Synthesis of Sulfaphenazole Derivatives and Their Use as Inhibitors and Tools for Comparing the Active Sites of Human Liver Cytochromes P450 of the 2C Subfamily

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Twenty-three new derivatives of sulfaphenazole (SPA) were synthesized to further explore the topology of the active sites of human liver cytochromes P450 of the 2C subfamily and to find new selective inhibitors of these cytochromes. These compounds are derived from SPA by replacement of the NH_2 and H (of the SO_2NH function) substituents of SPA with various R_1 and R_2 groups, respectively. Their inhibitory effects were studied on recombinant CYP 2C8, 2C9, 2C18, and 2C19 expressed in yeast. High affinities for CYP 2C9 ($IC_{50} < 1$ μM) were only observed for SPA derivatives having the SO_2NH function and a relatively small R_1 substituent ($R_1 = NH_2$, CH_3). Any increase in the size of R_1 led to a moderate decrease of the affinity, and the N-alkylation of the SO_2NH function of SPA to a greater decrease of this affinity. The same structural changes led to opposite effects on molecular recognition by CYP 2C8 and 2C18, which generally exhibited similar behaviors. Thus, contrary to CYP 2C9, CYP 2C8 and 2C18 generally prefer neutral compounds with relatively large R_1 and R_2 substituents. CYP 2C19 showed an even lower affinity for anionic compounds than CYP 2C8 and 2C18. However, as CYP 2C8 and 2C18, CYP 2C19 showed a much better affinity for neutral compounds derived from N-alkylation of SPA and for anionic compounds bearing a larger R_1 substituent. One of the new compounds (R_1 = methyl, R_2 = propyl) inhibited all human CYP 2Cs with IC_{50} values between 10 and 20 μM, while another one (R_1 = allyl, R_2 = methyl) inhibited all CYP 2Cs except CYP 2C9, and a third one ($R_1 = R_2$ = methyl) inhibited all CYP 2Cs except CYP 2C8. Only 2 compounds of the 25 tested derivatives were highly selective toward one human CYP 2C; these are SPA and compound **1** ($R_1 = CH_3$, R_2 = H), which acted as selective CYP 2C9 inhibitors. However, some SPA derivatives selectively inhibited CYP 2C8 and 2C18. Since CYP 2C18 is hardly detectable in human liver, these derivatives could be interesting molecules to selectively inhibit CYP 2C8 in human liver microsomes. Thus, compound **11** ($R_1 = NH_2$, $R_2 = (CH_2)_2CH(CH_3)_2$) appears to be particularly interesting for that purpose as its IC_{50} value for CYP 2C8 is low (3 μM) and 20-fold smaller than those found for CYP 2C9 and 2C19.

Introduction

Cytochrome P450 dependent monooxygenases play a key role in the oxidative metabolism of exogenous and endogenous compounds. Most metabolic oxidations of xenobiotics such as drugs are catalyzed by P450s of the first three families (CYP 1, 2, and 3).[1,2] In fact, P450s of the 3A and 2C subfamilies are the major enzymes in human liver.[1−3] From the four members of the 2C subfamily, only CYP 2C8, 2C9, and 2C19 are expressed at a significant level in human liver and are involved in the metabolism of many drugs.[1−3] Although CYP 2C8, 2C9, and 2C19 exhibit more than 80% sequence identity, they show very different substrate specificities.[3] Good substrates of CYP 2C9 most often are anionic or polar compounds such as diclofenac,[4] tienilic acid,[5] or tolbutamide.[6] CYP 2C8 appears to be able to oxidize much larger substrates such as paclitaxel,[7,8] retinoic acid,[9] benzo[*a*]pyrene,[10] and cerivastatine.[11] CYP 2C19 is responsible for the oxidative metabolism of many drugs including (*S*)-mephenytoin[12] and omeprazole.[13,14] By contrast, very few substrates of CYP 2C18 are presently known even though this enzyme has been described to catalyze the oxidation of tienilic acid,[15] diazepam,[16] and warfarin[17] with a low efficiency. Recently, a selective, efficiently oxidized substrate of CYP 2C18 has been reported; it is an alcohol derived from tienilic acid.[18]

To interpret or to predict various problems that may occur with some drugs, such as drug−drug interactions or consequences of genetic polymorphism, it is crucial to determine which human liver P450 is involved in the metabolism of a new drug. A classical approach is the use of human liver microsomes in the presence of specific inhibitors of the different human liver P450s. For that purpose, it is necessary to possess highly selective inhibitors for each human liver P450.

Sulfaphenazole (SPA) is a reasonably selective inhibitor of CYP 2C9.[2,3,19] Its potent ($K_i \approx 0.3$ μM) and selective inhibitory effects toward CYP 2C9[19] appear to be due to two strong interactions, a π-stacking interaction of its phenyl substituent with an aromatic residue of the protein, and an ionic (or hydrogen-bonding)

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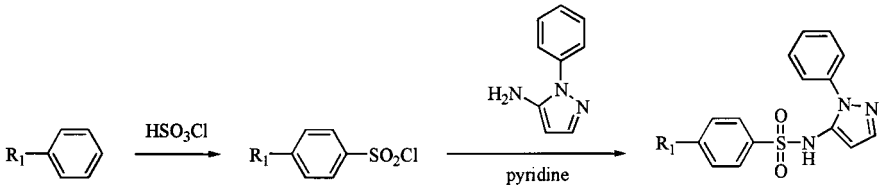
Scheme 1



R_1	compound
NH_2	SPA
CH_3	**1**
Br	**2**
(mNO_2)phenyl	**3**

R_1	compound
CH_2-CH=CH_2	**4**
(mNH_2)phenyl	**5**

interaction between its SO_2N^- anionic site and a cationic (or hydrogen-bonding) residue of CYP 2C9 (ref 19; N. T. Ha-Duong, C. Marques-Soares, S. Dijols, M. A. Sari, P. M. Dansette, D. Mansuy, manuscript in preparation). To further explore the origins of the selectivity of SPA as a CYP 2C9 inhibitor within the human 2C subfamily, and to find new selective inhibitors for the other members of the 2C subfamily, we have synthesized a series of new derivatives of SPA and compared their inhibitory effects toward recombinant CYP 2C8, 2C9, 2C18, and 2C19.

Chemistry

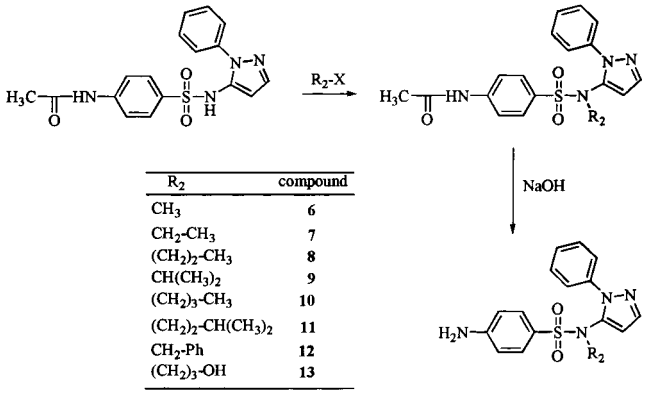
To further explore the topology of the active site not only of CYP 2C9 but also of CYP 2C8, 2C18, and 2C19, and to find new selective inhibitors of these human P450 2Cs, many SPA derivatives with various R_1 and R_2 substituents that replace the NH_2 and H (of SO_2NH) groups of SPA were synthesized. Three series of SPA derivatives have been synthesized.

The compounds of the first series, **1**−**5**, derive from SPA by replacement of its NH_2 substituent with R_1 groups of various sizes and polarities (R_1 = CH_3, CH_2-CH=CH_2, Br, *m*-NO_2Ph, and *m*-NH_2Ph). Compounds **1**−**3** were obtained from reaction of (2-phenyl-2*H*-pyrazol-3-yl)amine with benzenesulfonyl chlorides bearing the appropriate *p*-R_1 substituent (Scheme 1). Compounds **4** and **5** were derived from **2** and **3**, respectively, by classical procedures (Scheme 1).

Compounds of the second series, **6**−**13**, simply derive from SPA by N-alkylation of its sulfonamide function. They were prepared by direct N-alkylation of acetylamino-SPA using the appropriate alkyl halide and Na_2CO_3 in DMF, followed by deprotection of the acetylamino function (Scheme 2).

Finally, the compounds of the third series, **14**−**24**, bear both substituents R_1 and R_2 different from those found in SPA. Some of them, **18** and **20**−**24**, were directly obtained by N-alkylation of the SO_2NH function of compounds **3**, **4**, and **1** with the appropriate alkyl halide (Scheme 3). Such a direct N-alkylation of para-substituted (2-phenyl-2*H*-pyrazol-3-yl)arenesulfonamides (with R_1 = Br, *m*-NO_2Ph, $(CH_2)_2NHCOCH_3$, and $(CH_2)_3NHCOCH_3$) with 3-bromopropanol gave secondary products, not easily separated by column chromatography. Thus compounds **14, 15**, and **19** were prepared in two steps, the N-alkylation of the sulfonamide

