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Analogs of a potent maxi-K potassium channel opener with an improved inhibitory profile toward cytochrome P450 isozymes

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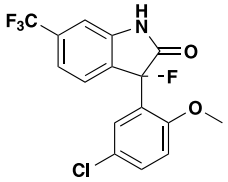
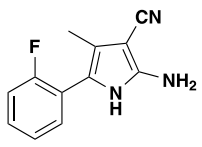
Abstract—Quinolinone **1** is a potent maxi-K potassium channel opener. In an effort to design analogs of **1** with a better inhibitory profile toward the CYP2C9 isozyme, the two acidic sites were chemically modified independently to generate a number of analogs. These analogs were evaluated as maxi-K channel openers in vitro using *Xenopus laevis* oocytes expressing cloned *h*Slo maxi-K channels. Compounds **15**, **17**, and **19** showed potent activity as maxi-K channel openers and were further evaluated for inhibition of the activity of the CYP2C9 isozyme. Compounds **17** and **19** showed diminished inhibitory potency against 2C9 and also against a panel of other more common CYP isozymes.

Potassium (K^+) channels are important regulators of critical functions in cells, and particularly in electrically excitable cells such as neurons and muscle cells. There are many subfamilies of K^+ channels, reflecting their genetic diversity and evolutionary importance and their many functions. One specialized function of one K^+ channel family, the calcium-activated K^+ channels, is to sense the intracellular concentration of the divalent cation calcium (Ca^{2+}) and to open in response to increases in its concentration. This acts to hyperpolarize cell membranes and reduce further Ca^{2+} entry.[1a] In mammalian cells, the calcium-activated K^+ channels are ubiquitous and are classified according to their conductance values. Based on conductance, these channels are classified as SK (small conductance), IK (intermediate conductance), and BK (maxi-K or large conductance) channels.[1a,b]

Agents that can modulate the activity of maxi-K ion channels present a number of therapeutic opportunities for conditions such as stroke, traumatic brain injury, and urinary incontinence[1a,b] that could be responsive to an increase in K^+ flux through the actions of maxi-K openers.[2] The maxi-K opener **BMS-204352**, a fluoroxindole, was previously shown to be neuroprotective and demonstrated efficacy in rodent models of acute focal stroke.[3] Another maxi-K opener **NS-8**, a pyrrole derivative, was effective in in vivo rodent models of urinary incontinence.[4] Recently, it was demonstrated in rats that intracavernous injection of *h*Slo DNA was capable of altering nerve-stimulated penile erection. A significant elevation in intracavernous pressure lasting for at least 2 months post-injection was observed in this study,[5] suggesting that maxi-K openers may also provide therapeutic benefit in sexual dysfunction.



BMS-204352



NS-8

Several chemotypes have been identified as maxi-K channel openers in our laboratories with potential utility in the aforesaid therapeutic areas. Among these, the 3-substituted-4-arylquinolin-2-ones were found to be a distinct class of compounds exhibiting significant activity as maxi-K openers.[6a–d] Quinolinone **1** was identified as a potent opener of maxi-K channels.[6a] During the

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course of profiling **1** for potential liabilities, it was found that **1** inhibited the cytochrome P450 (CYP) 2C9 isoform with an IC_{50} of 1.7 μM. Herein, we describe our efforts to ameliorate the CYP2C9 inhibition while maintaining the favorable maxi-K properties associated with **1**.

Cytochrome P450 (CYP) enzymes play a major role in metabolizing drug molecules. Many lead candidate molecules in pharmaceutical development fail due to the potent inhibition of one or more isozymic forms of CYP enzymes. Among the several hundreds of these enzymes, the 2C and 3A subfamilies are the major isoforms present in human liver.[7] The 2C9 isozyme of the 2C family is predominantly expressed in human liver.[8] It was felt that an approach with an understanding of binding of drugs as substrates to the active site of CYP2C9 may help us design analogs of **1** with diminished inhibitory activity toward CYP2C9.

