CYP2C9 Structure−Metabolism Relationships: Optimizing the Metabolic Stability of COX-2 Inhibitors

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The cytochrome P450 (CYP) family is composed of a large group of monooxygenases that mediate the metabolism of xenobiotics and endogenous compounds. CYP2C9, one of the major isoforms of the CYP family, is responsible for the phase I metabolism of a variety of drugs. The aim of the present investigation is to use rational design together with MetaSite, a metabolism site prediction program, to synthesize compounds that retain their pharmacological effects but that are metabolically more stable in the presence of CYP2C9. The model compound for the study is the nonsteroidal anti-inflammatory drug celecoxib, a COX-2 selective inhibitor and known CYP2C9 substrate. Thirteen analogs of celecoxib were designed, synthesized, and evaluated with regard to their metabolic properties and pharmacologic effects. The docking solutions and the predictions from MetaSite gave useful information leading to the design of new compounds with improved metabolic properties.

Introduction

An important aspect of the drug discovery process is the optimization of the metabolic properties of test compounds. Hence, it should be of value to be able to predict those structural modifications that are likely to lead to altered metabolic properties while retaining the desired pharmacological profile. This paper uses rational design together with MetaSite, a metabolism site prediction program, to synthesize analogs of celecoxib (Figure 1) that retain COX-2 inhibiting properties but that are metabolically more stable in the presence of CYP2C9. Structural characteristics for a compound to be selectively recognized by CYP2C9 include the presence of an anionic site and a hydrophobic zone between the substrate hydroxylation site and the anionic site.[1] CYP2C9 catalyzes the oxidation of several important drugs including diclofenac and warfarin.[2,3] It is reasonable to speculate that COX-2 inhibitors that are poor CYP2C9 substrates will display improved clearance and half-life properties. In the present study we explore computational methods to exploit the recently published crystal structures of mammalian CYP2C9[4,5] in an effort to improve our understanding of the metabolic pathways of this class of compounds that are catalyzed by CYP enzymes.

Celecoxib is a COX-2 selective inhibitor known to be rapidly metabolized to the hydroxymethyl metabolite by CYP2C9.[6,7] The compound has a fairly rigid core structure with three apparent interaction points: R^1, R^2, and R^3 (Figure 1). Celecoxib and other nonsteroidal anti-inflammatory drugs (NSAIDs[a]) mediate their anti-inflammatory actions primarily through the inhibition of cyclooxygenase (COX), the enzyme that catalyzes the conversion of arachidonic acid to prostaglandin. COX exists in at least two isoforms, COX-1, a constitutive enzyme, and COX-2, an inducible isoform.[8] The selective inhibition of COX-2 leads to improved anti-inflammatory properties with fewer gastrointestinal side effects.[9,10] However, COX-2 inhibitors recently have been shown to cause other serious side effects such as an increased risk of adverse cardiovascular events.[11]

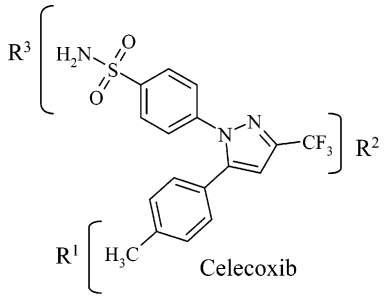

Figure 1. Celecoxib (4-[5-(4-methylphenyl)-3-trifluoromethyl-1*H*-pyrazol-1-yl]-benzenesulfonamide).

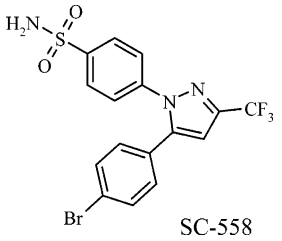

Figure 2. SC-558 (4-[5-(4-bromophenyl)-3-trifluoromethyl-1*H*-pyrazol-1-yl]-benzenesulfonamide).

According to the crystal structure of COX-2 in which the active site is occupied by the selective NSAID celecoxib analogue SC-558 (Figure 2), the active site is mainly hydrophobic. The amino acids in close proximity of the selective COX-2 inhibitor, SC-558, and the GRID molecular interaction fields (MIFs) for hydrophobic (DRY), hydrogen-bond acceptors

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[a] Abbreviations: COX, cyclooxygenase; CSA, camphorsulfonic acid; CYP, cytochrome P450; MIF, molecular interaction field; NSAID, nonsteroidal anti-inflammatory drug; PGE_2, prostaglandin E_2; SAR, structure−activity relationship; TBHP, *t*-butyl hydroperoxide.

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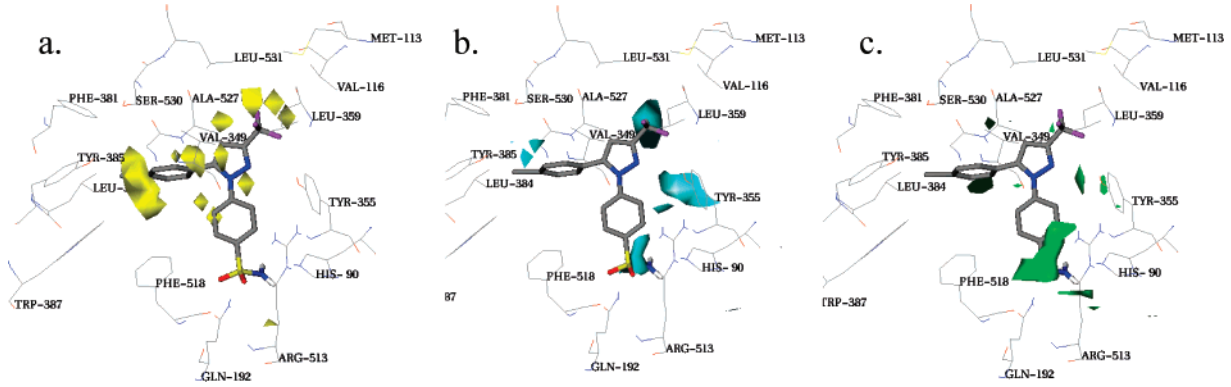


Figure 3. MIFs calculated in the COX-2 (PDB: 1CX2) crystal using three different probes (a) DRY (−0.2 kcal/ mol), (b) O (−4.0 kcal/mol), and (c) N1 (−6.0 kcal/mol) are viewed together with the cocrystallized compound, SC-558.

(O), and hydrogen-bond donors (N1) are shown in Figure 3. The bromine atom is surrounded by Phe381, Leu384, Tyr385, Trp387, Phe518, and Ser530, with additional contributions from the backbone atoms of Gly526 and Ala527. The trifluoromethyl group is surrounded by Met113, Val116, Val349, Tyr355, Leu359, and Leu531. The sulfonamide moiety interacts with His90, Gln192, and Arg513. The NH group in the sulfonamide moiety forms a hydrogen bond to the carbonyl oxygen of Phe518.[12] Retaining the pharmacological activity (COX-2 inhibition) was approached by taking advantage of these previously published data describing which amino acids are of importance for the affinity to COX-2.[12] The structure−activity relationship (SAR) around the 1,5-diarylpyrazolyl class of compounds published by Penning et al.[13] was also considered.

MetaSite[14,15] is a tool that can be applied to identify the potential sites of metabolism in a compound (hot spots). This information can be useful when attempting to design analogs with improved metabolic properties. MetaSite is based on two factors: protein ligand similarity analysis and chemical reactivity of the substrate. MetaSite performs a similarity analysis of the protein−cavity interaction profile and the potential substrate. The chemical reactivity of fragments toward oxidation, representing the activation energy of the conversion of substrate to metabolite, is precomputed and stored within the program. Hence, if the similarity search gives a high score for a fragment, the chemical reactivity of that fragment is included in the final "hot spot" prediction.[14,16]

The rational design of analogs to celecoxib with potentially improved metabolic properties was aided by docking celecoxib into the active site of CYP2C9 (PDB: 1R9O)[4] using the docking program GLUE[17] (see below). The site of metabolism and the molecular contribution to the exposure of a reactive atom to the heme moiety (activity contribution plot) were determined by MetaSite. The interactions with COX-2 needed to keep COX-2 inhibition were investigated using the crystal structure of COX-2 cocrystallized with the selective NSAID celecoxib analogue SC-558 (Figure 3).