Scheme 2



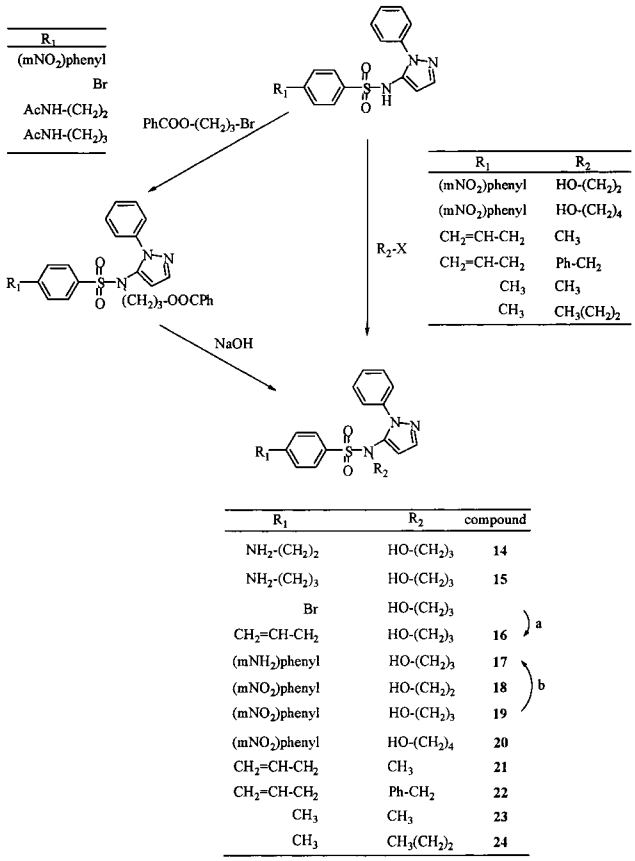
R_2	compound
CH_3	**6**
CH_2-CH_3	**7**
$(CH_2)_2$-CH_3	**8**
$CH(CH_3)_2$	**9**
$(CH_2)_3$-CH_3	**10**
$(CH_2)_2$-$CH(CH_3)_2$	**11**
CH_2-Ph	**12**
$(CH_2)_3$-OH	**13**

function of the precursor with benzoic acid 3-bromopropyl ester, followed by deprotection of the alcohol (and amino, in the case of **14** and **15**) function with NaOH. Compound **16** was prepared by treatment of its bromo precursor with allyltributyltin in DMF in the presence of $Pd(PPh_3)_4$ under argon ((a) in Scheme 3). Reduction of **19** into **17** was carried out using $Na_2S_2O_4$ as reducing agent[20] ((b) in Scheme 3), since the classical method of reduction of nitroaromatic molecules using H_2 in the presence of Ni or Pd led to longer reaction times and lower yields.

Biochemistry

The inhibitory effects of compounds **1**−**24** toward human liver CYP 2Cs were studied on microsomes of yeast strain W(R)fur1[21] expressing each human P450 of the 2C subfamily. The assays used for studying P450 2C activities were based on the 5-hydroxylation of 2-aroylthiophenes, the 5-hydroxy-2-aroylthiophene metabolites being easily detected by UV−vis spectroscopy at 390 nm.[22] Tienilic acid and 2-[2,3-dichloro-4-(2-thenoyl)phenoxy]ethanol were used as substrates for CYP 2C9[5] and CYP 2C8,[18] respectively. 3-[2,3-Dichloro-4-(2-thenoyl)phenoxy]propan-1-ol was used as a substrate for measuring the activities of CYP 2C18 and 2C19.[18] In this paper, our objective was not to study the detailed mechanism of inhibition of CYP 2Cs by the various SPA derivatives, but more to compare their efficiency as inhibitors of CYP 2C8, 2C9, 2C18, and 2C19. Thus, determination of the IC_{50} values was done by measuring the effects of the inhibitors on the activity of each CYP 2C using a substrate concentration equal

Scheme 3[a]



R_1	R_2	compound	
NH_2-$(CH_2)_2$	HO-$(CH_2)_3$	**14**	
NH_2-$(CH_2)_3$	HO-$(CH_2)_3$	**15**	
Br	HO-$(CH_2)_3$		a
CH_2=CH-CH_2	HO-$(CH_2)_3$	**16**	
(mNH_2)phenyl	HO-$(CH_2)_3$	**17**	b
(mNO_2)phenyl	HO-$(CH_2)_2$	**18**	
(mNO_2)phenyl	HO-$(CH_2)_3$	**19**	
(mNO_2)phenyl	HO-$(CH_2)_4$	**20**	
CH_2=CH-CH_2	CH_3	**21**	
CH_2=CH-CH_2	Ph-CH_2	**22**	
CH_3	CH_3	**23**	
CH_3	$CH_3(CH_2)_2$	**24**	

[a] Reagents: (a) Allyltributyltin, $Pd[PPh_3]_4$, in DMF, under argon. (b) $Na_2S_2O_4$, 60 °C, 2 h, under argon.

Table 1. Comparison of the Inhibitory Effects of SPA Derivatives Bearing Various R_1 Substituents (See Scheme 1) toward Recombinant CYP 2C8, 2C9, 2C18, and 2C19 Expressed in Yeast

		IC_{50}[a] (μM)			
compd	R_1	CYP 2C8	CYP 2C9	CYP 2C18	CYP 2C19
SPA	NH_2	130	0.6	60	>500[b]
1	CH_3	220	0.6	170	100
3	*m*-NO_2Ph	7	4	5	65
4	$CH_2CH{=}CH_2$	10	1	150	450
5	*m*-NH_2Ph	8	12	20	95

[a] IC_{50} values were measured from increasing concentrations of potential inhibitors added to the assay incubation mixture including yeast microsomes expressing CYP 2C in 0.1 M Tris buffer, pH 7.4, substrate, and an NADPH-generating system. The experimental details are described in the Experimental Section. [b] CYP 2C activity higher than 50% of control activity at the indicated inhibitor concentration. Values are the mean from three experiments.

to K_M[5,18] (see the Experimental Section). However, it is noteworthy that the few compounds, such as SPA,[19] **1**, or **4** (data not shown), for which the type of inhibition of CYP 2C9 was determined on the basis of Lineweaver−Burk or Dixon plots were found to be competitive inhibitors.

Table 1 illustrates the effects of replacing the NH_2 substituent of SPA with R_1 groups of different sizes and polarities on the inhibition of CYP 2C activities. SPA itself and its derivative bearing a relatively small substituent ($R_1 = CH_3$) are selective inhibitors of CYP 2C9 with IC_{50} values 2 orders of magnitude lower than those observed for CYP 2C8, 2C18, and 2C19. Increasing the space occupied by R_1 leads to a decrease of selectivity toward CYP 2C9, as already with R_1 = allyl, the IC_{50} value for CYP 2C8 reaches 10 μM. Further increases of the R_1 size, as in compounds **3** and **5**, lead to strong inhibitory effects not only toward CYP 2C8 but also toward CYP 2C18, and even to moderate effects toward CYP 2C19. In fact compound **3** acts as a reasonable inhibitor for all human CYP 2Cs, with IC_{50} values of 7, 4, 5, and 65 μM for CYP 2C8, 2C9, 2C18, and 2C19, respectively.

Table 2. Comparison of the Inhibitory Effects of Various N-Alkylated SPA Derivatives (See Scheme 2) toward Recombinant CYP 2C8, 2C9, 2C18, and 2C19 Expressed in Yeast

		IC_{50}[a] (μM)			
compd	R_2	CYP 2C8	CYP 2C9	CYP 2C18	CYP 2C19
SPA	H	130	0.6	60	>500[b]
6	CH_3	50	100	15	200
7	CH_2CH_3	13	90	7	225
8	$(CH_2)_2CH_3$	8	55	8	250
9	$CH(CH_3)_2$	30	80	3	130
10	$(CH_2)_3CH_3$	4	50	6	60
11	$(CH_2)_2CH$-$(CH_3)_2$	3	60	9	60
12	CH_2Ph	15	55	7	45

[a] IC_{50} values were measured from increasing concentrations of potential inhibitors added to the assay incubation mixture including yeast microsomes expressing CYP 2C in 0.1 M Tris buffer, pH 7.4, substrate, and an NADPH-generating system. The experimental details are described in the Experimental Section. [b] CYP 2C activity higher than 50% of control activity at the indicated inhibitor concentration. Values are the mean from three experiments.

N-alkylation of the SO_2NH function of SPA has a dramatic effect on the inhibition of CYP 2C9 (Table 2), as it leads to IC_{50} values 2 orders of magnitude larger than that of SPA (50−100 μM instead of 0.6 μM). These results are in agreement with previous reports indicating that an ionic (or hydrogen-bonding) interaction of the SO_2N^- (or SO_2NH) function of SPA with an amino acid residue of the CYP 2C9 active site is very important in SPA recognition by CYP 2C9.[19,23] In parallel with this spectacular decrease of the inhibitory effects toward CYP 2C9, N-alkylation of SPA leads to a marked increase of the inhibitory effects toward CYP 2C8 and 2C18. Thus, compounds **8**, **10**, and **11** ($R_2 = (CH_2)_2CH_3$, $(CH_2)_3CH_3$, and $(CH_2)_2CH(CH_3)_2$ respectively) exhibit IC_{50} values of 8, 4, and 3 μM toward CYP 2C8 and 8, 6, and 9 μM toward CYP 2C18. In fact, CYP 2C8 and CYP 2C18 exhibit a similar behavior toward the N-alkylated SPA derivatives **6**−**12**, with a large 10-fold decrease of the IC_{50} values on passing from SPA to **7** and a further smaller decrease of IC_{50} on increasing the length of R_2. Compounds **6**−**12** are less efficient inhibitors of CYP 2C9 and 2C19, as they show IC_{50} values between 45 and 250 μM for those two enzymes. However, their inhibitory potency also increases in parallel with the size of R_2. As a consequence, compounds **10**−**12** inhibit the four human CYP 2Cs with IC_{50} values smaller than 60 μM.