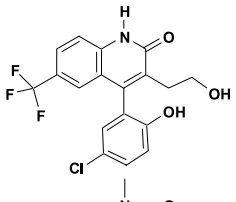
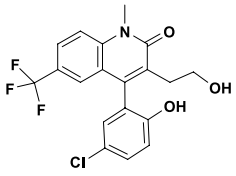
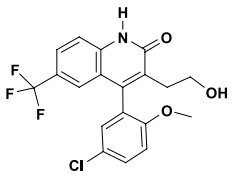
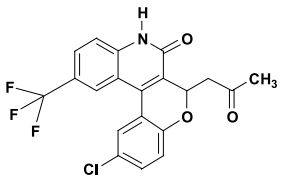
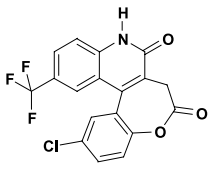
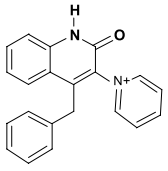
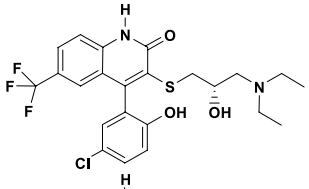
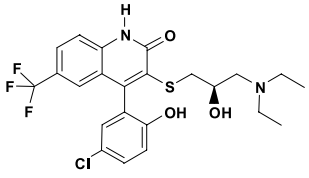
Mancy et al.[9] examined a number of drugs that were substrates of CYP2C9 and found that they were all protic acids with a pK_a between 4.5 and 8.1. It was proposed[9] that at physiological pH, the acidic site in these molecules forms an anionic species capable of interacting with a cationic site located at the substrate binding pocket of cytochrome P4502C9. The importance of a second pi-stacking anchor site was subsequently recognized based on the studies done with warfarin.[10]

Determination of the aqueous pK_a of **1** indicated that the phenolic proton is more acidic (pK_a = 8.69 ± 0.2) than that of the amide (pK_a = 12.19 ± 0.2).[11] Examination (Table 1) of the data related to CYP2C9 inhibition accumulated in our quinolinone database with compounds **1**–**8**[6a–d] revealed the following observations:

(a) Generally, modification of the *p*-chlorophenol by alkylation or acylation resulted in diminished inhibition toward CYP2C9 relative to **1**. However, the *p*-chlorophenol moiety is also important for maxi-K activity.[6a–d]
(b) Quinolinones containing functionalities positively charged at physiological pH (compounds **6**–**8**) are also poor inhibitors of CYP2C9 perhaps due to the presence of a repelling cationic site on 2C9.
(c) Introduction of a small alkyl substituent such as Me on N^1 (compound **2**) did not improve CYP inhibition but retained the maxi-K activity (see Table 2).

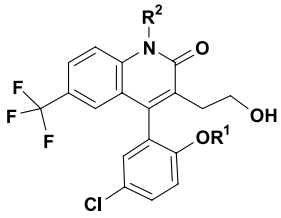
Assuming that these quinolinones interact with the same binding pocket described by Mancy et al.,[9] a priori, it was not known which acidic site in the quinolinone would interact with the cationic site of CYP2C9 and which aromatic residue would interact with the second pi-stacking site. It seemed, therefore, appropriate to modify the two acidic sites independently, incorporating polar functionalities that are neutral, basic, and acidic. The objective of these changes would be to disrupt CYP2C9 recognition while preserving the maxi-K channel opening ability.

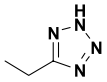
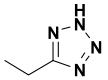
Table 1. IC_{50} values for CYP2C9 inhibition