Results and Discussion

Modeling of Celecoxib Metabolism. GLUE, a GRID-based docking program,[17] was used in this study to analyze ligand−receptor interactions. Celecoxib was docked into the active site of CYP2C9 using the available crystal structure (PDB: 1R9O), without crystallographic water molecules. Multiple docking solutions were obtained, but only one docking solution had the carbon atom in the methyl group within 3 Å from the ferryl oxygen (Figure 4). MetaSite predicted that the methyl group in celecoxib was most likely to be metabolized by CYP2C9 (Figure 5a) and celecoxib is known to be hydroxylated at the methyl group by CYP2C9,[7] hence, all other docking solutions could be discarded.

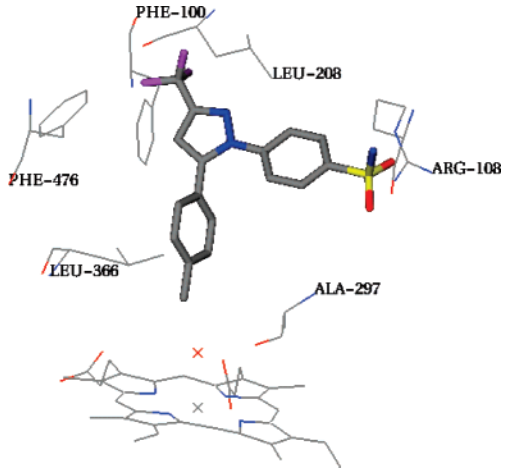


Figure 4. Celecoxib docked in the active site of the crystal structure of CYP2C9 (PDB: 1R9O).

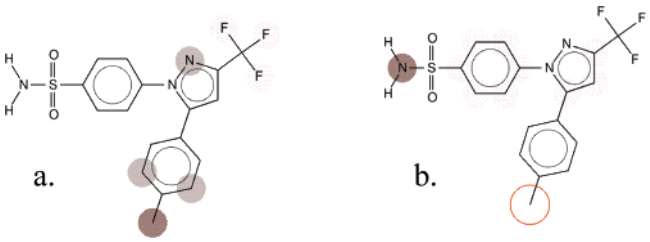


Figure 5. Plots of MetaSite predictions. (a) Site of metabolism: The groups in celecoxib that most likely will be metabolized by CYP2C9 are marked in dark brown; the darker the color the higher is the probability of metabolism to occur. (b) Contribution plot: The atoms in celecoxib with molecular contribution to the exposure of the metabolic "hot spot" (red circle) to the heme moiety of CYP2C9 are marked dark brown; the darker the color the higher the probable contribution.

The carbon atom in the methyl group is docked 2.9 Å from the ferryl oxygen. This is a productive mode because it can lead to metabolite formation. Amino acids within 3 Å from the trifluoromethyl group are Leu208, Phe476, and Phe100. A bulky group in this position may fail to fit into the pocket and thus decrease the affinity for CYP2C9 of the new celecoxib analog. The sulfonamide is docked 2.7 Å from the Arg108. This docking solution indicates that altering the interaction between the sulfonamide group and the Arg108 may decrease the interaction

between the new celecoxib analog and CYP2C9. The contribution plot from MetaSite also indicates the importance of this interaction (Figure 5b).

The site of metabolism predictions were performed by the program MetaSite. These analyses involve the calculation of two sets of descriptors, one set of descriptors for the CYP enzyme and one for the potential substrate. The resulting analysis provided a "chemical fingerprint" of the enzyme and the substrate. Chemical fingerprints are distance-based descriptors that are calculated from MIFs computed by GRID. Moreover, as mentioned earlier, the program considers the chemical reactivity of the compound by taking into consideration the activation energy in the hydrogen abstraction step that ultimately leads to metabolite formation. These hydrogen abstraction processes have been simulated by ab initio calculations of small fragments from druglike substrates for human CYPs and stored in MetaSite.[15] When a fragment in the target molecule is recognized as one in the MetaSite database, all atoms in that fragment are assigned the corresponding reactivity value. The final ranking for potential metabolic sites is the product of the similarity analysis and the chemical reactivity. The information used for the prediction was the structure of CYP2C9 and several conformations of the compound of interest. MetaSite correctly predicted that the methyl group in celecoxib was most likely to be metabolized by CYP2C9 (Figure 5a). The prediction was achieved without any input of information of known metabolites.

MetaSite can also provide an activity contribution plot that shows the molecular contribution to the exposure of a reactive atom toward the heme moiety.[15] The program locks the group that has been predicted to be metabolized by the CYP close to the heme moiety. Subsequently, the program allows the potential substrate to move within the active site, keeping the group fixed, and computes the energy of interaction between the compound and CYP. Interactions considered during the computation are hydrophobic, charge, and hydrogen-bond interactions. The molecular moieties in the potential substrate, which have the strongest impact for directing the metabolic hot spot toward the heme moiety, are determined. In the analysis of celecoxib, the activity contribution plot points out the nitrogen in the sulfonamide moiety as the responsible atom for orienting the methyl group toward the heme (Figure 5b).

One advantage achieved with the MetaSite program compared to docking is that no time-consuming visual inspection of the potential interactions between the compound and the metabolizing enzyme is needed. The site of metabolism is predicted and shown in schematic plots. The main disadvantage is that no information about which amino acids that interact with the compound in the active site is achieved.

Design of Celecoxib Analogs. Two approaches were combined for the design of new analogs of celecoxib with the potential for improved metabolic stability. One approach was rational design using information obtained from crystal structures. The crystal structure of COX-2 (PDB: 1CX2)[12] was available cocrystallized with the celecoxib analog SC-558 (Figure 2). No celecoxib analog cocrystallized with CYP2C9 was available, therefore, the rational design was performed by using the docking pose of celecoxib in the active site of CYP2C9 (PDB: 1R9O).[4] The other approach was to use the site of metabolism prediction tool, MetaSite, that predicts which group in a compound is most likely to be metabolized by CYP2C9. The program also predicts which atom may be responsible for the positioning of metabolically susceptible groups close to the heme moiety. Structural changes were made in three positions

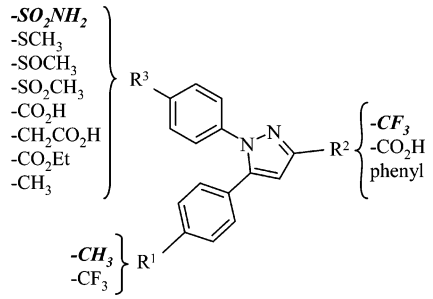
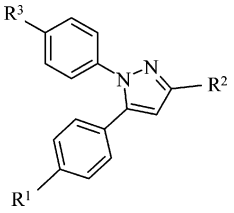


Figure 6. Synthesized analogs of celecoxib (substituents highlighted in bold).