Previous studies on tienilic acid derivatives have shown that replacement of the anionic moiety of tienilic acid, $-OCH_2COOH$, with a neutral substituent containing an alcohol function, $-O(CH_2)_3OH$, led to a very good selective substrate of CYP 2C18.[18] On the basis of these results, a series of SPA derivatives bearing an $R_2 = -CH_2(CH_2)_nOH$ chain, with n = 1, 2, or 3, have been

Table 3. Comparison of the Inhibitory Effects of Various Polar SPA Derivatives (See Schemes 2 and 3) toward Recombinant CYP 2C8, 2C9, 2C18, and 2C19 Expressed in Yeast

			IC_{50}[a] (μM)			
compd	R_1	R_2	CYP 2C8	CYP 2C9	CYP 2C18	CYP 2C19
13	NH_2	$(CH_2)_3OH$	22	>100[b]	30	150
14	$(CH_2)_2NH_2$	$(CH_2)_3OH$	>250[b]	10	65	10
15	$(CH_2)_3NH_2$	$(CH_2)_3OH$	>200[b]	50	30	15
16	$CH_2CH{=}CH_2$	$(CH_2)_3OH$	35	80	18	10
17	*m*-NH_2Ph	$(CH_2)_3OH$	13	12	3	40
18	*m*-NO_2Ph	$(CH_2)_2OH$	>250[b]	20	9	165
19	*m*-NO_2Ph	$(CH_2)_3OH$	>250[b]	28	8	200
20	*m*-NO_2Ph	$(CH_2)_4OH$	>250[b]	15	4	>200[b]

[a] IC_{50} values were measured from increasing concentrations of potential inhibitors added to the assay incubation mixture including yeast microsomes expressing CYP 2C in 0.1 M Tris buffer, pH 7.4, substrate, and an NADPH-generating system. The experimental details are described in the Experimental Section. [b] CYP 2C activity higher than 50% of control activity at the indicated inhibitor concentration. Values are the mean from three experiments.

Table 4. Comparison of the Inhibitory Effects of Hydrophobic SPA Derivatives (See Scheme 3) toward Recombinant CYP 2C8, 2C9, 2C18, and 2C19 Expressed in Yeast

			IC_{50}[a] (μM)			
compd	R1	R2	CYP 2C8	CYP 2C9	CYP 2C18	CYP 2C19
21	$CH_2CH{=}CH_2$	CH_3	20	>200[b]	10	20
22	$CH_2CH{=}CH_2$	CH_2Ph	30	>200[b]	125	20
23	CH_3	CH_3	180	10	12	20
24	CH_3	$(CH_2)_2CH_3$	15	20	20	10

[a] IC_{50} values were measured from increasing concentrations of potential inhibitors added to the assay incubation mixture including yeast microsomes expressing CYP 2C in 0.1 M Tris buffer, pH 7.4, substrate, and an NADPH-generating system. The experimental details are described in the Experimental Section. [b] CYP 2C activity higher than 50% of control activity, even with 200 μM potential inhibitor. Values are the mean from three experiments.

synthesized as potential good inhibitors of CYP 2C18. Table 3 shows the results obtained with such compounds, **13**−**20**. Some of these compounds such as **17**−**20** are good inhibitors of CYP 2C18, with IC_{50} values between 3 and 9 μM; however, they are not selective, as all of them except **13** also inhibit CYP 2C9, and some of them, **16** and **17**, inhibit all human CYP 2Cs with IC_{50} values between 3 and 80 μM. It is noteworthy that compounds **14**−**16** are relatively good inhibitors of CYP 2C19, with IC_{50} values between 10 and 15 μM, which have never been reached with compounds **1**−**13**. Compound **13** derives from SPA by N-alkylation of its SO_2NH function and may be compared to compounds **6**−**12**. As expected, **13**, **8,** and **10**, which bear R_2 groups of similar size (R_2 = $CH_2CH_2CH_2OH$, $CH_2CH_2CH_3$, and $CH_2CH_2CH_2CH_3$, respectively), exhibit similar inhibitory properties with a marked preference for CYP 2C8 and 2C18. Addition of **13** to yeast microsomes expressing CYP 2C8 leads to the appearance of a difference visible spectrum characterized by a peak at 430 nm and a trough at 405 nm. This difference spectrum is typical of cytochrome P450 Fe(III) bound to a nitrogenous ligand, and indicates that **13** binds to CYP 2C8 iron via its NH_2 function, with a K_s value for dissociation of the CYP 2C8−**13** complex of 2 ± 1 μM (data not shown). Conversely, addition of 100 μM **13** to yeast microsomes expressing CYP 2C9 does not lead to any significant difference spectrum. These spectral results are in agreement with the inhibitory properties of **13** toward CYP 2C8 (relatively low IC_{50} value of 22 μM, which would correspond to a K_i value of 11 μM in the case of competitive inhibition) and CYP 2C9 (IC_{50} > 100 μM) (Table 3).

Finally, four compounds bearing two hydrophobic alkyl substituents R_1 and R_2 were synthesized and compared as inhibitors of human CYP 2Cs (Table 4). Interestingly, these four hydrophobic compounds are strong inhibitors of CYP 2C19, with IC_{50} values between 10 and 20 μM. Compound **24** acts as a good inhibitor for all human CYP 2Cs with IC_{50} values around 15 μM, whereas compound **21** inhibits all human CYP 2Cs with IC_{50} values at the same level except CYP 2C9, and compound **23** inhibits all human CYP 2Cs except CYP 2C8.

Discussion

The aforementioned results provide some data about the structural factors that are important for molecules to be recognized by each human CYP 2C.

High affinities ($IC_{50} \approx 1$ μM) for CYP 2C9 were only observed for SPA derivatives having the intact SO_2NH function and a relatively small R_1 substituent (R_1 = NH_2, CH_3, or $CH_2CH{=}CH_2$). Any increase in the size of R_1 leads to a moderate decrease of the affinity (Table 1) and the N-alkylation of the SO_2NH function of SPA (Table 2) or of **1** (see **23** and **24** in Table 4) to a greater decrease of the affinity in general (IC_{50} between 10 and >200 μM).

The same structural changes have opposite effects on molecular recognition by CYP 2C18, since an increase in the size of R_1 (compounds **3** and **5** of Table 1) or the N-alkylation of SPA (Table 2) generally results in a marked decrease of IC_{50} values from 60 to 5 or 3 μM (if one excepts compound **4**). Thus, contrary to CYP 2C9, CYP 2C18 generally prefers neutral compounds with relatively large R_1 and R_2 substituents. This conclusion is in agreement with previous data on the interactions of another series of compounds related to tienilic acid with CYP 2C9 and 2C18.[18]

In a general manner, CYP 2C8 exhibits a behavior toward these structural changes of SPA similar to that of CYP 2C18. The only exceptions to this rule are concerned with primary amines **14** and **15**, and com-

pounds **18**−**20**. CYP 2C19 shows an even lower affinity for anionic compounds than CYP 2C8 and 2C18 (see SPA, **1**, and **4**). However, as CYP 2C8 and 2C18, it exhibits a better affinity for neutral compounds derived from N-alkylation of the SO_2NH function of SPA (Table 2) or for anionic compounds bearing a larger R_1 substituent (Table 1). Several SPA derivatives with R_1 different from NH_2 and R_2 different from H lead to IC_{50} values as low as 10 μM.

Coming back to the search of new inhibitors for human CYP 2Cs, we have found two molecules that inhibit all human CYP 2Cs with IC_{50} values smaller than 40 μM. These are compounds **17** (IC_{50} = 13, 12, 3, and 40 μM for CYP 2C8, 2C9, 2C18, and 2C19, respectively) and **24** (IC_{50} = 15, 20, 20, and 10 for CYP 2C8, 2C9, 2C18, and 2C19, respectively). Such molecules might be useful to evaluate the contribution of the whole human subfamily 2C in the oxidative metabolism of a xenobiotic in various microsomes or tissues, provided that they do not inhibit the CYPs of the other subfamilies. In that context, compound **21** might also be interesting as it inhibits all human CYP 2Cs at a reasonable level (IC_{50} < 20 μM) except CYP 2C9 (Table 4). In the same idea, compound **23** inhibits all human CYP 2Cs with IC_{50} < 20 μM, except CYP 2C8, for which an IC_{50} of 180 μM was found. Further experiments are required to know whether compounds **17**, **24**, **21**, and **23** do not act as inhibitors of human CYPs of subfamilies different from 2C.

Finally, when one looks for a selective inhibitor for one of the human CYP 2Cs among the 24 compounds that have been synthesized, one only finds one molecule leading to an IC_{50} value for a given CYP 2C 2 orders of magnitude smaller than the IC_{50} values for the three other CYP 2Cs. This is compound **1**, which acts as a specific inhibitor of CYP 2C9, with IC_{50} values for CYP 2C8, 2C18, and 2C19 more than 200 times higher than that for CYP 2C9. Compound **1** is even slightly more selective toward CYP 2C9 than SPA itself. It is noteworthy that some of the described molecules, such as **7**−**12**, are much better inhibitors of CYP 2C8 and 2C18 than of CYP 2C9 and 2C19 (Table 2). If one takes into account that CYP 2C18 is hardly detectable in human liver microsomes,[24,25] these molecules could be interesting to selectively inhibit CYP 2C8 in these microsomes. Compound **11** seems to be the most interesting molecule for that purpose, as its IC_{50} value for CYP 2C8 is low (3 μM) and 20-fold lower than the IC_{50} values found for CYP 2C9 and 2C19.

Experimental Section

Chemicals. All chemicals used were of the highest quality commercially available. Tienilic acid was provided by Anphar-Rolland (Chilly-Mazarin, France). 3-[2,3-Dichloro-4-(2-thenoyl) phenoxy]propan-1-ol and 2-[2,3-dichloro-4-(2-thenoyl)phenoxy]ethanol were prepared by previously described procedures.[18,26] SPA was purchased from Sigma.

Physical Measurements. UV−vis spectra were recorded on Kontron Uvikon 860 and 820 spectrophotometers equipped with a diffusion sphere. UV characteristics of compounds **2**−**24** reported in the following are λ_{max} (indicated as λ followed by its value (nm) in the text) and ϵ ($mM^{-1}\cdot cm^{-1}$) (values in parentheses). ^{1}H NMR spectra were recorded at 27 °C on a Bruker ARX-250 instrument; chemical shifts are reported downfield from $(CH_3)_4Si$, and coupling constants are in hertz. The abbreviations s, d, t, q, m, br s, and dd are used for singlet, doublet, triplet, quadruplet, multiplet, broad singlet, and doublet of doublets, respectively. Mass spectra (MS) were obtained with chemical ionization (CI) using NH_3 or CH_4 (HRMS) on a Nermag R1010 apparatus. Elemental analyses were carried out at the Centre Regional de Microanalyse, Paris.