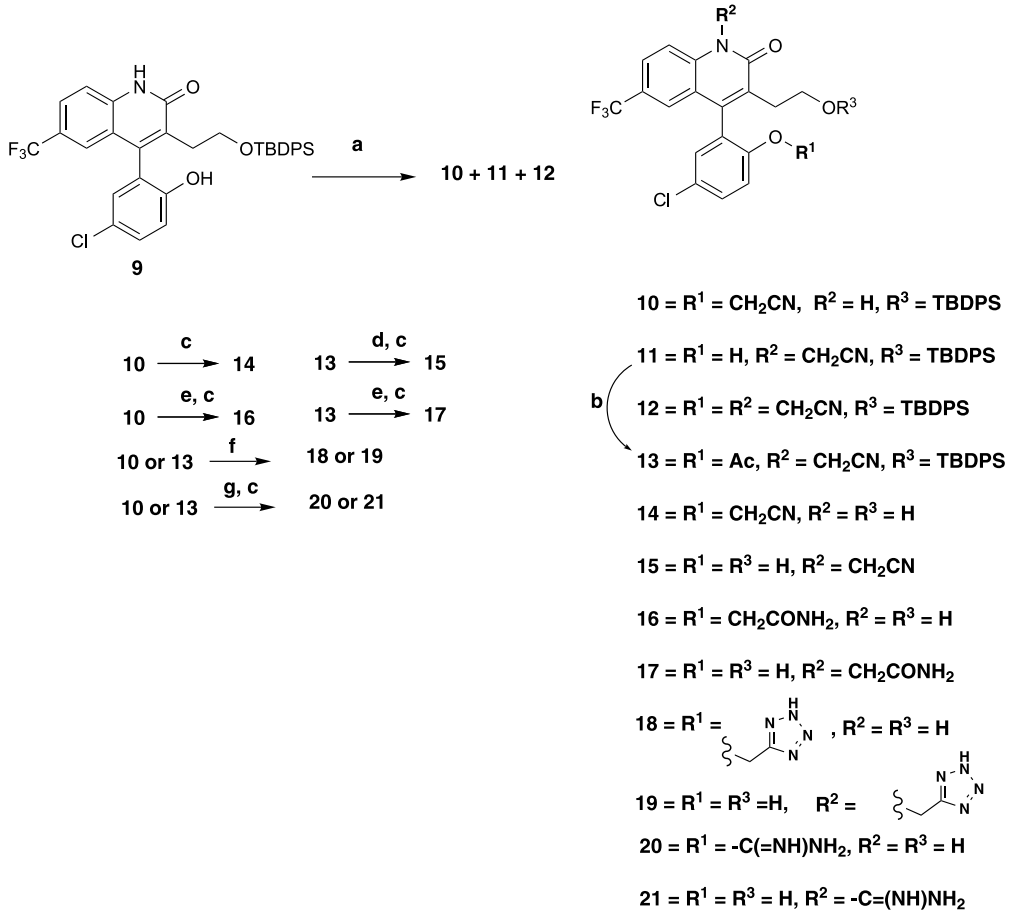
Compound	Structure	IC_{50} (μM)
1		1.7
2		1.1
3		5.47
4		19.49
5		12.45
6		>100
7		37.66
8		50.68

The nitrile functionality served as a good handle to prepare the desired three types of derivatives.[12] The nitrile nitrogen can serve as a neutral H bond acceptor. Nitriles can undergo facile transformation into carboxylic acids or acidic amides, the degree of acidity depending on the environment around the amide bond. They can also be elaborated into moderately acidic tetrazoles and basic amidines. The synthesis of amides and amidines is

Table 2. Effect of selected test compounds on maxi-K-mediated outward current in *h*Slo injected *X. laevis* oocytes



Compound	R^1	R^2	% Increase of current at 20 μM
1	H	H	252.6 ± 8.2
2	H	Me	296.1 ± 12.6
3	Me	H	211.2 ± 20.3
14	CH_2CN	H	148.9 ± 6.8
15	H	CH_2CN	236.6 ± 13.0
16	CH_2CONH_2	H	124.0 ± 4.7
17	H	CH_2CONH_2	262.5 ± 24.5
18		H	111.0 ± 1.1
19	H		205.8 ± 9.2
20	$CH_2C(=NH)NH_2$	H	118.3 ± 5.8
21	H	$CH_2C(=NH)NH_2$	105.8 ± 5.3



Scheme 1. Reagents and conditions. (a) $ICH_2CN/K_2CO_3/Me_2CO$/reflux. (b) Ac_2O/Et_3N/DCE. (c) TBAF/THF. (d) $ICH_2CN/K_2CO_3/Me_2CO$. (e) Na_2O_2/H_2O_2/MeOH. (f) NaN_3/DMF/NH_4Cl. (g) NaOMe/MeOH/NH_4Cl.

Table 3. Evaluation of active quinolinones for inhibition of activity of CYP isozymes[17]—IC_{50} values (μM)

Compound	1A2/CEC	2C9/7-MFC	2C19/CEC	2D6/AMMC	3A4/BFC	3A4/BzRES
1	30	1.7	5.2	35	8.1	>100
15	>40	3.9	24	23	13	18
17	>100	16	8.2	>100	26	50
19	>100	19	12	>100	30	13

generally amenable to parallel synthesis for rapid generation of analogs for evaluation. For the synthesis of the three classes of analogs, the key intermediate turned out to be the *tert*-butyldiphenylsilyl (TBDPS) ether (**9**) obtained by standard silylation procedure.[6c] Alkylation of this silyl derivative with iodoacetonitrile resulted in the formation of *O*-alkyl derivative **10** and *N*-alkyl derivative **11** in 9:1 ratio (35% yield), along with *N*,*O*-dialkyl derivative **12** (41% yield). These silyl-protected cyanomethyl derivatives were separated by silica gel chromatography and used for subsequent derivatization (Scheme 1).

