.3 mmol), 10% Pd/C (0.03 g), NEt_3 (0.14 mL, 1 mmol) and THF (12 mL) was hydrogenated at 200 psi overnight. The mixture was filtered over Celite and the solvent was eliminated to afford a crude product. This was chromatographed on silica gel (hexane−AcOEt mixtures) to afford **41c** as a white solid (0.095 g, 69%): mp 216−217 °C; H NMR ($CDCl_3$ d TMS) 2.38 (s, 3 H), 2.68 (s, 3 H), 3.05 (s, 3 H), 7.22 (m, 2 H), 7.54 (m, 2 H), 7.90 (m, 4 H), 8.10 (s, 1 H). Anal. ($C_{21}H_{18}FN_3O_2S\cdot H_2O$) C, H, N, S.

3-(4-Fluorophenyl)-2-(4-methylsulfonylphenyl)imidazo-[1,2-*a*]pyrazine (43a). The same procedure described for **41b** starting from 2-aminopyrazine (**42a**) was used to obtain **43a:** mp 229−231 °C; ^{1}H NMR ($CDCl_3$ d TMS) 3.04 (s, 3 H), 7.33 (m, 2 H), 7.47 (m, 2 H), 8.87 (m, 6 H), 9.19 (s, 1 H). Anal. ($C_{19}H_{14}FN_3O_2S\cdot0.5H_2O$) C, H, N, S.

Analogously, **43b**,**c** and **45** were obtained using, respectively, 2-amino-3,5-dimethylpyrazine (**42b**, obtained from 2-chloro-3,5-dimethylpyrazine in a manner similar to that described in ref 34), 2-amino-3-butylpyrazine (**42c**)[35] and 2-amino-4-methylpyrimidine (**44**). **43b**: mp 239−242 °C. Anal. ($C_{21}H_{18}FN_3O_2S\cdot0.25H_2O$) C, H, N, S. **43c**: Anal. ($C_{23}H_{22}FN_3O_2S\cdot0.25H_2O$) C, H, N, S. **45**: mp 272−273 °C; Anal. ($C_{20}H_{16}FN_3O_2S$) C, H, N, S.

Inhibition of COX-1 and COX-2 Activities in HWB.[27] The tests were performed with heparinized human whole blood from healthy donors who had not taken NSAIDs for 1 week nor alcohol or xanthines within 24 h of blood collection. COX-1 activity was measured in spontaneously clotting human blood for 5 h at 37 °C and stimulated with A23187 (5 μM) for 30 min longer. The reaction was stopped at 0 °C with EGTA and MeOH. After centrifugation the supernatant was removed and stored at −70 °C. TXB2 levels were determinated by enzyme immunoassay kit (RPN 220, Amersham). COX-2 activity was stimulated by adding lipopolysaccharide 026:B6 (25 μL/mL) at 37 °C for 24 h. Aspirin (20 μg/mL) was used to inhibit COX-1 activity. After centrifugation, the samples were stored at −70 °C. PGE_2 levels were determinated by enzyme immunoassay kit (RPN 222, Amersham).

Inhibition of COX-1 and COX-2 Activities in Human Cell Lines. The production of PGE_2 after AA stimulation by lines expressing h-COX-1 (U-937 from human histiocytic lymphoma) and h-COX-2 (143.98.2 from human osteosarcoma) was used as a cell-based assay to evaluate COX-1 and COX-2 inhibition, respectively. Briefly, osteosarcoma cells were cultured in 1 mL of media in 24-well multidishes until confluent (2×10^5 cells/well during 24 h). U-937 cells were grown in flasks and resuspended to a final density of 3×10^6 cells/mL/well. The cells were resuspended in HBSS without Ca^{2+}/Mg^{2+}. 10 μL of a DMSO solution of test compound or DMSO vehicle was added. The samples were then incubated for 15 min at 37 °C (5% CO_2 and 95% humidity) before the addition of AA (final concentration: 10 μM) for 10 min. The reactions were stopped by the addition of indomethacine (8 mM, 30 μL). PGE_2 levels in supernatant were determined by specific EIA (Amersham). All assays were performed in triplicate.

Inhibition of Carrageenan-Induced Rat Paw Edema. Male Sprague−Dawley rats (175−200 g) were used. Edemas were produced by injecting 0.1 mL of a solution of 1% λ-carrageenan in the hindpaw. Paw volumes were measured by water displacement with a plethysmometer (UGO BASILE) before and 1, 2, 3, 4 and 5 h after treatment. The compounds were administered orally as a suspension in carboxymethyl cellulose and Tween-80 at 1% (10 mL/kg), 0.5 h before carrageenan injection and after being hydrated with H_2O (5 mL). The percentages of inhibition were calculated by comparing the areas under the curve of treated and control animals.

Air-Pouch Model of Inflammation: Determination of PGE_2 in Exudate and Stomach. Male Lewis rats (175−200 g) were used. Air cavities were produced by a subcutaneous injection of sterile air (20 mL) into the intracapsular area. Every 2 days air (10 mL) was injected into the cavity to keep the space open. 7 days after the first injection, λ-carregeenan (Sigma) in saline (2 mL of a 1% solution) was injected into the air pouch to produce an inflammatory reaction. The compounds were administered by oral route as a suspension in carboxymethyl cellulose and Tween-80 at 1% (10 mL/kg), 0.5 h before carrageenan injection. The animals were killed 6 h later and the exudate volume was measured. Cells were pelleted by centrifugation at 1200*g* for 5 min at 4 °C, and PGE_2 was determined in the supernatant by specific ELISA. Immediately after the exudates were collected, the stomachs were excised and frozen at −70 °C. On the day of analysis, stomachs were homogenized in 70% ethanol. The homogenates were centrifuged (1000*g*, 10 min, 4 °C) and the supernatants were dried under N_2 stream and resuspended in ELISA buffer for PGE_2 determination.

Acknowledgment. We thank M. Carmen Torres, Concepción González, Consol Ferreri, Guadalupe Martínez, Nuria Recasens, Teresa Gamero, José Antonio García, Ana Ester Sanahuja, and Assumpta Oliveras

for their excellent technical assistance and Jordi Belloc for the GC, MS, and HPLC analyses.

Supporting Information Available: Combustion analyses and ^{1}H NMR spectra for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM0009383