Synthesis of Sulfaphenazole Derivatives. 4-Methyl-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide, 1. 1 was prepared as described previously:[27] mp 142 °C (lit.[27] mp 142−143 °C).

4-Bromo-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide, 2. 2 was prepared by reaction of 2-phenyl-2*H*-pyrazol-3-ylamine (1.26 mmol) with 4-bromobenzenesulfonyl chloride (1.37 mmol) in the presence of pyridine. Recrystallization from CH_2Cl_2 gave compound **2** (61% yield) as pale yellow crystals: mp 185−186 °C; UV (MeOH) λ 235 (22); ^{1}H NMR ($CDCl_3$) δ 7.55 (d, 1H, J = 2), 7.53−7.43 (m, 4H), 7.37 (m, 3H), 7.11 (m, 2H), 6.55 (br s, 1H, NH), 6.27 (d, 1H, J = 2); MS (CI, NH_3) m/z 378 ([M + H]$^+$, 97), 163 (18). Anal. ($C_{15}H_{12}BrN_3O_2S$) C, H, N.

3′-Nitrobiphenyl-4-sulfonic Acid (2-Phenyl-2*H*-pyrazol-3-yl)amide, 3. 3-Nitrobiphenyl (920 mg, 4.62 mmol) was progressively added to chlorosulfonic acid (2 mL). The reaction mixture was stirred for 1 h at room temperature, followed by thin-layer chromatography (TLC) (SiO_2, cyclohexane/CH_2Cl_2, 50/50). The solution was dripped into ice, extracted with CH_2Cl_2, and dried over $MgSO_4$, and the solvent was evaporated. The product was purified by column chromatography (SiO_2, cyclohexane/CH_2Cl_2, 50/50) and obtained in a 65% yield: ^{1}H NMR ($CDCl_3$) δ 7.70 (t, 1H, J = 8), 7.85 (d, 2H, J = 8.6), 7.93 (d, 1H, J = 8), 8.16 (d, 2H, J = 8.6), 8.31 (d, 1H, J = 8), 8.48 (s, 1H); IR (cm^{-1}) 1348, 1164. The previous compound (870 mg, 2.92 mmol) was added to 2-phenyl-2*H*-pyrazol-3-ylamine (465 mg, 2.92 mmol) in anhydrous pyridine (4.5 mL). After the mixture was heated under reflux at 95 °C for 1 h, pyridine was evaporated; the residue was dissolved in 1 N HCl, extracted with CH_2Cl_2, and dried over $MgSO_4$. Purification by column chromatography (SiO_2, CH_2Cl_2/EtOAc, 90/10), and two recrystallizations from CH_2Cl_2 and Et_2O, led to a crystalline product in a 47% yield: mp 164−165 °C ; UV (MeOH) λ 253 (37); ^{1}H NMR ($CDCl_3$) δ 6.30 (d, 1H, J = 1.9), 6.70 (br s, 1H, NH), 7.16 (m, 2H), 7.35 (m, 3H), 7.56 (d, 1H, J = 1.9), 7.63 (d, 2H, J = 8.6), 7.67 (t, 1H, J = 8), 7.77 (d, 2H, J = 8.6), 7.90 (d, 1H, J = 8), 8.28 (d, 1H, J = 8), 8.43 (s, 1H); MS (CI, NH_3) m/z 421 ([M + H]$^+$, 100), 391 (39), 163 (26). Anal. ($C_{21}H_{16}N_4O_4S$) C, H, N.

4-Allyl-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide, 4. Reaction of **2** (210 mg) with allyltributyltin (0.19 mL) in DMF was performed in the presence of tetrakis(triphenylphosphine)palladium(0) under argon at 110 °C for 7.5 h. Recrystallization from ether of the crude product obtained after purification by column chromatography (SiO_2, CH_2Cl_2/Et_2O, 5%) led to 102 mg of compound **4** as pale yellow crystals: mp 115−116 °C; UV (MeOH) λ 229 (18); ^{1}H NMR ($CDCl_3$) δ 7.58 (d, 2H, J = 8.3), 7.53 (d, 1H, J = 1.9), 7.35 (m, 3H), 7.23 (d, 2H, =8.3), 7.07 (m, 2H), 6.47 (br s, 1H, NH), 6.25 (d, 1H, J = 1.9), 5.91 (m, 1H, H_a, J = 6.7, J_{ab} = 10.1, $J_{ab'}$ = 16.9), 5.14 (m, 1H, H_b, J_{ba} = 10.1, $J_{bb'}$ = 1.6), 5.08 (m, 1H, H_b, J_{ba} = 16.9, $J_{bb'}$ = 1.6), 3.43 (d, 2H, J = 6.7); MS (CI, NH_3) m/z 340 ([M + H]$^+$, 100), 357 (16), 163 (50). Anal. ($C_{18}H_{17}N_3O_2S$) C, H, N.

3′-Aminobiphenyl-4-sulfonic Acid (2-Phenyl-2*H*-pyrazol-3-yl)amide, 5. To a solution of compound **3** (91 mg, 0.22 mmol) dissolved in hot EtOH (10 mL) was added Pd black (7.5 mg). This solution was hydrogenated under H_2 (1 atm) for 20 h. After filtration over Celite, the solvent was evaporated. The product was purified by column chromatography (SiO_2, CH_2Cl_2/EtOAc, 70/30). The solvent was evaporated, and the obtained oil was dissolved in Et_2O and then evaporated. Compound **5** was obtained as a foam in a 65% yield: UV (MeOH) λ 245 (29), 310 (4); ^{1}H NMR ($CDCl_3$) δ 7.67 (d, 2H, J = 8.5), 7.56 (d, 2H, J = 8.5), 7.55 (d, 1H, J = 1.9), 7.33 (m, 3H), 7.25 (t, 1H, J = 7.8), 7.10 (m, 2H), 6.94 (d, 1H, J = 7.8), 6.85 (t,

1H, $J = 1.7$), 6.73 (dd, 1H, $J = 7.8$, $J = 1.7$), 6.5 (br s, 1H, NH), 6.30 (d, 1H, $J = 1.9$), 3.79 (br s, 2H, NH_2); MS (CI, NH_3) m/z 391 ($[M + H]^+$, 100), 163 (24). Anal. ($C_{21}H_{18}N_4O_2S$) C, H, N.

General Procedure for the Preparation of the N-Alkylated Compounds 6−13. 4-(Acetylamino)-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide was prepared as previously described.[19] The appropriate alkyl halide (1.5−7.5 equiv) was added to a solution of 4-(acetylamino)-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide (0.42 mmol) in 1.5 mL of anhydrous DMF containing 100 mg of Na_2CO_3. After 1 h to 3 days at 20−80 °C, DMF was evaporated and the residue was dissolved in CH_2Cl_2. The organic phase was washed with water and dried over $MgSO_4$. After purification by column chromatography (SiO_2), the intermediate acetylated compound was heated under reflux in 3 mL of 3 N NaOH and 3−7 mL of EtOH for 2−2.5 h. EtOH was evaporated, and the solution was diluted with water and extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$, and the final product was purified by crystallization.

4-Amino-*N*-methyl-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide, 6. 6 was prepared in an 84% yield using the general procedure described above. Reaction with methyl iodide (1.6 equiv) was done at 80 °C for 1 h. Product **6** was purified by crystallization from CH_2Cl_2/Et_2O: mp 210−211 °C; UV (MeOH) λ 271 (22); ^{1}H NMR ($CDCl_3$) δ 7.61−7.56 (m, 3H), 7.47−7.30 (m, 5H), 6.63 (d, 2H, $J = 8.7$), 5.94 (d, 1H, $J = 1.9$), 4.17 (br s, 2H, NH_2), 3.01 (s, 3H, CH_3); MS (CI, NH_3) m/z 329 ($[M + H]^+$, 100), 174 (16). Anal. ($C_{16}H_{16}N_4O_2S$) C, H, N.

4-Amino-*N*-ethyl-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide, 7. 7 was prepared in a 67% yield using the general procedure described above. Reaction with ethyl bromide (7.5 equiv) was performed at room temperature for 39 h. Product **7** was recrystallized from CH_2Cl_2/cyclohexane: mp 209−210 °C; UV (MeOH) λ 271 (29); ^{1}H NMR ($CDCl_3$) δ 7.66 (d, 2H, $J = 8.7$), 7.60 (d, 1H, $J = 2$), 7.50−7.32 (m, 5H), 6.64 (d, 2H, $J = 8.7$), 5.93 (d, 1H, $J = 2$), 4.15 (br s, 2H, NH_2), 3.35 (q, 2H, $J = 7.2$), 0.88 (t, 3H, $J = 7.2$); MS (CI, NH_3) m/z 343 ($[M + H]^+$, 100), 188 (26). Anal. ($C_{17}H_{18}N_4O_2S$) C, H, N.

4-Amino-*N*-propyl-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide, 8. The title compound was prepared in an 81% yield using the general procedure described above. Reaction with propyl iodide (2.2 equiv) was performed at 60 °C for 24 h. Product **8** (83 mg) was obtained after recrystallization from CH_2Cl_2/Et_2O: mp 169−170 °C; UV (MeOH) λ 271 (27); ^{1}H NMR ($CDCl_3$) δ 7.67 (d, 2H, $J = 7.1$), 7.59 (d, 1H, $J = 2$), 7.50 (d, 2H, $J = 8.7$), 7.47−7.35 (m, 3H), 6.65 (d, 2H, $J = 8.7$), 5.91 (d, 1H, $J = 2$), 4.16 (br s, 2H, NH_2), 3.21 (t, 2H, $J = 7.4$), 1.21 (m, 2H), 0.63 (t, 3H, $J = 7.4$); MS (CI, NH_3) m/z 357 ($[M + H]^+$, 100), 202 (18). Anal. ($C_{18}H_{20}N_4O_2S \cdot {}^1/_8H_2O$) C, H, N.