Table 1. Summary of Celecoxib and Its Synthesized Analogs Metabolism in CYP2C9 after a 30 Min Incubation and Percent Inhibition of PGE_2 Formation in COX-1 and COX-2



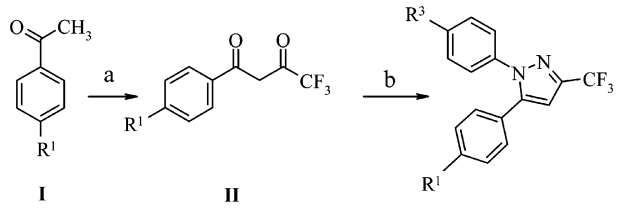
				% metabolized	% inhibition of control value	
cmpd	R^1	R^{2g}	R^3	CYP2C9 (1 μM)	COX-2 (100 μM)	COX-1 (100 μM)
celecoxib	$-CH_3$	$-CF_3$	$-SO_2NH_2$	94	96	96
1	$-CF_3$	$-CF_3$	$-CO_2H$	29	28	6
2	$-CH_3$	$-CF_3$	$-CO_2H$	29	67	26
3	$-CH_3$	phenyl	$-SO_2NH_2$	49	100	92
4	$-CH_3$	$-CF_3$	$-SCH_3$	87	95	99
5	$-CH_3$	$-CF_3$	$-SOCH_3$	48	82	99
6	$-CH_3$	$-CF_3$	$-SO_2CH_3$	81	99	92
7	$-CF_3$	$-CF_3$	$-CO_2Et$	2	55	96
8	$-CH_3$	$-CF_3$	$-CO_2Et$	88	55	87
9	$-CF_3$	$-CF_3$	$-SO_2NH_2$	0	100	86
10	$-CH_3$	$-CF_3$	$-CH_3$	30	94	98
11	$-CF_3$	$-CF_3$	$-CH_2CO_2H$	4	96	93
12	$-CH_3$	$-CF_3$	$-CH_2CO_2H$	10	88	95
13c	$-CH_3$	$-CO_2H$	$-SO_2NH_2$	61	66	43

of celecoxib, namely, R^1, R^2, and R^3 (Figure 6). The design of the celecoxib analogs was challenging due to the fact two enzymes must be considered.

Modifications in the R^1 position were of interest because MetaSite had predicted correctly that the methyl group was most likely to be metabolized by CYP2C9. The initial structural change was made in the R^1 position in which the CH_3 group of celecoxib (Figure 1) was replaced with a CF_3 group to give **9** (for the structures of compounds see Table 1). We suspected that this relatively minor structural alteration would not result in loss of COX-2 inhibitory activity but would block the principal CYP2C9-catalyzed oxidation.

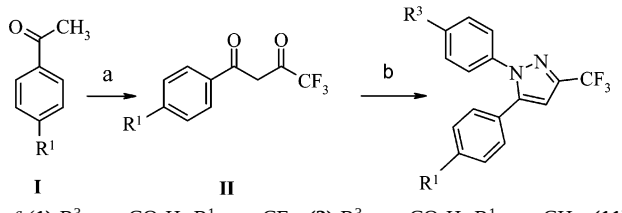
Structural knowledge gained from the crystal structures of COX-2 (Figure 3) and CYP2C9 (Figure 4) guided the modifications in the R^2 position. In the cocrystallized structure, the trifluoromethyl group of SC-558 resides in a large pocket that is constructed from several hydrophobic amino acids (Val116, Leu117, Tyr355, Phe357, and Leu359) and a positively charged amino acid (Arg120). The dockings of celecoxib into the active site of CYP2C9 indicated that a bulky group in the R^2 position could cause a clash with Phe100. Hence, analog **3**, containing a phenyl group at C(3) of the pyrazolyl moiety, was synthesized to investigate if this modification would lead to a less-productive interaction with CYP2C9. The 3-position of the pyrazolyl ring

Scheme 1[a]



[a] **(4)** $R^3 = -SCH_3$; $R^1 = -CH_3$; **(7)** $R^3 = -CO_2Et$; $R^1 = -CF_3$; **(8)** $R^3 = -CO_2Et$; $R^1 = -CH_3$; **(9)** $R^3 = -SO_2NH_2$; $R^1 = -CF_3$; **(10)** $R^3 = -CH_3$; $R^1 = -CH_3$. Reagents and conditions: (a) NaH, dry THF, ethyl trifluoroacetate; (b) R^3-phenylhydrazine·HCl, EtOH, reflux.

Scheme 2[a]



[a] **(1)** $R^3 = -CO_2H$; $R^1 = -CF_3$; **(2)** $R^3 = -CO_2H$; $R^1 = -CH_3$; **(11)** $R^3 = -CH_2CO_2H$; $R^1 = -CF_3$; **(12)** $R^3 = -CH_2CO_2H$; $R^1 = -CH_3$. Reagents and conditions: (a) NaH, dry THF, ethyl trifluoroacetate; (b) R^3-phenylhydrazine·HCl, acetonitrile, reflux.

(R^2) is known to have very few steric restrictions in COX-2.[13] Therefore, the COX-2 inhibition properties should be retained. Due to the fact that there is an Arg120 in the pocket of COX-2, a compound containing a carboxylic acid group (compound **13c**) was also synthesized in an effort to increase stabilizing polar interactions between the substrate and the enzyme.

Modifications in the R^3 position were of interest because according to the MetaSite prediction the sulfonamide moiety of celecoxib may be responsible for the positioning of the methyl group close to the heme moiety. The changes in this position had to be performed with some caution. The sulfonamide group is critical for the selectivity for COX-2 inhibition because it binds to a pocket that is more restricted in COX-1 than in COX-2. This pocket is unoccupied in complexes of COX-2 with nonselective inhibitors.[12] The docking of celecoxib in CYP2C9 indicated an interaction between the sulfonamide group and Arg108. In an attempt to disrupt this interaction, the sulfonamide group of celecoxib was replaced with a methyl group to give compound **10**. Other structural changes were also performed in this position. Three compounds, the sulfide (**4**), the sulfoxide (**5**), and the sulfone (**6**), representing different oxidation states on the sulfur atom, were studied. This series of compounds was synthesized to evaluate the influence of the hydrogen-bonding interactions between the oxygens and Arg108 in CYP2C9.

In compound **2**, a carboxyl group has replaced the sulfonamide group. Compound **8** is the corresponding ethyl ester. These two compounds provided another opportunity to evaluate the influence of potential hydrogen-bond interactions in the active site of CYP2C9 that are implicated from the docking studies. For comparison, the corresponding two analogs with a trifluoromethyl group in the R^1 position were also examined. The resulting analogs are compound **1**, with a carboxyl group in the R^3 position, and compound **7**, with an ethoxycarbonyl group in the R^3 position. A further investigation of the effects of a carboxyl group in the R^3 position was pursued with compounds **11** and **12**. These two compounds have an additional methylene unit between the aromatic ring and the carboxyl group. These analogs provided an opportunity to evaluate the importance of conformational flexibility on both CYP2C9 and COX-2 activity. Moreover, the influence of the distance between the carboxyl group and the methyl group could be studied by comparing compounds **1** and **2** in which the carboxyl group is attached directly to the phenyl ring.

