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The Synthesis and Structure–Activity Relationships of 4-Aryl-3-aminoquinolin-2-ones: A New Class of Calcium-Dependent, Large Conductance, Potassium (Maxi-K) Channel Openers Targeted for Post-Stroke Neuroprotection

Piyasena Hewawasam,[a,*] Wenhong Fan,[a] Jay Knipe,[c] Sandra L. Moon,[b] Christopher G. Boissard,[b] Valentin K. Gribkoff[b] and John E. Starrett, Jr.[a]

[a]*Department of Chemistry, The Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492, USA*

[b]*Department of Neuroscience, The Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492, USA*

[c]*Department of Pharmacokinetics and Metabolism, The Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492, USA*

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Abstract—A series of 4-aryl-3-aminoquinoline-2-one derivatives was synthesized and evaluated as activators of the cloned maxi-K channel *mSlo* (*hSlo*) expressed in *Xenopus laevis oocytes* using electrophysiological methods. A brain penetrable activator of maxi-K channels was identified and shown to be significantly active in the MCAO model of stroke.

Stroke is currently recognized as a major cause of adult disability and death affecting more than 700,000 per year in the United States alone and over 2 million annually worldwide.[1] Strokes are classified into two types: ischemic stroke, which results from blockade of arterial blood flow, and hemorrhagic stroke which is caused by the rupture (aneurysm) of a blood vessel in the brain. Acute ischemic stroke represents the most common form with approximately 80% of all strokes. Thrombolytic agents have been developed and utilized to restore blood flow to ischemic neuronal tissue and tissue plasminogen activator has been proven to be effective in a limited patient population.[2] In the last decade, numerous neuroprotective mechanisms have been examined including antagonists of AMPA/kainate[3–5] and *N*-methyl-D-aspartate (NMDA) excitatory amino acid receptors[6] and inhibitors of neuronal adenosine reuptake.[7,8] However, to date value of these potential neuroprotective agents has not been realized due to either a lack of demonstrated clinical efficacy or because of poor side-effect profiles.[9] Thus, post-stroke neuroprotection represents one of the most prominent unmet medical needs in the clinical arena. During ischemic stroke, neurons at risk are exposed to abnormally high levels of intracellular calcium (Ca^{2+}), initiating a neurotoxic cascade.[10,11] To protect neurons at risk we have relied on a strategy designed to reduce abnormally high levels of Ca^{2+} entry, by the opening of large-conductance, calcium-activated potassium (maxi-K) channels and thereby reducing the excessive release of excitatory amino acids[12] and controlling neuronal hyperexcitability.[13,14]

Large-conductance, Ca^{2+}-activated potassium (maxi-K or BK) channels are present in a variety of excitable cell types including neurons and smooth muscle cells[15,16] and play a key role in regulating cell membrane potential and neuronal excitability.[17] As a consequence, modulators of maxi-K channels have emerged as potentially useful therapeutic agents for various disease states associated with both the central nervous system

*Corresponding author. Fax: +1-203-677-7702; e-mail: hewawasp@bms.com

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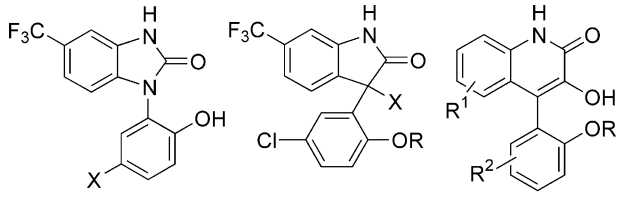
and smooth muscle.[18,19] Maxi-K channel opening stimulated by Ca^{2+} in the presence of a pharmacological opener would act to limit further Ca^{2+} entry, thus interrupting the potentially fatal neurotoxic cascade initiated by abnormally high Ca^{2+} entry, a circumstance encountered during neuronal ischemia.[10,11,14,17] Therefore, a maxi-K opener would not be expected to significantly affect channels in non-ischemic tissue in which intracellular levels of Ca^{2+} are low thereby reducing the potential for undesirable side effects.