4-Amino-*N*-isopropyl-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide, 9. 2-Iodopropane (0.1 mL, 1 mmol) was added to a solution of 4-(acetylamino)-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide (150 mg, 0.42 mmol) in 1.5 mL of anhydrous DMF containing 100 mg of Na_2CO_3. After 24 h at 50 °C, the solvent was evaporated and the residue dissolved in CH_2Cl_2. The organic phase was washed with water and dried over $MgSO_4$. After purification by column chromatography (SiO_2, CH_2Cl_2/EtOAc, 70/30), 141 mg of *N*-acetyl-**9** was obtained in an 84% yield: ^{1}H NMR ($CDCl_3$) δ 8.76 (br s, 1H, NH), 7.73 (d, 2H, $J = 7$), 7.68 (s, 4H), 7.64 (d, 1H, $J = 1.9$), 7.43−7.30 (m, 3H), 5.96 (d, 1H, $J = 1.9$), 4.18 (m, 1H, CH), 2.10 (s, 3H, CH_3), 0.68 (br s, 3H, CH_3), 0.58 (br s, 3H, CH_3). The previous compound (141 mg, 0.35 mmol) was heated under reflux in 2.4 mL of 3 N NaOH and 7 mL of EtOH for 2 h. EtOH was evaporated, and the solution was diluted with water and extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$. Compound **9** was recrystallized from CH_2Cl_2/cyclohexane, and obtained (99 mg) in a 79% yield: mp 223−224 °C; UV (MeOH) λ 271 (27); ^{1}H NMR ($CDCl_3$) δ 7.77 (d, 2H, $J = 7.5$), 7.64 (d, 1H, $J = 2$), 7.59 (d, 2H, $J = 8.7$), 7.47−7.32 (m, 3H), 6.67 (d, 2H, $J = 8.7$), 5.99 (d, 1H, $J = 2$), 4.20 (m, 1H), 4.16 (br s, 2H, NH_2), 0.75 (br s, 3H), 0.58 (br s, 3H); MS (CI, NH_3) m/z 357 ($[M + H]^+$, 100), 202 (82). Anal. ($C_{18}H_{20}N_4O_2S$) C, H, N.

4-Amino-*N*-butyl-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide, 10. 1-Bromobutane (0.1 mL, 0.93 mmol) was added to a solution of 4-(acetylamino)-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide (150 mg, 0.42 mmol) in 1.5 mL of anhydrous DMF containing 100 mg of Na_2CO_3. After 38 h at 50 °C, the solvent was evaporated and the residue dissolved in CH_2Cl_2. The organic phase was washed with water and dried over $MgSO_4$. After purification by column chromatography (SiO_2, CH_2Cl_2/EtOAc, 70/30), 160 mg of *N*-acetyl-**10** was obtained in a 92% yield: ^{1}H NMR ($CDCl_3$) δ 7.66 (m, 4H), 7.62−7.59 (m, 3H), 7.53 (br s, 1H), 7.47−7.33 (m, 3H), 5.87 (d, 1H, $J = 2$), 3.26 (t, 2H, $J = 7.2$), 2.20 (s, 3H), 1.22 (m, 2H), 1.05 (m, 2H), 0.67 (t, 3H, $J = 7.2$). The previous compound (160 mg, 0.38 mmol) was heated under reflux in 3 mL of 3 N NaOH and 6 mL of EtOH for 2 h. EtOH was evaporated, and the solution was diluted in water and extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$. After recrystallization from Et_2O/cyclohexane, **10** was obtained (125 mg) in an 89% yield: mp 111−112 °C; UV (MeOH) λ 271 (31); ^{1}H NMR ($CDCl_3$) δ 7.67 (d, 2H, $J = 7.2$), 7.59 (d, 1H, $J = 2$), 7.49 (d, 2H, $J = 8.7$), 6.65 (d, 2H, $J = 8.7$), 5.91 (d, 1H, $J = 2$), 4.16 (br s, 2H, NH_2), 3.24 (t, 2H, $J = 7.2$), 1.18 (m, 2H), 1.05 (m, 2H), 0.68 (t, 3H, $J = 7.2$); MS (CI, NH_3) m/z 371 ($[M + H]^+$, 100), 216 (92). Anal. ($C_{19}H_{22}N_4O_2S$) C, H, N.

4-Amino-*N*-(3-methylbut-1-yl)-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide, 11. 1-Bromo-3-methylbutane (0.11 mL, 0.92 mmol) was added to a solution of 4-(acetylamino)-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide (150 mg, 0.42 mmol) in 1.5 mL of anhydrous DMF containing 100 mg of Na_2CO_3. After 30 h at 60 °C and 60 h at room temperature, the solvent was evaporated and the residue dissolved in CH_2Cl_2. The organic phase was washed with water and dried over $MgSO_4$. After purification by column chromatography (SiO_2, CH_2Cl_2/EtOAc, 70/30), 160 mg of *N*-acetyl-**11** was obtained in an 84% yield: ^{1}H NMR ($CDCl_3$) δ 9.01 (br s, 1H), 7.67−7.55 (m, 7H), 7.40−7.27 (m, 3H), 5.85 (d, 1H, $J = 2$), 3.24 (t, 2H, $J = 6.6$), 2.06 (s, 3H), 1.20 (m, 1H), 1.04 (q, 2H, $J = 6.6$), 0.63 (d, 6H, $J = 6.6$). The previous compound (150 mg, 0.35 mmol) was heated under reflux in 2.4 mL of 3 N NaOH and 3 mL of EtOH for 2 h. EtOH was evaporated, and the solution was diluted in water and extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$. **11** was recrystallized from Et_2O/cyclohexane, and obtained (118 mg) in an 88% yield: mp 143−144 °C; UV (MeOH) λ 271 (27); ^{1}H NMR ($CDCl_3$) δ 7.66 (d, 2H, $J = 7.1$), 7.59 (d, 1H, $J = 2$), 7.49 (d, 2H, $J = 8.7$), 6.65 (d, 2H, $J = 8.7$), 5.92 (d, 1H, $J = 2$), 4.16 (br s, 2H, NH_2), 3.27 (t, 2H, $J = 6.6$), 1.27 (m, 2H), 1.09 (q, 2H, $J = 6.6$), 0.68 (d, 6H, $J = 6.6$); MS (CI, NH_3) m/z 385 ($[M + H]^+$, 100), 230 (61). Anal. ($C_{20}H_{24}N_4O_2S$) C, H, N.

4-Amino-*N*-benzyl-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide, 12. Benzyl bromide (0.075 mL, 0.63 mmol) was added to a solution of 4-(acetylamino)-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide (150 mg, 0.42 mmol) in 1.5 mL of anhydrous DMF containing 70 mg of Na_2CO_3. After 3.5 h at 80 °C, the solvent was evaporated and the residue dissolved in CH_2Cl_2. The organic phase was washed with water and dried over $MgSO_4$. After purification by column chromatography (SiO_2, CH_2Cl_2/EtOAc 70/30), 160 mg of *N*-acetyl-**12** was obtained in an 85% yield: ^{1}H NMR ($CDCl_3$) δ 7.70 (s, 4H), 7.53 (br s, 2H), 7.26 (m, 3H), 7.14 (m, 3H), 7.07 (t, 2H, $J = 7.2$), 6.83 (d, 2H, $J = 7.2$), 5.87 (d, 1H, $J = 2$), 4.38 (s, 2H,), 2.23 (s, 3H). The previous compound (140 mg, 0.31 mmol) was heated under reflux in 3 mL of 3 N NaOH and 3 mL of EtOH for 2 h. EtOH was evaporated, and the solution was diluted in water and extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$. **12** was recrystallized from Et_2O/cyclohexane, and obtained (103 mg) in an 81% yield: mp 153−154 °C; UV (MeOH) λ 272 (27); ^{1}H NMR ($CDCl_3$) δ 7.54−7.51 (m, 3H), 7.26−7.11 (m, 7H), 7.03 (t, 1H, $J = 7.3$), 6.82 (d, 2H, $J = 7.3$), 6.68 (d, 2H, $J = 8.7$), 5.93 (d, 1H, $J = 1.9$), 4.36 (s, 2H), 4.20

(br s, 2H, NH_2); MS (CI, NH_3) *m/z* 405 ($[M + H]^+$, 100), 250 (71). Anal. ($C_{22}H_{20}N_4O_2S$) C, H, N.

4-Amino-*N*-(3-hydroxypropyl)-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide, 13. 3-Bromopropanol (0.35 mL, 3.9 mmol) was added to a solution of 4-(acetylamino)-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide (210 mg, 0.59 mmol) in anhydrous DMF (1.5 mL) containing 70 mg of Na_2CO_3. After 16 h at 50 °C, DMF was evaporated and the residue was dissolved in CH_2Cl_2. The organic phase was washed with water and dried over $MgSO_4$. Purification by column chromatography (SiO_2, CH_2Cl_2/EtOAc, 40/60, and 2% MeOH and then 4% MeOH) gave 146 mg of *N*-acetyl-**13** in a 59% yield: mp 162−163 °C; ^{1}H NMR ($CDCl_3$) δ 2.22 (s, 3H), 1.38 (m, 2H), 3.41 (t, 2H, J = 6), 3.28 (m, 2H), 5.88 (d, 1H, J = 2), 7.48−7.38 (m, 4H), 7.65−7.60 (m, 3H), 7.74−7.66 (m, 4H). The previous compound (125 mg, 0.3 mmol), 3 mL of 2 N NaOH, and 4 mL of EtOH were heated under reflux for 2.5 h. The reaction mixture was cooled, water was added, and the solution was extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$ and evaporated, and **13** was recrystallized from CH_2Cl_2 (79% yield): mp 170−171 °C; UV (MeOH) λ 271 (22); ^{1}H NMR ($CDCl_3$) δ 1.36 (m, 2H), 1.66 (t, 1H, J = 6), 3.26 (q, 2H, J = 6), 3.39 (t, 2H, J = 6), 4.21 (br s, 2H, NH_2), 5.91 (d, 1H, J = 2), 6.68 (d, 2H, J = 8.7), 7.41 (m, 3H), 7.54 (d, 2H, J = 8.7), 7.60 (d, 1H, J = 2), 7.67 (dd, 2H, J = 8.2 and 1.5); MS (CI, NH_3) *m/z* 373 ($[M + H]^+$, 100), 218 (18) 160 (17). Anal. ($C_{18}H_{20}N_4O_3S$) C, H, N: calcd 58.05, found 57.60.