Synthesis. The general method employed for the synthesis of the celecoxib analogs (Schemes 1 and 2) is patterned after the route described by Penning et al.[13]

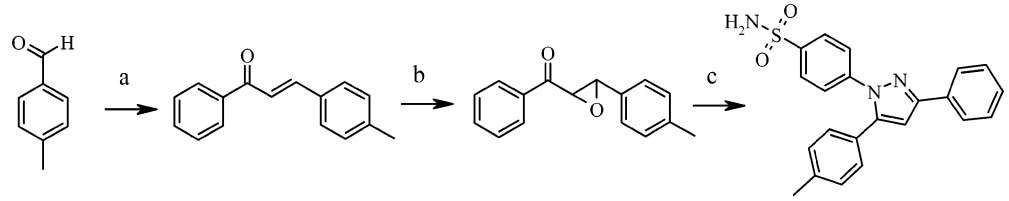
Claisen condensation of a *para*-substituted acetophenone derivative (**I**) with ethyl trifluoroacetate in the presence of NaH gave the 1,3-diketo adduct (**II**) in good yield. The reaction of 1,3-diketones with phenylhydrazine has been reported to yield a mixture of 1,5-diarylpyrazolyl and 1,3-diarylpyrazolyl products.[13] However, Penning et al. showed that the reaction proceeds regiospecifically with the hydrochloride salt of phenylhydrazine in refluxing ethanol to give exclusively the 1,5-diaryl analogs. When a bulky alkyl group is present on the diketone, the major product is usually the 1,5-diarylpyrazole.[18] This is probably due to the fact that the attack of the $-NH_2$ end of the phenylhydrazine is favored at the less sterically hindered carbonyl group.[19] In the present study, the reaction was highly regioselective for the 1,5-diaryl analogs, except for **3**, a compound with a phenyl group instead of the trifluoromethyl group in the R^2 position. With two equally large groups on the diketone, no regioselectivity was achieved. To obtain the desired isomer **3** in pure form, the synthetic route presented in Scheme 3[13] was pursued.

An aldol condensation between 4-methylbenzaldehyde (**III**) and acetophenone gave the chalcone (**IV**), which subsequently was further oxidized to the epoxide (**V**). Reaction of the epoxide with phenylhydrazine provided exclusively the 1,5-diarylpyrazolyl isomer (**3**). Compounds **5** and **6** were prepared by oxidation of the sulfide (**4**) (Scheme 4). Oxidation of (**4**) by *t*-butyl hydroperoxide (TBHP) in the presence of a catalytic amount of camphorsulfonic acid (CSA) gave the sulfoxide (**5**) in good yield.[20] The sulfone (**6**) was prepared by oxidation of **4** with oxone.[21]

The carboxylic acid **13c** (Scheme 5) was synthesized starting with a Claisen condensation of 4-methylacetophenone and diethyl oxalate in the presence of sodium ethoxide.[22] The resulting 2,4-diketo ester was heated under reflux in ethanol with 4-sulfamoylphenylhydrazine followed by hydrolysis[23] of the resulting ester to yield the desired carboxylic acid **13c**.

Metabolism Studies in CYP2C9. Celecoxib and all synthesized analogs were examined for their metabolic properties using an *in vitro* assay employing recombinant CYP2C9. The test compounds (1 μM) were incubated with CYP2C9 over a period of 30 min. The relative amounts of the parent compounds remaining after the incubation gives an indication on how stable the compounds are in the test system. The different analogs with chemical modifications in the R^1, R^2, and R^3 positions allowed an analysis of the relationship between the structure and the metabolic properties (Table 1). Celecoxib is known to be metabolized mainly at the methyl group.[7] The trifluoromethyl analog **9** proved to be stable metabolically. To investigate whether changes in the R^2 position would prevent the interaction with CYP2C9, analogs **3** and **13c** containing a phenyl and a carboxyl group, respectively, were studied. The docking solution of celecoxib in the active site of CYP2C9 indicated that there would be no space for a phenyl group in this position. Although **3** was a CYP2C9 substrate, the phenyl group decreased the extent of metabolism at 30 min to only 49% compared to 94% for celecoxib. Because the compound was metabolized, it could fit into the active site, but the decreased metabolism indicates that the interactions were weakened. Analog **13c** with a carboxyl

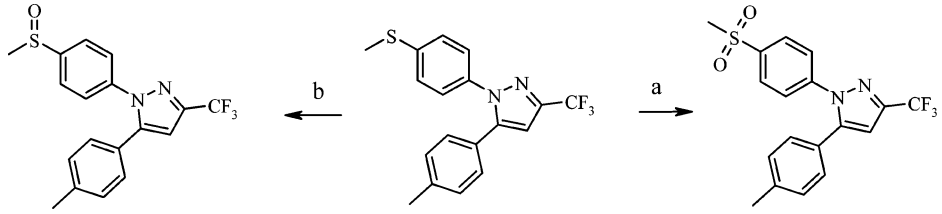
Scheme 3[a]



III **IV** **V** **3**

[a] Reagents and conditions: (a) MeOH, NaOH (50% w/v aq solution), acetophenone; (b) 30% H_2O_2, 4 M NaOH, EtOH, acetone; (c) (4-sulfamoylphenyl)hydrazine·HCl, EtOH.

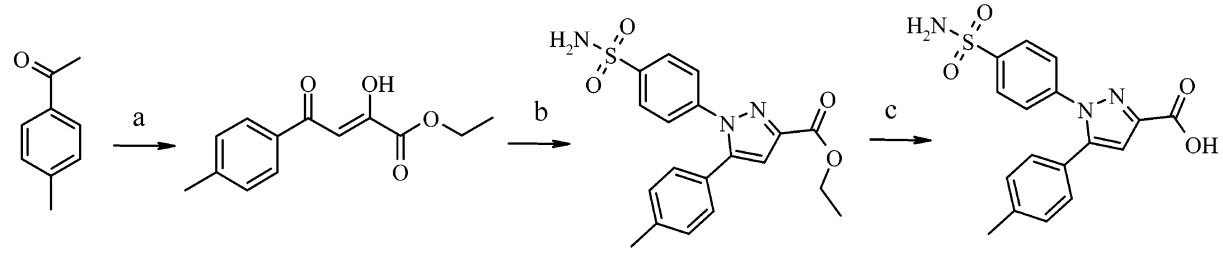
Scheme 4[a]



5 **4** **6**

[a] Reagents and conditions: (a) THF/MeOH (1:1), oxone; (b) dichloromethane, TBHP, CSA.

Scheme 5[a]



13a **13b** **13c**

[a] Reagents and conditions: (a) EtOH, diethyl oxalate; (b) (4-sulfamoylphenyl)hydrazine·HCl, EtOH, reflux; (c) THF/MeOH/LiOH (2 M, 1:1:1), RT.

group in the R^2 position provided information on whether or not this replacement could lead to additional hydrogen bond formation in the active site. The metabolism was decreased (61% consumed), indicating that if additional hydrogen bonds were formed, they did not enhance the positioning of the methyl relative to the heme moiety in a productive way. In the case of compound **10**, a methyl group replaces the sulfonamide group of celecoxib. This modification led to a 70% decrease in metabolism, suggesting a poor fit of this compound in the active site of CYP2C9. The decreased metabolism also emphasizes the importance of the interactions between the sulfonamide moiety and CYP2C9.

Finally, the three compounds representing different oxidation states on the sulfur atom were studied. The sulfonamide group was replaced by a sulfide (**4**), a sulfoxide (**5**), or a sulfone (**6**). The analysis of the docking of celecoxib into the active site of CYP2C9 indicated that the oxygen atoms attached to sulfur could result in strong interactions with Arg108. From these observations, the sulfone (**6**) was expected to be metabolized to a greater extent than the sulfoxide (**5**) and the sulfoxide (**5**) to a greater extent than the sulfone (**4**). The results from the *in vitro* assay showed no decrease in the extent of metabolism for compound **4** and only a modest decrease to 48% consumed for **5**. Removing apparently important interactions with the enzyme had only a minor or no influence on the metabolism. Hence, these results indicate that the compounds hydrophobic core structure has a major impact on the total interaction with CYP2C9.