Deprotection of protecting silyl and acetyl groups from nitriles **10** and **13** gave nitriles **14** and **15** (~72% and 46% yield, respectively). Amides **16** and **17** were obtained from the corresponding nitriles by alkaline peroxide-mediated hydrolysis[13] and desilylation in an overall yield of 42%. Elaboration of nitriles **10** and **13** to the corresponding tetrazoles **18** and **19** occurred by the cycloaddition of azide ion in DMF.[14] Interestingly, this process also resulted in concomitant desilylation. The synthesis of amidines **20** and **21** was performed via the corresponding methyl imidates.[15]

The target compounds thus obtained were evaluated in *Xenopus laevis* oocytes, expressing the cloned *h*Slo maxi-K channel for their ability to open the maxi-K channel.[16] Table 2 illustrates the percent increase of *h*Slo current obtained with the target compounds tested at 20 μM. In this assay, compounds demonstrating a value ⩾130% increase of measured maxi-K current are considered significant openers of the maxi-K channel.

Modification of the phenolic OH by alkylation with substituents containing neutral or acidic or basic functionalities, as in analogs **14**, **16**, **18**, and **20**, led to diminished activity as maxi-K openers, confirming the importance of phenolic hydroxyl for channel opening ability.[6b] The N^1 position was also modified to introduce the same substituents to obtain analogs **15**, **17**, **19**, and **21**. In this series, nitrile **15**, the weakly acidic amide **17**, and the tetrazole **19** were found to be potent maxi-K openers, whereas the basic amidine **21** turned out to be inactive.

Thus, the maxi-K channel tolerated neutral and weakly acidic substituents on N^1. Nitrile **15** displayed only 2-fold reduction in CYP2C9 inhibition (IC_{50} = 3.9 μM) compared to **1** (IC_{50} = 1.7 μM). However, compounds **17** and **19** showed 9- and 11-fold, respectively, reduced inhibition of CYP2C9 (Table 3). Further evaluation with other CYP isozymes showed that the profile across a panel of CYP enzymes was generally better than **1**, with IC_{50} values for the common isoforms being in the range of 8–100 μM.

Of the two acidic sites present in the quinolinone moiety, the phenolic OH is important for activity as maxi-K opener. Modification of the second N^1 acidic site in the quinolinones can result in disruption of recognition by CYP2C9 without loss of activity as in maxi-K opener.

In conclusion, we have demonstrated that modification of the acidic site in the quinolinone **1** can be used as an approach to overcome the CYP2C9 enzyme inhibition while maintaining maxi-K activity.

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was used for samples with poor solubility in water alone; in this case a minimum of three different water/methanol mixtures were analyzed and the aqueous pK_a was determined by extrapolation to 0% cosolvent. All data processing were done with Sirius RefinementPro software.
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17. Crespi, C. L.; Miller, V. P.; Penman, B. W. *Med. Chem. Res.* **1998**, *8*, 457, The described assay was slightly modified. The capacity of test compounds to inhibit cDNA-derived cytochrome P450 enzymes in microsomes prepared from baculovirus-infected insect cells was measured using either 3-cyano-7-ethoxycoumarin (CYP1A2 and CYP2C19), 7-methoxy-4-trifluoromethylcoumarin (CYP2C9) or 3-[2-(*N*,*N*-diethyl-*N*-methylamino)ethyl]-7-methoxy-4-methylcoumarin (CYP2D6) as substrates. CYP3A4 was tested with multiple substrates; 7-benzyloxy-4-trifluoromethylcoumarin (BFC) and resorufin benzyl ether (BR). The inhibition study consisted of the determination of a 50% inhibitory concentration (IC_{50}) for the test substance and each enzyme. A single concentration of each model substrate (approximately the apparent K_m with the exception of BFC, which is tested below the apparent K_m) and multiple test substance concentrations, separated by approximately 1/2 log, were tested in duplicate. Metabolism of the model substrate was assayed by the production of 7-hydroxy-3-cyanocoumarin, 3-[2-(*N*,*N*-diethylamino)ethyl]-7-hydroxy-4-methylcoumarin, 7-hydroxy-4-trifluoromethylcoumarin or resorufin metabolites, and measured via fluorescence detection. Assays were conducted in 96-well microtiter plates. Incubations were performed with microsomes prepared from baculovirus-infected insect cells containing cDNA-derived cytochrome P450 enzymes, and utilized an NADPH generating system. The IC_{50} values were calculated utilizing XLfit curve-fitting software.