4-(2-Aminoethyl)-*N*-(3-hydroxypropyl)-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide, 14. In a 50 mL round-bottomed flask, fitted with a reflux condenser and a $CaCl_2$ tube, containing 3 mL of chlorosulfonic acid cooled in an ice bath, was added dropwise *N*-acetyl-2-phenylethylamine (1.5 g, 9.2 mmol). The solution was stirred for 1 h at room temperature, 1.5 h at 50 °C, and then 1.5 h at 70 °C, and the reaction was followed by TLC (SiO_2, CH_2Cl_2/EtOAc, 2/1). The reaction mixture was poured onto ice, extracted with CH_2Cl_2, and dried over $MgSO_4$. The residue was dissolved in CH_2Cl_2. An insoluble fraction was filtered. When cyclohexane was added to the solution, a white precipitate was formed, leading to 1.78 g of 4-[2-(acetylamino)ethyl]benzenesulfonyl chloride in a 74% yield: ^{1}H NMR ($CDCl_3$) δ 7.97 (d, 2H, J = 8.4), 7.43 (d, 2H, J = 8.4), 5.48 (br s, 1H), 3.53 (q, 2H, J = 7), 2.95 (t, 2H, J = 7), 1.95 (s, 3H). This product (800 mg, 3 mmol) was added to a solution of 636 mg of (2-phenyl)-2*H*-pyrazol-3-ylamine (4 mmol) in 6 mL of anhydrous pyridine. After 5 min at room temperature, the reaction mixture was heated at 95 °C for 2 h. Pyridine was evaporated, and the residue was dissolved in CH_2Cl_2, with a little volume of MeOH, and washed with 1 N HCl. The aqueous phase was extracted with CH_2Cl_2 and the organic phase dried over $MgSO_4$ and evaporated. Purification by column chromatography (SiO_2, CH_2Cl_2/MeOH, 5%) and recrystallization from MeOH/acetone led to 0.3 g of 4-(2-(acetylamino)ethyl]-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide in a 26% yield: mp 174−175 °C; ^{1}H NMR (CD_3SOCD_3) δ 10.32 (s, 1H), 7.90 (m, 1H),7.54 (d, 1H, J = 1.7), 7.57 (d, 2H, J = 8.2) 7.44 (m, 5H), 7.36 (d, 2H, J = 8.2), 5.80 (d, 1H, J = 1.7), 3.28 (m, 2H), 2.77 (t, 2H, J = 7), 1.78 (s, 3H). Benzoic acid 3-bromopropyl ester (334 mg, 1.37 mmol) was added to a solution of 4-[2-(acetylamino)ethyl]-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide (200 mg, 0.52 mmol) in 2 mL of anhydrous DMF containing 66 mg of Na_2CO_3. After 24 h at 80 °C, the solvent was evaporated and the residue was dissolved in CH_2Cl_2. The organic phase was washed with water and dried over $MgSO_4$. After purification by column chromatography (SiO_2, CH_2Cl_2, and then MeOH, 2%), 244 mg of protected **14** was obtained in an 86% yield: ^{1}H NMR ($CDCl_3$) δ 7.92 (d, 2H, J = 7.5), 7.68 (d, 2H, J = 8.4), 7.65−7.61 (m, 3H), 7.56 (t, 1H, J = 7.5), 7.48−7.39 (m, 5H), 7.28 (d, 2H, J = 8.4), 5.99 (d, 1H, J = 1.9), 5.64 (m, 1H), 3.93 (t, 2H, J = 6.1), 3.55−3.44 (m, 4H), 2.88 (t, 2H, J = 6.9), 1.94 (s, 3H), 1.68 (m, 2H). This compound (244 mg, 0.45 mmol) was heated under reflux in 5 mL of NaOH (5 N) and 5 mL of EtOH for 24 h. After evaporation of EtOH, the solution was diluted with water and extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$. After purification by column chromatography (SiO_2, CH_2Cl_2/MeOH, 20%, and then 1+% NH_3(aq)), 65 mg of **14** was obtained in a 36% yield: ^{1}H NMR ($CDCl_3$) δ 7.65 (d, 2H, J = 8.2) 7.59 (m, 2H), 7.55 (d, 1H, J = 1.7), 7.41−7.35 (m, 3H), 7.30 (d, 2H, J = 8.2), 5.84 (d, 1H, J = 1.7), 3.37 (t, 2H, J = 6.1), 3.18 (t, 2H, J = 6.1), 2.92 (t, 2H, J = 6.6), 2.77 (t, 2H, J = 6.6), 1.34 (m, 2H). Anal. ($C_{20}H_{24}N_4O_3S$) C, H, N.

4-(3-Aminopropyl)-*N*-(3-hydroxypropyl)-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide, 15. A solution of acetic anhydride (1.5 mL) in CH_2Cl_2 (3 mL) was added dropwise at 0 °C to a solution of 3-phenylpropylamine (2 mL, 14 mmol) in CH_2Cl_2 (15 mL) in the presence of triethylamine (2.2 mL). After 15 min, the reaction mixture was allowed to warm to room temperature for the night. The solution was washed with an aqueous HCl solution (pH 4) and extracted with CH_2Cl_2. The organic phases were gathered, washed with water until neutralization, and dried over $MgSO_4$. The solvent was evaporated, leading to 2.48 g of *N*-acetyl-3-phenylpropylamine, as an oil, in a quantitative yield: ^{1}H NMR ($CDCl_3$) δ 7.30−7.14 (m, 5H), 5.46 (br s, 1H), 3.27 (q, 2H, J = 7.2), 2.64 (t, 2H, J = 7.2), 1.82 (m, 2H), 1.92 (s, 3H). *N*-Acetyl-3-phenylpropylamine (2.17 g, 12 mmol) was then added dropwise to 4 mL of chlorosulfonic acid contained in a 50 mL round-bottomed flask fitted with a reflux condenser and a $CaCl_2$ tube and cooled in ice. The solution was stirred for 1 h at room temperature and for 1.5 h at 70 °C. The mixture was then poured onto ice, extracted with CH_2Cl_2, and dried over $MgSO_4$. Evaporation of the solvent led to 440 mg of crude 4-[3-(acetylamino)propyl]benzenesulfonyl chloride (13% yield): ^{1}H NMR ($CDCl_3$) δ 7.93 (d, 2H, J = 8.1), 7.41 (d, 2H, J = 8.1), 5.60 (br s, 1H), 3.31 (t, 2H, J = 7.2), 2.76 (t, 2H, J = 7.2), 1.87 (m, 2H), 1.97 (s, 3H). A 435 mg sample of this arylsulfonyl chloride was then added to a solution of (2-phenyl-2*H*-pyrazol-3-yl)amine (250 mg, 1.57 mmol) in 2.5 mL of anhydrous pyridine. After 5 min at room temperature, the mixture was heated at 95 °C for 1 h. Pyridine was evaporated, and the residue was dissolved in 1 N NaOH, washed with CH_2Cl_2, acidified with 1 N HCl, and extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$ and evaporated. After purification by column chromatography (SiO_2, EtOAc, and MeOH, 1%, and then MeOH, 2%), 110 mg of 4-[3-(acetylamino)propyl]-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide was obtained in a 17% yield : ^{1}H NMR ($CDCl_3$) δ 7.59 (d, 2H, J = 8.4), 7.53 (s, 1H), 7.35 (m, 3H), 7.23 (m, 2H), 7.10 (m, 2H), 6.51 (br s, 1H), 6.23 (s, 1H), 5.45 (br s, 1H), 3.27 (q, 2H, J = 7.2), 2.69 (t, 2H, J = 7.2), 1.95 (s, 3H), 1.82 (m, 2H). Benzoic acid 3-bromopropyl ester (130 mg, 0.54 mmol) was then added dropwise to a solution of this product (110 mg, 0.27 mmol) in 1 mL of anhydrous DMF containing 34 mg of Na_2CO_3. After 20 h at 80 °C, the solvent was evaporated and the residue was dissolved in CH_2Cl_2. The organic phase was washed with water and dried over $MgSO_4$. After purification by column chromatography (SiO_2, CH_2Cl_2, and MeOH, 2%), 93 mg of protected **15** was obtained in a 61% yield: ^{1}H NMR ($CDCl_3$) δ 7.92 (d, 2H, J = 7.4),7.68−7.24 (m, 13H), 5.93 (s, 1H), 5.45 (br s, 1H, NH), 3.97 (t, 2H, J = 6.4), 3.45 (t, 2H, J = 6.4), 3.29 (q, 2H, J = 7), 2.70 (t, 2H, J = 7), 1.96 (s, 3H), 1.87 (m, 2H), 1.66 (m, 2H). The previous compound (93 mg, 0.16 mmol) was heated under reflux in 2 mL of 5 N NaOH and 2 mL of EtOH for 24 h. After evaporation of EtOH, the solution diluted in water was extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$. **15** was recrystallized from CH_2Cl_2, and obtained (34 mg) in a 49% yield: mp 133−134 °C; UV (MeOH) λ 233 (25); ^{1}H NMR ($CDCl_3$) δ 7.70−7.60 (m, 5H), 7.44−7.32 (m, 5H), 5.87 (d, 1H, J = 1.9), 3.42 (t, 2H, J = 6), 3.28 (t, 2H, J = 6), 2.75 (m, 4H), 1.80 (m, 2H), 1.42 (m, 2H); MS (CI, NH_3) *m/z* 415 ($[M + H]^+$, 86), 218 (100). Anal. ($C_{21}H_{26}N_4O_3S{\cdot}H_2O$) C, H, N.