All compounds with a carboxylic acid group in the R^3 position (**1**, **2**, **11**, and **12**) showed a clear decrease in the metabolism. For compounds **1** (29%) and **11** (4%), one possible explanation for this behavior could be that the methyl group has been replaced with a trifluoromethyl group, which is not susceptible for metabolism. The decreased metabolism of the other two compounds, **2** (29%) and **12** (10%), indicates that the carboxylic acid group does not direct the methyl toward the heme moiety to the same extent as the sulfonamide group. Structural characteristics of CYP2C9 substrates include the presence of an anionic site and an hydrophobic zone between the substrate hydroxylation site and the anionic site.[1] The different distances between the carboxyl group and the methyl group in compounds **2** and **12** may have an influence on the metabolism. Compound **12** was metabolized to a lesser extent than compound **2**, suggesting that the increased conformational flexibility and distance between the methyl group and the carboxyl group may be unfavorable for metabolism. The ethoxycarbonyl analog **8** underwent CYP2C9-catalyzed oxidation almost to the same extent as celecoxib (88% vs 94%). The corresponding ethoxycarbonyl analog **7**, that also bears a trifluoromethyl group in the R^1 position, instead proved to be a very poor substrate (2%

conversion at 30 min). This observation leads us to conclude that the difference in the metabolism of these two compounds was not due to the metabolism of the ester.

COX-1 and COX-2 Inhibition. Celecoxib and all synthesized analogs were analyzed for their effect on the activity of human COX-1/COX-2. The results are presented in Table 1 and expressed as percent inhibition of the control enzyme activity. The inhibition of COX-1 and COX-2 was determined at a compound concentration of 100 μM. Celecoxib has an IC_{50} of 0.04 μM for COX-2 and 15 μM for COX-1,[13] hence, the selectivity cannot be captured in this study.

Replacing the methyl group in the R^1 position of celecoxib with a trifluoromethyl group to obtain **9** did not change the inhibition of COX-1 and COX-2. Compounds **7** and **8** have an ester functionality in the R^3 position but differ in the R^1 position. Compound **8** has a methyl group in the R^1 position, while compound **7** has a trifluoromethyl group. These two compounds had similar COX-1 and COX-2 inhibition. Hence, modifications in the R^1 position did not affect the inhibition of the two isoforms.

The active site of COX-2 is mainly hydrophobic, therefore replacing the CF_3 group with a phenyl group would most likely increase the interactions. The in vitro result verified this hypothesis because **3** showed potent inhibitory effects on both enzymes. Altering the R^2 position to a carboxylic acid as in **13c** decreased both the COX-2 and the COX-1 inhibition.

Changing celecoxib in the R^3 position to a sulfide (**4**), a sulfoxide (**5**), or a sulfone (**6**) did not effect COX-1 and COX-2 inhibition. Four analogs with a carboxyl group in the R^3 position, two analogs with an additional methylene unit between the aromatic ring and the carboxyl group (**11** and **12**) and two with the carboxyl group directly attached to the ring (**1** and **2**) were compared for their effects on COX-1 and COX-2. The carboxymethyl analogs retained their inhibitor properties for both isoforms of COX, whereas the inhibitory capacity decreased for the other two compounds, especially the COX-1 inhibition. Compounds **7** and **8**, the corresponding ethyl esters of **1** and **2**, showed a dramatic and selective increased inhibition of COX-1 compared to the carboxy analogs. Interestingly, compound **10**, which lacks the sulfonamide moiety, inhibited both COX-1 and COX-2 to the same extent as celecoxib. The sulfonamide moiety is known to be important for the COX-2 selectivity. Consequently, if compound **10** were to be tested at a lower concentration, even though it has a low IC_{50}, one might expect to see no selectivity between the two isoforms.

To be considered a potent COX-2 inhibitor, a compound should have an IC_{50} value lower than 1 μM. Optimizing the compounds toward these properties, that is, selectivity and potency, is not within the scope of this study. The inhibition data of the COX enzymes demonstrate that most compounds retain their inhibitory capacity, thus, the design toward improved metabolic properties has not led to structures lacking the pharmacophore for COX-2 inhibitors.

Conclusions

When a compound is optimized toward the pharmacological target of interest it is important to take its metabolic properties into account as early as possible in the drug discovery process. Maintaining the pharmacological activity during the design of more metabolically stable compounds is a challenging task. The aim of the present investigation is to use rational design together with MetaSite, a metabolism site prediction program, to synthesize compounds that retain their pharmacological effects but that are metabolically more stable in the presence of CYP2C9. Another issue, which is not considered in this study, is the inhibition potential of the compound toward the metabolizing enzyme. Modifying the metabolic "hot spot" may decrease the rate of metabolism of a compound, but because other steric and hydrophobic properties are retained, the outcome may be a metabolically stable compound that still fits into the enzyme's active site. The compound may no longer be a substrate for the isoform, but there is a possibility that it will display inhibitor properties instead of substrate properties. Ongoing studies of this issue and further details will be disclosed in future publications. The design of the celecoxib analogs was challenging due to the fact that two enzymes had to be considered. Previously published data about the amino acids that are important for the affinity of inhibitors to the COX-2 enzyme and published SAR of COX-2 inhibitors were also considered when designing the new compounds.[12,13,24] When this information was taken into account, it was possible to retain the COX-2 inhibition properties in the new analogs. Dockings and the predictions from MetaSite gave useful information leading to the design of new compounds with improved metabolic properties. MetaSite provided two approaches to alter the metabolic properties either to modify the metabolic "hot spot" or to modify the chemical group responsible for directing the "hot spot" toward the heme moiety. Which group in the compound that should be modified depends on the compound's pharmacophore. Both approaches were successful in terms of decreasing the metabolism in CYP2C9 compared to the parent compound celecoxib. The synthesized compounds increased the knowledge about the active site of the two enzymes, COX-2 and CYP2C9. Combining the structural information gained from the docking studies with MetaSite predictions is an excellent approach to design pharmacologically active compounds with improved metabolic properties.

Experimental Section

Modeling of Celecoxib Metabolism. All calculations were performed in a Linux environment on a 32 MB personal computer. The software utilized in the computational analysis was GRID v. 21 and MetaSite v. 2.5 (Molecular Discovery Ltd., http://moldiscovery.com), SYBYL 6.9 (Tripos Associates Inc., St. Louis, MO), and MacroModel v. 7.0.110 (Schrödinger, NY).

Celecoxib was submitted to the program MetaSite that is a fully automated procedure. The CYP2C9 structure used in MetaSite is the homology model[25] based on the CYP2C5 (PDB: 1DT6[26]) structure. The MIF for CYP2C9 obtained from the GRID package are precomputed and stored inside the software. Once the structures of the compounds are provided, the semiempirical calculations, pharmacophoric recognition, descriptor handling, similarity computation, and the reactivity computation are all carried out automatically.[14,15]

The human CYP2C9 crystal structure complexed with flurbiprofen at 2.0 Å resolution was used in the docking study (PDB: 1R9O). This is a wild-type enzyme, except that the N-terminal membrane insertion peptide has been removed to increase solubility for crystallization. The modifications done to the protein were to delete the water molecules and to remove the ligand from the binding site. An additional modification was to add a dummy atom 2.0 Å above the iron. This will decrease the importance of the electrostatic interactions and hinder the docked compounds to interact directly with the iron in the heme. The dummy atom used was OES, which represents an explicit tetrahedral ester oxygen atom that does not accept any hydrogen bonds. The PDB file had to be modified before being imported into GLUE, which is performed by Greater that converts the PDB format to the format of the input files (kout files) required to run the docking procedure.