Electrophysiological and pharmacological properties of a variety of synthetic and naturally occurring maxi-K channel openers has recently been reviewed.[18–20] Among small molecule maxi-K channel openers, the benzimidazolone derivatives[21–23] NS-004 (**1**) and NS-1619 (**2**) have been studied in some detail, both in vitro and in vivo.[24–27] More recently, a series of 3-aryloxindole derivatives with maxi-K channel opening activity has been disclosed.[14,28,29] The oxindole derivatives, **3a**–**b**[29] and (+)-**4** (MaxiPost)[14] identified from this series have been evaluated in a rat model of stroke that involved permanent occlusion of the middle cerebral artery (MCAO model).

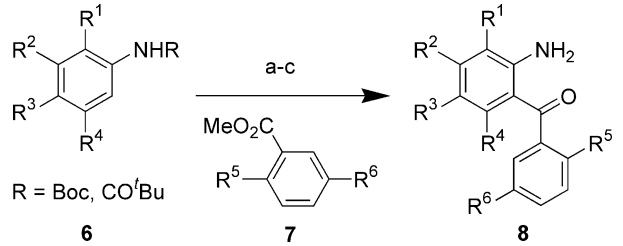


1 (NS-004), X = Cl
2 (NS-1619), X = CF_3

3a, X = R = H
3b, X =OH, R = H
(+)-4, X = F, R = CH_3

5, R = H, CH_3

As part of a broad-based effort directed towards the identification of brain penetrable activators of neuronal maxi-K channels that would be useful as neuroprotective agents, we synthesized a series of 4-aryl-3-aminoquinolin-2-one derivatives. Recently, a series of 4-aryl-3-hydroxyquinolin-2-ones (**5**)[30] has been disclosed as maxi-K channel openers, but these openers lack brain penetrability mainly due to the presence of an ionizable 3-hydroxyl moiety.[31] We reasoned that replacement of the 3-hydroxyl moiety of **5** with non-ionizable amino groups would enhance the brain penetrability. The alkylsulfonamido group has been widely used as a bioisosteric replacement for the hydroxyl group.[32]



R = Boc, CO^tBu

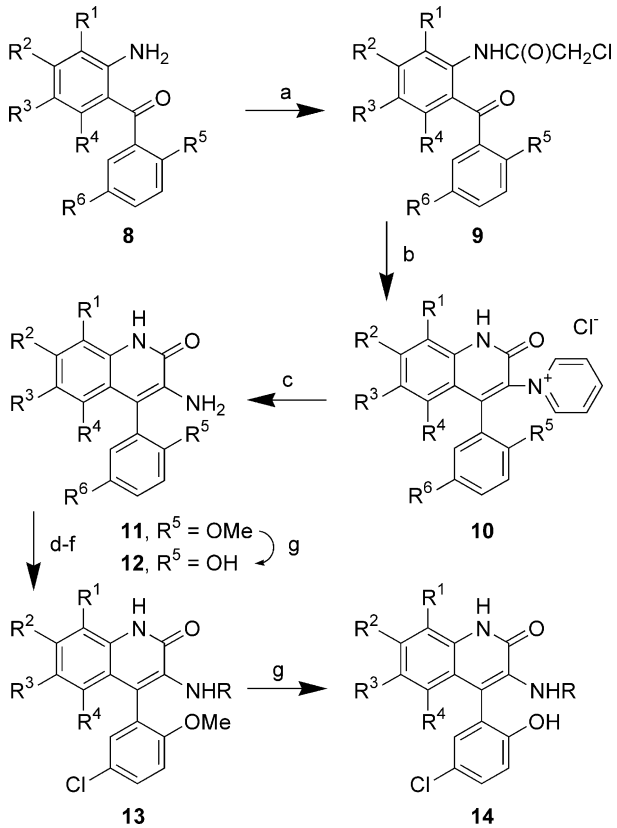
6 **7** **8**

Scheme 1. (a) ^{t}BuLi (2.2 equiv), THF or ether, −78 to −40 °C; (b) add **7** at −40 °C then warm to 0 °C; 45–82%; (c) 3–6 N HCl, EtOH, reflux; 93–97%.