4-Allyl-*N*-(3-hydroxypropyl)-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide, 16. Benzoic acid 3-bromopropyl ester (515 mg, 2.12 mmol) was added to a solution of 4-bromo-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide, **2** (400 mg, 1.06 mmol), in 4 mL of anhydrous DMF containing 500 mg of Na_2CO_3. After 16 h at 80 °C, DMF was evaporated and the residue was dissolved in CH_2Cl_2. The organic phase was

washed with water and dried over $MgSO_4$. After purification by column chromatography (SiO_2, CH_2Cl_2, and then Et_2O, 3%), 440 mg of the expected benzoate was obtained in a 77% yield: mp 123−124 °C; ^{1}H NMR ($CDCl_3$) δ 1.73 (m, 2H), 3.48 (t, 2H, $J = 7.2$), 4.02 (t, 2H, $J = 6$), 5.96 (d, 1H, $J = 2$), 7.46−7.41 (m, 5H), 7.62−7.57 (m, 7H), 7.63 (d, 1H, $J = 2$), 7.93 (d, 2H, $J = 8$). The previous compound (400 mg, 0.74 mmol) was boiled in 10 mL of EtOH and 5 mL of 2 N NaOH for 2 h. After evaporation of EtOH, dilution in water, and extraction with CH_2Cl_2, 250 mg of product was obtained (78% yield): ^{1}H NMR ($CDCl_3$) δ 1.44 (m, 2H), 3.32 (q, 2H, $J = 6$), 3.45 (t, 2H, $J = 6$), 5.89 (d, 1H, $J = 2$), 7.68−7.59 (m, 7H), 7.44 (m, 3H). A solution containing 240 mg (0.55 mmol) of this compound, 43 mg of $Pd[PPh_3]_4$, and 6 mL of anhydrous DMF was deoxygenated under argon for 0.5 h. A 0.22 mL sample of allyltributyltin was then added dropwise with a syringe. The reaction mixture was stirred under argon at room temperature for 0.5 h and then at 110 °C for 16 h. The solution was cooled, and the solvent was evaporated under vacuum. After purification by column chromatography (SiO_2, CH_2Cl_2, and then Et_2O, 10%), and recrystallization from Et_2O, 190 mg of compound **16** was obtained (86% yield): mp 114−115 °C; UV (MeOH) λ 234 (23); ^{1}H NMR ($CDCl_3$) δ 7.70 (d, 2H, $J = 8.3$), 7.65−7.60 (m, 2H), 7.60 (d, 1H, $J = 2$), 7.48−7.38 (m, 3H), 7.35 (d, 2H, $J = 8.3$), 5.96 (m, 1H), 5.87 (d, 1H, $J = 2$), 5.13 (m, 2H), 3.48 (d, 2H, $J = 6.7$), 3.42 (m, 2H), 3.29 (q, 2H, $J = 6$), 1.39 (m, 2H); MS (CI, NH_3) *m/z* 398 ([M + H]$^+$, 100), 218 (76). Anal. ($C_{21}H_{23}N_3O_3S$) C, H, N.

3′-Aminobiphenyl-4-sulfonic Acid (3-Hydroxypropyl)-(2-phenyl-2*H*-pyrazol-3-yl)amide, 17. Compound **19** (195 mg, 0.41 mmol) was dissolved in a minimum volume of acetone, and then a half-volume of water was added. The reaction mixture was deoxygenated under argon. A 1.42 g sample of $Na_2S_2O_4$ was added, and the solution was heated at 60 °C under argon for 2 h. The reaction was followed by TLC (SiO_2, CH_2Cl_2/EtOAc, 90/10). The mixture was diluted with water, extracted with EtOAc, and dried over $MgSO_4$, and the solvents were evaporated. Compound **17** was purified on preparative TLC plates (SiO_2, 1 mm, CH_2Cl_2/MeOH, 90/10), and obtained as a resin in a 38% yield : UV (MeOH) λ 250 (30); ^{1}H NMR ($CDCl_3$) δ 1.43 (m, 2H), 3.32 (m, 2H), 3.50 (m, 2H), 3.80 (s, 2H, NH_2), 5.93 (s, 1H), 6.75 (d, 1H, $J = 7.7$), 6.91 (s, 1H), 7.00 (d, 1H, $J = 7.7$), 7.26 (t, 1H, $J = 7.7$), 7.30−7.50 (m, 3H), 7.62−7.66 (m, 3H), 7.69 (d, 2H, $J = 8.5$), 7.80 (d, 2H, $J = 8.5$); MS (CI, NH_3) *m/z* 449 ([M + H]$^+$, 100), 218 (48). Anal. ($C_{24}H_{24}N_4O_3S$) C, H, N.

3′-Nitrobiphenyl-4-sulfonic Acid (2-Hydroxyethyl)(2-phenyl-2*H*-pyrazol-3-yl)amide, 18. Compound **3** (120 mg, 0.29 mmol) was added to 1 mL of DMF containing 90 mg of Na_2CO_3 and 0.06 mL of 2-chloroethanol (0.89 mmol). The solution was stirred at 80 °C. After 1 day, the reaction was not finished. A 3 equiv sample of NaI was then added, and the reaction was heated for 24 h at 80 °C. The solvent was evaporated, and the residue was diluted with water and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4. After purification by column chromatography (SiO_2, CH_2Cl_2/EtOAc, 10%), 60 mg of an oil was obtained, which crystallized slowly, leading to **18** in a 45% yield: mp 168−169 °C; UV (MeOH) λ 259 (42); ^{1}H NMR ($CDCl_3$) δ 8.49 (s, 1H), 8.29 (d, 1H, $J = 8$), 7.94 (d, 1H, $J = 8$), 7.89 (d, 2H, $J = 8.5$), 7.76 (d, 2H, $J = 8.5$), 7.73−7.63 (m, 4H), 7.42−7.54 (m, 3H), 5.96 (d, 1H, $J = 1.9$), 3.49 (m, 4H), 1.33 (t, 1H, $J = 5.8$); MS (CI, NH_3) *m/z* 465 ([M + H]$^+$, 67), 204 (100). Anal. ($C_{23}H_{20}N_4O_5S$) C, H, N.

3′-Nitrobiphenyl-4-sulfonic Acid (3-Hydroxypropyl)-(2-phenyl-2*H*-pyrazol-3-yl)amide, 19. To a solution of compound **3** (180 mg, 0.43 mmol) in DMF (2 mL) was added benzoic acid 3-bromopropyl ester (208 mg, 0.86 mmol), in the presence of Na_2CO_3 (55 mg, 0.52 mmol). The solution was stirred at 80 °C for 1 day. After evaporation of DMF, the residue was dissolved in water and CH_2Cl_2, then extracted with CH_2Cl_2, and dried over $MgSO_4$, and the solvents were evaporated. The benzoate of **19** was purified by column chromatography (SiO_2, CH_2Cl_2/EtOAc, 90/10), and obtained in an 88% yield: ^{1}H NMR ($CDCl_3$) δ 1.74 (m, 2H), 3.53 (t, 2H, $J = 6.6$), 4.02 (t, 2H, $J = 6.6$), 6.02 (d, 1H, $J = 1.8$), 7.37−7.48 (m, 5H), 7.54 (t, 1H, $J = 7.3$), 7.62−7.72 (m, 6H), 7.86 (d, 2H, $J = 8.5$), 7.93, (m, 3H), 8.29 (d, 1H, $J = 8.2$), 8.44 (s, 1H). This compound (215 mg, 0.37 mmol) was dissolved in EtOH (10 mL) and added to a solution of 148 mg of NaOH (10 equiv) in 5 mL of water. The mixture was heated under reflux. At the end of the reaction, EtOH was evaporated, the mixture was diluted with H_2O, extracted with CH_2Cl_2, and dried over $MgSO_4$, and the solvents were evaporated. Compound **19** was obtained as a resin in an 85% yield: UV (MeOH) λ 259 (49); ^{1}H NMR ($CDCl_3$) δ 1.46 (m, 2H), 3.34 (m, 2H), 3.51 (t, 2H, $J = 6.6$), 5.94 (d, 1H, $J = 1.8$), 7.35−7.50 (m, 3H), 7.63−7.67 (m, 3H), 7.69 (t, 1H, $J = 8$), 7.77 (d, 2H, $J = 8.4$), 7.89 (d, 2H, $J = 8.4$), 7.95 (d, 1H, $J = 8$), 8.29 (d, 1H, $J = 8$), 8.49 (s, 1H); MS (CI, NH_3) *m/z* 479 ([M + H]$^+$, 100), 449 (22), 218 (60). Anal. ($C_{24}H_{22}N_4O_5S$) C, H, N.

3′-Nitrobiphenyl-4-sulfonic Acid (4-Hydroxybutyl)(2-phenyl-2*H*-pyrazol-3-yl)amide, 20. Compound **3** (120 mg, 0.29 mmol) was added to 1 mL of DMF containing 90 mg of Na_2CO_3, 0.1 mL of 4-chlorobutanol (1 mmol), and 130 mg of NaI (3 equiv). The solution was stirred at 80 °C. After 1 day, the solvent was evaporated and the residue was diluted in water and extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$. After purification by column chromatography (SiO_2, CH_2Cl_2/EtOAc, 20%) and then on preparative TLC plates (SiO_2), 34 mg of **20** was obtained as a white-green powder in a 24% yield: mp 145−146 °C; UV (MeOH) λ 259 (38); ^{1}H NMR ($CDCl_3$) δ 8.49 (s, 1H), 8.29 (d, 1H, $J = 8$), 7.95 (d, 1H, $J = 8$), 7.86 (d, 2H, $J = 8.5$), 7.75 (d, 2H, $J = 8.5$), 7.71−7.64 (m, 3H), 7.63 (d, 1H, $J = 1.9$), 7.48−7.38 (m, 3H), 5.94 (d, 1H, $J = 1.9$), 3.41 (m, 4H), 1.33 (m, 4H), 1.06 (br s, 1H); MS (CI, NH_3) *m/z* 493 ([M + H]$^+$, 73), 463 (41), 232 (100). Anal. ($C_{25}H_{24}N_4O_5S$) C, H, N.