The box size used was 20 × 20 × 20 Å, centered round the crystallographic ligand coordinates. The probes, representing the

different atoms,[27] were selected manually when performing the binding site precalculations in GLUE. These probes were selected manually when performing the binding site precalculations in GLUE. Default values were used when performing the docking, and the result was analyzed visually. The structure of celecoxib was drawn in Sybyl. Conformation search followed by energy minimization was performed in MacroModel (v. 7.0.110). Ten conformations were docked into the active site of CYP2C9 using the docking program GLUE.[17]

Synthesis. General. Unless otherwise noted, all solvents, chemicals, and reagents were obtained commercially and used without purification. Reactions were routinely monitored by thin-layer chromatography (TLC) on silica-plated aluminum sheets (silica gel 60 F254, E. Merck), detecting spots by UV light. ^{1}H and ^{13}C NMR spectra were obtained on a JEOL JNM-EX 400 spectrometer at 400 and 100 MHz, respectively, in $CDCl_3$ or CD_3-OD solutions, as indicated. Peak positions are given in parts per million (δ) downfield from tetramethylsilane as internal standard, and J values are given in Hz. Melting points were measured in a Büchi Melting Point B-540 apparatus and are uncorrected. Chromatography was performed on silica (silica gel 60 (0.040−0.063 mm), E. Merck) using flash chromatography. Preparative high performance liquid chromatography (HPLC) was performed on a Kromasil-C8 analytical column (250 mm × 4.6 mm i.d., 10 μm, Phenomenex, England, U.K.). The mobile phase consisted of 0.1% formic acid in acetonitrile/water (60/40). The microwave reactions were carried out in a Biotage Initiator instrument. Elemental analyses were performed at H. Kolbe Mikroanalytisches laboratorium, Mülheim an der Ruhr, Germany, and were within ±0.2% of the theoretical values for C, H, and N.

4,4,4-Trifluoro-1-(4-methylphenyl)butane-1,3-dione (A).[28] 4-Methyl-acetophenone (360 μL, 2.7 mmol) was dissolved in dry THF (15 mL) under nitrogen atmosphere and NaH (60% in mineral oil, 130 mg, 3.24 mmol) was added in three lots maintaining the temperature between −5 and 0 °C. After stirring at this temperature for 30 min, ethyl trifluoroacetate (390 μL, 3.24 mmol) was added via syringe and the reaction mixture was allowed to stir at ambient temperature for 5 h. The reaction mixture was poured into ice water, acidified with HCl (2 M), and extracted with ethyl acetate (2 × 50 mL). The combined organic layer was washed with water (2 × 50 mL), dried, and concentrated. The solid residue was washed with hexane and dried under high vacuum to provide a solid mass that was redissolved in dichloromethane and dried under high vacuum to give the diketone **A** (590 mg, 95%). ^{1}H NMR ($CDCl_3$) δ 7.84 (d, J = 8.2 Hz, 2H), 7.30 (d, J = 8.0 Hz, 2H), 6.54 (s, 1H), 2.43 (s, 3H).

4,4,4-Trifluoro-1-(4-trifluoromethylphenyl)butane-1,3-dione (B).[28] 4-Trifluoromethyl-acetophenone (813 mg, 4.3 mmol) was reacted with NaH (60% in mineral oil, 216 mg, 5.4 mmol) and ethyl trifluoroacetate (643 μL, 5.4 mmol) as described above for **A**. The crude product (1.29 g, 100%) was used without further purification.

[4-Methylthio-phenyl]hydrazine Hydrochloride (C).[29] Aqueous HCl (37%, 8 mL) and 4-methylthio-aniline (490 μL, 4.0 mmol) were added to a 50 mL three-necked round bottomed flask. The slurry was heated to 120 °C until a solution was formed. The solution was cooled down to −5 °C and a solution of $NaNO_2$ (290 mg, 4.2 mmol) in H_2O (2 mL) was added dropwise at −5 °C. The mixture was stirred at −5 °C for 15 min and was then added at −20 °C to a solution of $SnCl_2 \times 2H_2O$ (2.7 g, 12.0 mmol) in aqueous HCl (37%) (3 mL). After stirring at −20 °C for 25 min and at −5 °C for another 25 min, the precipitate was collected by filtration and successively washed with cool H_2O and Et_2O to give **C** (574 mg, 75%).

4-Sulfamoylphenyl Hydrazine Hydrochloride (D).[29] Aqueous HCl (37%, 12 mL), sulfanilamide (1.16 g, 6.73 mmol), and $NaNO_2$ (489 mg, 7.09 mmol) in H_2O (3 mL) were reacted as described for **C** to afford pure **D** (1.29 g, 86%).

2-(4-Hydrazinophenyl) Acetic Acid Hydrochloride (E).[29] Aqueous HCl (37%, 10 mL), 4-aminophenylacetic acid (1.10 g, 6.70 mmol), and $SnCl_2 \cdot 2H_2O$ (4.55 g, 20.2 mmol) were reacted as described above. The precipitate of **E** was collected by filtration and washed with cool EtOH to give pure MA11 (1.32 g, 96%).

3-(4-Methylphenyl)-1-phenyl-2-propen-1-one (F).[30] Aqueous NaOH (50%, 0.5 mL) was added to a stirred solution of 4-methyl-benzaldehyde (590 μL, 5.0 mmol) and acetophenone (585 μL, 5.0 mmol) in MeOH (20 mL). The mixture was stirred overnight at RT. The precipitate was filtrated to give **F** (866 mg, 78%).

2,3-Epoxy-3-(4-methylphenyl)-1-phenylpropanone (G).[13] To a solution of **F** (603 mg, 2.71 mmol) in EtOH (15 mL) and acetone (5 mL) at 50 °C was added H_2O_2 (30%, 2 mL) and NaOH (4 M, 1.5 mL). After stirring for 3 h, the resulting precipitate was filtered off and dried to obtain **G** (643 mg, 99%) as a white solid.

4-[3-Trifluoromethyl-5-(4-trifluoromethylphenyl)-1*H*-pyrazol-1-yl]-benzoic Acid (1).[13] 4-Hydrazinobenzoic acid HCl (69.8 mg, 0.37 mmol) was added to a stirred solution of **B** (93.4 mg, 0.33 mmol) in acetonitrile (15 mL). The mixture was heated at reflux for 20 h. After cooling to room temperature, the reaction was concentrated under vacuum. The residue was taken up in EtOAc (25 mL), washed with water (10 mL) and brine (10 mL), dried over $MgSO_4$, filtered, and concentrated under vacuum. Purification with flash chromatography (dichloromethane/MeOH 99:1) gave **1** (87 mg, 66%). Purification by preparative HPLC yielded (50 mg, 40%) **1**: ^{1}H NMR (CD_3OD) δ 7.02 (s, 1H), 7.33 (d, J = 8.0, 2H), 7.43 (d, J = 8.0, 2H), 7.62 (d, J = 8.0, 2H), 8.02 (d, J = 8.0, 2H). Anal. ($C_{18}H_{10}F_6N_2O_2$) C, H, N.

4-[3-Trifluoromethyl-5-(4-methylphenyl)-1*H*-pyrazol-1-yl]-benzoic Acid (2).[13] 4-Hydrazinobenzoic acid HCl (52.8 mg, 0.28 mmol) and **A** (57.6 mg, 0.25 mmol) in acetonitrile (15 mL) were reacted as described for **1** to give **2** (62 mg, 71%): ^{1}H NMR (CD_3-OD) δ 2.27 (s, 3H), 6.85 (s, 1H), 7.08 (s, 4H), 7.24 (d, J = 8.0, 2H), 8.03 (d, J = 8.0, 2H). An analytical sample was further purified with preparative LC to get 99.9% purity. Anal. ($C_{18}H_{13}F_3N_2O_2$) C, H, N.