4-Allyl-*N*-methyl-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide, 21. Methyl iodide (0.01 mL, 0.16 mmol) was added to a solution of 4-allyl-*N*-(2-phenyl-2*H*-pyrazol-3-yl)-benzenesulfonamide, **4** (34 mg, 0.1 mmol), in 0.3 mL of anhydrous DMF containing Na_2CO_3 (12 mg, 0.11 mmol). After 45 min at 80 °C, DMF was evaporated and the residue was diluted with water and extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$. After purification by column chromatography (SiO_2, CH_2Cl_2, and then Et_2O, 2%), 35 mg of **21** was obtained in a quantitative yield as an oil, which crystallized after a few days: mp 85−86 °C; ^{1}H NMR ($CDCl_3$) δ 7.61 (d, 2H, $J = 8.3$), 7.59−7.53 (m, 2H), 7.57 (d, 1H, $J = 2$), 7.48−7.34 (m, 3H), 7.31 (d, 2H, $J = 8.3$), 5.94 (m, 1H), 5.88 (d, 1H, $J = 2$), 5.14 (m, 1H), 5.11 (m, 1H), 3.46 (d, 2H, $J = 6.7$), 3.04 (s, 3H); HRMS (CI, CH_4) (M + H)$^+$ calcd for $C_{19}H_{20}O_2N_3S$ 354.1276, found 354.1274.

4-Allyl-*N*-benzyl-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide, 22. Benzyl bromide (0.013 mL, 0.11 mmol) was added to a solution of 4-allyl-*N*-(2-phenyl-2*H*-pyrazol-3-yl)-benzenesulfonamide, **4** (24.6 mg, 0.073 mmol), in 0.3 mL of anhydrous DMF containing Na_2CO_3 (10 mg, 0.1 mmol). After 2 h at 80 °C, DMF was evaporated and the residue was dissolved in water and extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$. After purification by column chromatography (SiO_2, CH_2Cl_2/Et_2O, 1%), 26 mg of **22** was obtained as an oil, which crystallized after a few days in an 83% yield: mp 94−95 °C; UV (MeOH) λ 235; ^{1}H NMR ($CDCl_3$) δ 7.70 (d, 2H, $J = 8.3$), 7.53 (d, 1H, $J = 1.9$), 7.35 (d, 2H, $J = 8.3$), 7.30−7.10 (m, 6H), 7.04 (t, 2H, $J = 7.3$), 6,82 (d, 2H, $J = 7.3$), 5.97 (m, 1H), 5.86 (d, 1H, $J = 1.9$), 5.18−5.09 (m, 2H), 4.39 (s, 2H), 3.50 (d, 2H, $J = 6.6$); HRMS (CI, CH_4) (M + H)$^+$ calcd for $C_{25}H_{24}O_2N_3S$ 430.1589, found 430.1592.

4-Methyl-*N*-methyl-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide, 23. Methyl iodide (0.022 mL, 0.35 mmol) was added to a solution of 4-methyl-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide (75 mg, 0.24 mmol) in 0.5 mL of anhydrous DMF containing 40 mg of Na_2CO_3. After 3 h at 70 °C, DMF was evaporated and the residue was dissolved in CH_2Cl_2. The organic phase was washed with water and dried over $MgSO_4$. After purification by column chromatography (SiO_2,

CH_2Cl_2/Et_2O, 3%), 76 mg of **23** was obtained in a 97% yield: mp 121−122 °C; UV (MeOH) λ 232 (22); ^{1}H NMR ($CDCl_3$) δ 2.44 (s, 3H), 3.03 (s, 3H), 5.88 (d, 1H, $J = 2$), 7.29 (d, 2H, $J = 8.1$), 7.39 (t, 1H, $J = 7.2$), 7.44 (t, 2H, $J = 7.2$), 7.60−7.56 (m, 5H); MS (CI, NH_3) m/z 328 ([M + H]$^+$, 100), 174 (36). Anal. ($C_{17}H_{17}N_3O_2S$) C, H, N.

4-Methyl-*N*-propyl-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide, 24. 1-Iodopropane (0.03 mL, 0.3 mmol) was added to a solution of 4-methyl-*N*-(2-phenyl-2*H*-pyrazol-3-yl)benzenesulfonamide (45 mg, 0.14 mmol) in 0.5 mL of anhydrous DMF containing 30 mg of Na_2CO_3. After 18 h at 60 °C, DMF was evaporated and the residue was dissolved in CH_2Cl_2. The organic phase was washed with water and dried over $MgSO_4$. After purification by column chromatography (SiO_2, CH_2Cl_2), 45 mg of **24** was obtained (88% yield). Recrystallization from ether/cyclohexane led to 32 mg of pure **24**: mp 127−128 °C; UV (MeOH) λ 233 (20); ^{1}H NMR ($CDCl_3$) δ 7.66−7.58 (m, 5H), 7.42 (m, 3H), 7.30 (d, 2H, $J = 8.1$), 5.86 (d, 1H, $J = 1.9$), 3.22 (t, 2H, $J = 7.5$), 2.44 (s, 3H), 1.24 (m, 2H), 0.64 (t, 3H, $J = 7.5$); MS (CI, NH_3) m/z 356 ([M + H]$^+$, 100), 202 (41). Anal. ($C_{19}H_{21}N_3O_2S$) C, H, N.

Yeast Transformation, Cell Culture, and Preparation of the Yeast Microsomal Fraction. The expression system used for human liver P450s was based on a yeast strain, W(R)-fur1, previously described,[21] in which yeast cytochrome P450 reductase was overexpressed. Transformation by the pYeDP60 vector containing one of the human liver CYP 2C8, 2C9, 2C18, and 2C19[28−31] cDNAs was then performed according to a general construction method of yeast strain W(R)fur1 expressing various human liver cytochrome P450s.[32,33] Yeast culture and microsome preparations were performed by using previously described techniques.[34] Microsomes were homogenized in 50 mM Tris buffer (pH 7.4) containing 1 mM EDTA and 20% glycerol (v/v), aliquoted, frozen under liquid N_2, and stored at −80 °C until use. The P450 contents of yeast microsomes were 40, 90, 40, and 20 pmol P450/mg of protein for CYP 2C8, 2C9, 2C18, and 2C19, respectively.

The microsomal P450 content was determined according to the method of Omura and Sato.[35] The protein content in microsomal suspensions was determined by the Lowry procedure[36] using bovine serum albumin as the standard.

Enzyme Activity Assay. 5-Hydroxylation of 2-Aroylthiophenes. Quantitation of 5-hydroxy-2-aroylthiophenes was based on a spectrophotometric method[22] adapted to yeast microsomes expressing CYP 2C9 in the case of tienilic acid.[5] Incubations for metabolic activity with yeast microsomes were carried out at 28 °C, using glass tubes in a shaking bath. The incubation mixtures contained the yeast microsomal suspension, providing 0.2, 0.2, 0.1, and 0.075 μM P450 for CYP 2C8, 2C9, 2C18, and 2C19, respectively, the substrate, and an NADPH-generating system (1 mM NADP$^+$, 10 mM glucose 6-phosphate, and 2 units of glucose 6-phosphate deshydrogenase/mL) diluted in 0.1 M Tris buffer, pH 7.4, containing 1 mM EDTA and 8% glycerol (final concentrations). Activity assays were routinely initiated ($t_0 = 0$ min) by incorporation of the NADPH-generating system into the incubation mixture after 3 min of separate preincubation at 28 °C for temperature equilibration. At t_0 and regularly thereafter, aliquots (140 μL) were taken and the reaction was quickly stopped by treatment with 70 μL of a cold CH_3CN/CH_3COOH (10/1) mixture.

As previously reported,[18] the 2-aroylthiophene substrates used for measuring CYP 2C8 and CYP 2C9 dependent activities were 3-[2,3-dichloro-4-(2-thenoyl)phenoxy]ethanol and tienilic acid, respectively. In the case of CYP 2C18 and CYP 2C19, 3-[2,3-dichloro-4-(2-thenoyl)phenoxy]propan-1-ol was used as substrate.[18] IC_{50} values of sulfaphenazole derivatives were determined after addition of variable concentrations of these derivatives to incubates containing 250, 6, 15, and 100 μM substrate of CYP 2C8, 2C9, 2C18, and 2C19, respectively. These concentrations correspond to the K_M values of the hydroxylation of these substrates.[18] The studied compound dissolved in CH_3OH or DMSO was added to the incubation mixture (final solvent concentration 1%). The residual activity was plotted versus the inhibitor concentration. IC_{50} values were estimated by curve fit analyses.

Study of Substrate Binding to Yeast-Expressed CYP 2C9 by Difference Visible Spectroscopy. Difference visible spectra produced by sulfaphenazole derivatives were recorded at room temperature with a Kontron 820 spectrophotometer. Yeast microsomes were suspended in 50 mM Tris, 1 mM EDTA, pH 7.4, to obtain a P450 concentration of 0.13 μM. The solution was equally divided between two 500 μL quartz cuvettes (1 cm path length), and a baseline was recorded. Aliquots (1−5 μL) of solutions containing the studied compound were added to the sample cuvette, the same volume of solvent being added to the reference cuvette. The difference spectra were recorded between 380 and 520 nm.[37]

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