4-[5-(4-Methylphenyl)-3-phenyl-1*H*-pyrazol-1-yl]-benzene-sulfonamide (3). Compounds **D** (315 mg, 1.40 mmol) and **G** (334 mg, 1.40 mmol) were added to a 10 mL microwave reaction vessel containing a stirrer, EtOH (5 mL), and 3 drops of acetic acid. The vessel was sealed and the mixture was heated in the microwave for 20 min at 180 °C and then allowed to cool to room temperature. The reaction mixture was partitioned between water (50 mL) and EtOAc (100 mL), and the aqueous layer was extracted with EtOAc (3 × 100 mL). The combined extracts were dried over $MgSO_4$, filtered, and concentrated under vacuum. The crude product was purified by flash chromatography (EtOAc/hexane 5:5) to obtain **3** (117 mg, 21%): ^{1}H NMR ($CDCl_3$) δ 2.39 (s, 3H), 6.81 (s, 1H), 7.18 − 7.92 (m, 13H). Anal. ($C_{22}H_{19}N_3O_2S$) C, H, N.

4-[5-(4-Methylphenyl)-3-trifluoromethyl-1*H*-pyrazol-1-yl]-1-methylthio-benzene (4).[13] Compounds **C** (515 mg, 2.70 mmol) and **A** (564 mg, 2.45 mmol) in EtOH (15 mL) were reacted as described for **3** to give **4** (275 mg, 32%): ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 3H), 2.49 (s, 3H), 6.71 (s, 1H), 7.13 (d, J = 4.0, 4H), 7.22 (d, J = 4.0, 4H). Anal. ($C_{18}H_{15}F_3N_2S$) C, H, N.

4-[5-(4-Methylphenyl)-3-trifluoromethyl-1*H*-pyrazol-1-yl]-1-methylsulfinyl-benzene (5).[20] A mixture of **4** (60.6 mg, 0.17 mmol), TBHP (6 M in nonane, 58.3 μL, 0.35 mmol), and CSA (4.0 mg, 0.017 mmol) in dichloromethane was stirred at RT for 4 h. The reaction was monitored by TLC. When completed, the solution was directly poured onto the top of a silica gel column and eluted with hexane/ethyl acetate (7:3) to give pure **5** (64 mg, 87%): ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 3H), 2.72 (s, 3H), 6.73 (s, 1H), 7.10 (d, J = 8.0, 2H), 7.15(d, J = 8.0, 2H), 7.48 (d, J = 8.0, 2H), 7.64 (d, J = 8.0, 2H). Anal. ($C_{18}H_{15}F_3N_2OS$) C, H, N.

4-[5-(4-Methylphenyl)-3-trifluoromethyl-1*H*-pyrazol-1-yl]-1-methylsulfonyl-benzene (6).[21] Compound **4** (45.3 mg, 0.13 mmol) was dissolved in methanol (10 mL) and cooled to 0 °C. To this a solution of oxone (49.5%, 240 mg, 0.39 mmol) in H_2O (5 mL) was added. The resulting cloudy slurry was stirred for 4 h at room temperature, diluted with water (30 mL), and extracted with dichloromethane (3 × 50 mL). The combined organic layers were washed with H_2O (50 mL) and brine (50 mL), dried over $MgSO_4$, filtered, and concentrated under vacuum to give **6** (49 mg, 99%)

as a white solid: ^{1}H NMR ($CDCl_3$) δ 2.38 (s, 3H), 3.06 (s, 3H), 6.75 (s, 1H), 7.11 (d, J = 8.0, 2H), 7.18 (d, J = 8.0, 2H), 7.53 (d, J = 8.0, 2H), 7.93 (d, J = 8.0, 2H). Anal. ($C_{18}H_{15}F_3N_2O_2S$) C, H, N.

Ethyl 4-[5-(4-Trifluoromethylphenyl)-3-trifluoromethyl-1*H*-pyrazol-1-yl]-benzoate (7).[13] 4-Hydrazinobenzoic acid hydrochloride (181 mg, 0.96 mmol) and **B** (247 mg, 0.87 mmol) in 15 mL of EtOH were reacted as described for MAX. Purification with flash chromatography (MeOH/EtOAc/hexane 1:10:89) gave **7** (183 mg, 58%): ^{1}H NMR ($CDCl_3$) δ 1.40 (t, J = 8.0 3H), 4.39 (q, J = 8.0, 2H), 6.83 (s, 1H), 7.34−7.38 (m, 4H), 7.61 (d, J = 8.0, 2H), 8.06 (d, J = 8.0, 2H). Anal. ($C_{20}H_{14}F_6N_2O_2$) C, H, N.

Ethyl 4-[5-(4-Methylphenyl)-3-trifluoromethyl-1*H*-pyrazol-1-yl]-benzoate (8).[13] 4-Hydrazinobenzoic acid hydrochloride (181 mg, 0.96 mmol) and **A** (200 mg, 0.87 mmol) in 15 mL EtOH were reacted as described for **7** to afford **8** (144 mg, 48%): ^{1}H NMR ($CDCl_3$) δ 1.40 (t, J = 8.0, 3H), 2.36 (s, 3H), 4.38 (q, J = 8.0, 2H), 6.73 (s, 1H), 7.10 (d, J = 8.0, 2H), 7.15 (d, J = 8.0, 2H), 7.39 (d, J = 8.0, 2H), 8.03 (d, J = 8.0, 2H). Anal. ($C_{20}H_{17}F_3N_2O_2$) C, H, N.

4-[5-(4-Trifluoromethylphenyl)-3-trifluoromethyl-1*H*-pyrazol-1-yl]-benzenesulfonamide (9). 4-Sulfamoylphenylhydrazine HCl (399 mg, 1.71 mmol) was added to a 20 mL microwave reaction vessel containing a stirrer and a solution of **B** (440 mg, 1.55 mmol) in EtOH (15 mL). The vessel was sealed and the mixture was heated in the microwave for 20 min at 180 °C and then allowed to cool to room temperature. The reaction was concentrated under vacuum and the residue was taken up in EtOAc (200 mL), washed with water (50 mL × 4) and brine (50 mL × 2), dried over $MgSO_4$, filtered, and concentrated under vacuum. Purification with flash chromatography (EtOAc/hexane 1:9) afforded **9** (383 mg, 57% yield): ^{1}H NMR ($CDCl_3$) δ 6.85 (s, 1H), 7.38 (d, J = 8.0, 2H), 7.47 (d, J = 8.0, 2H), 7.66 (d, J = 8.0, 2H), 7.96 (d, J = 8.0, 2H). Anal. ($C_{17}H_{11}F_6N_3O_2S$) C, H, N.

1,5-Bis-(4-methylphenyl)-3-trifluoromethyl-pyrazole (10). *p*-Tolylhydrazine HCl (262 mg, 1.65 mmol) was added to a 20 mL microwave reaction vessel containing a stirrer and solution of **A** (345 mg, 1.50 mmol) in EtOH (15 mL). The vessel was sealed and the mixture was heated in the microwave for 10 min at 180 °C and then allowed to cool to room temperature. The reaction was concentrated under vacuum and the residue was taken up in EtOAc (200 mL), washed with water (2 × 100 mL) and brine (2 × 100 mL), dried over $MgSO_4$, filtered, and concentrated under vacuum. Purification with flash chromatography (dichloromethane/hexane 2:8) gave **10** (272 mg, 53%): ^{1}H NMR ($CDCl_3$) δ 2.36 (d, J = 8.0, 6H), 6.70 (s, 1H), 7.12 (d, J = 8.0, 4H), 7.17 (q, J = 8.0, 4H). Anal. ($C_{18}H_{15}F_3N_2$) C, H, N.

2-[4-(5-(4-Trifluoromethylphenyl)-3-trifluoromethyl-1*H*-pyrazol-1-yl)phenyl]-acetic Acid (11). Compound **E** (346 mg, 1.71 mmol) was added to a 20 mL microwave reaction vessel containing a stirrer and a solution of **B** (440 mg, 1.55 mmol) in acetonitrile (20 mL). The vessel was sealed and the mixture was heated in the microwave for 3 min at 200 °C and then allowed to cool to room temperature. The reaction was concentrated under vacuum and the residue was taken up in EtOAc (250 mL), washed with water (100 mL × 4) and brine (100 mL × 2), dried over $MgSO_4$, filtered, and concentrated under vacuum. Purification with flash chromatography (dichloromethane/methanol 98:2) gave **11** (204 mg, 29%): ^{1}H NMR (CD_3OD) δ 3.68 (s, 2H), 7.07 (s, 1H), 7.31 (d, J = 8.0, 2H), 7.43 (d, J = 8.0, 2H), 7.51 (d, J = 8.0, 2H), 7.68 (d, J = 8.0, 2H). Anal. ($C_{19}H_{12}F_6N_2O_2$) C, H, N.

2-[4-(5-(4-Methylphenyl)-3-trifluoromethyl-1*H*-pyrazol-1-yl)-phenyl]-acetic Acid (12). Compounds **E** (346 mg, 1.71 mmol) and **A** (357 mg, 1.55 mmol) in acetonitrile (20 mL) were reacted as described for **11** to give **12** (311 mg, 54%): ^{1}H NMR (CD_3OD) δ 2.39 (s, 3H), 3.69 (s, 2H), 6.89 (s, 1H), 7.18 (s, 4H), 7,29 (d, J = 8.0, 2H), 7,38 (d, J = 8.0, 2H). Anal. ($C_{19}H_{15}F_3N_2O_2$) C, H, N.

5-(4-Methylphenyl)-1-(4-sulfamoylphenyl)-1*H*-pyrazol-3-carboxylic Acid (13c). Synthesis of **13c** was a three-step process:

Ethyl 2-Hydroxy-4-oxo-4-(4-methylphenyl)-2-butenoate (13a).[22] A solution of diethyl oxalate (1627 μL, 12.0 mmol) and 4-methylacetophenone (801 μL, 6.0 mmol) in ethanol (5 mL) was added dropwise at 50 °C to a solution of sodium ethoxide (made from 275 mg, 12.0 mmol sodium) in ethanol (10 mL). The mixture was heated at reflux for 2 h. After cooling, the reaction mixture was poured into water (40 mL), acidified with HCl (37%, 1 mL), and extracted with diethyl ether (3 × 100 mL). The combined organic layer was washed with brine (30 mL), dried over $MgSO_4$, filtered, and concentrated under vacuum. The crude product was purified with flash chromatography (EtOAc/hexane 3:7) to give **13a** (1.33 g, 95%).

Ethyl 5-(4-Methylphenyl)-1-(4-sulfamoylphenyl)-1*H*-pyrazole-3-carboxylate (13b). 4-Sulfamoylphenylhydrazine HCl (351 mg, 1.50 mmol) was added to a 20 mL microwave reaction vessel containing a stirrer and a solution of **13a** (436 mg, 1.50 mmol) in EtOH (20 mL). The vessel was sealed and the mixture was heated in the microwave for 9 min at 180 °C and then allowed to cool to room temperature. The reaction was concentrated under vacuum and the residue was taken up in EtOAc (200 mL), washed with water (2 × 100 mL) and brine (2 × 100 mL), dried over $MgSO_4$, filtered, and concentrated under vacuum to give **13b** (566 mg, 86%).

5-(4-Methylphenyl)-1-(4-sulfamoylphenyl)-1*H*-pyrazol-3-carboxylic acid (13c). Compound **13b** (538 mg, 1.40 mmol) was added to a stirred solution of THF (50 mL), MeOH (50 mL), and LiOH (2 M, 50 mL) in a 250 mL round-bottomed flask and stirred for 15 h. NaOH (1 M, 200 mL) was added, and the mixture was extracted with EtOAc (200 mL). The aqueous phase was acidified with concd HCl (38 mL), pH 1.0, and extracted with EtOAc (300 mL), dried over $MgSO_4$, filtered, and concentrated under vacuum to give **13c** (466 mg, 87%): ^{1}H NMR (CD_3OD) δ 2.38 (s, 3H), 7.05 (s, 1H), 7.21 (q, J = 8.0, 4H), 7.53 (d, J = 8.0, 2H), 7.96 (d, J = 8.0, 2H). Anal. ($C_{17}H_{15}N_3O_4S$) C, H, N.

***In Vitro* Assay for Recombinant CYP2C9. Chemicals.** The chemicals used in the assays were NADPH purchased from Sigma Chemical Co. (St. Louis, MO). Celecoxib was purchased from AApin Chemicals Limited (Abingdon, U.K.). Tris-hydroxymethyl-aminomethane was purchased from ICN Biomedicals, Inc. (Irvine, CA). K_2HPO_4 and KH_2PO_4 were purchased from Kebo Lab (Stockholm, Sweden), and acetonitrile and formic acid were purchased from Merck (Darmstadt, Germany). Human CYP2C9HR Bactosomes expressing human CYP2C9 was purchased from Cypex, Ltd. (Dundee, Scotland, U.K.).

Incubations. The total reaction volume was 100 μL, and the experiments were performed in duplicates. Each reaction mixture contained 5 pmol CYP2C9 (Dundee, Scotland, U.K.), 1 mM NADPH, 0.1 mM KPO_4 buffer, pH 7.4, and 1 μM test compound. Test compounds were dissolved in acetonitrile, giving a final assay concentration of solvent of 2.5%. Time point samples were taken at 0 and 30 min. The reactions were started by the addition of NADPH after a preincubation of 10 min at 37 °C. Ice-cold acetonitrile (200 μL) was added to stop the reaction. After centrifugation at 4000 *g* for 20 min at 4 °C, 10 μL of supernatant was injected into the liquid chromatography/tandem mass spectrometry system.

LC/MS/MS. The HPLC system used included a HP 1100 serial LC pump, a column oven (Agilent Technologies Deutschland, Waldbronn, Germany), and a CTC HTS auto sampler (CTC Analytics, Zwingen, Switzerland). Chromatography was performed on a HyPURITY C18 analytical column (50 mm × 2.1 mm i.d., 5 μm, ThermoQuest, Runcorn, U.K.) with a HyPURITY C18 guard column (10 mm × 2.1 mm, 5 μm). The mobile phase consisted of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. For the metabolism study, a programmed linear gradient started at 30% B and increased to 90% B over 2.0 min, stayed there for 1.5 min, and then returned to 30% for 1.5 min to equilibrate the column at a flow rate of 0.75 mL/min. For compound **13c**, the time gradient was the same as for the other compounds, but the gradient started with 10% of solvent B instead of 30%.

The mass spectrometrical analyses were performed using an API4000 instrument (Applied Biosystems/MDS Sciex, CA). The mass spectrometer was operated in both positive and negative ion mode. The tuning parameters were optimized for the compounds by infusing a 50% acetonitrile/H_2O solution containing 1 μM of

analyte at a flow rate of 10 μL/min into the mobile phase (0.75 mL/min) using a T connection. The scan parameters for multiple reaction monitoring (MRM) of the 14 compounds investigated can be found in the Supporting Information. Instrument control, data acquisition, and data evaluation were performed using Analyst 1.4 software (Applied Biosystems/MDS sciex, CA).

***In Vitro* Assay for Recombinant COX-1 and COX-2.** The assays were performed according to Glaser et al.[31] by CEREP (Celle l'Evescault, France).[32] The assays evaluate the effects of compounds, at 100 μM, on the activity of the human COX-1/COX-2 quantified by measuring the formation of prostaglandin E_2 (PGE_2) from arachidonic acid using a recombinant enzyme isolated from Sf-9 cells. The results are expressed as a percent inhibition of the control enzyme activity.

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Supporting Information Available: Scan parameters for multiple reaction monitoring (MRM) of the 14 compounds investigated and elemental analyses of all synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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