As illustrated in Scheme 2, preparation of 4-aryl-3-aminoquinolin-2-ones required the 2-amino-benzophenones (**8**) as precursors.

The synthesis of 2-aminobenzophenones by a variety methods has been reviewed.[33] The majority of 2-aminobenzophenones (**8**) were prepared by the reaction of aryl esters (**7**) with *ortho*-lithiated protected aniline derivatives (**6**) via the formation of dianion species with *tert*-BuLi (2.2 equiv) followed by deprotection of either Boc (3 N HCl) or CO^tBu (6 N HCl) groups (Scheme 1). The pattern of substitution of the 2-aminobenzophenones prepared by this method was dependent upon the regiospecificity of the directed metalation.

As shown in Scheme 2, chloroacetylation of 2-aminobenzophenones (**8**) with chloroacetyl chloride in the presence of pyridine gave the corresponding *N*-(chloroacetyl)-2-aminobenzophenone derivatives (**9**). Upon heating a solution of **9** in anhydrous pyridine at reflux for 15–30 min, the initially formed α-pyridinium salt undergoes cyclodehydration to afford the *N*-[(4-arylquinolin-2-one)-3-yl]pyridinium chloride (**10**). Hydrazinolysis of **10** with hydrazine hydrate in ethanol at reflux for 1–2 h provided the desired 4-aryl-3-aminoquinolin-2-ones **11**. Finally, demethylation of the methyl ether moiety of **11** with BBr_3 afforded the desired phenols **12**. In order to mimic the 3-hydroxyl moiety present in **5**,



11, R^5 = OMe
12, R^5 = OH

8 **9** **10** **13** **14**

Scheme 2. (a) $ClCH_2COCl$, pyridine, CH_2Cl_2; 82–91%; (b) pyridine, reflux; 88–98%; (c) $NH_2NH_2{\cdot}H_2O$, EtOH, reflux; 76–89%; (d) R = SO_2CF_3; (1) $(CF_3SO_2)_2O$, pyridine, 23 °C; (2) NaOH, THF–MeOH; 70%; (e) R = SO_2Me; (1) $MeSO_2Cl$, pyridine, 50–60 °C; (2) NaOH, THF–MeOH; 62%; (f) R = Ac; (1) Ac_2O, pyridine, 60–70 °C; (2) NaOH, THF–MeOH; 82%; (g) BBr_3, CH_2Cl_2, 0–23 °C; 90–95%.

the 3-amino group was derivatized as both trifluoromethyl and methyl sulfonamides. Selective monosulfonylation of the amino group of **11** was not possible even with stoichiometric amounts of the corresponding sulfonylating agent. Alternatively, persulfonylation of **11** followed by selective deprotection with aqueous NaOH in THF–MeOH led to the formation of desired monosulfonylated 3-aminoquinolin-2-one derivatives **13**. The acetamide derivative was also prepared by a similar protocol using excess Ac_2O in refluxing pyridine. Finally, demethylation of the methyl ether moiety of **13** with BBr_3 afforded the desired phenols **14**. The 3-aminoquinolin-2-one derivatives prepared by these methods are compiled in Table 1 along with relevant physicochemical data.

The ability of the target compounds to open maxi-K channels and increase maxi-K-mediated whole-cell outward K^+-currents was assessed by using two-electrode voltage clamp recording from *Xenopus laevis* oocytes expressing cloned *mSlo*[34] (or *hSlo*[35]) maxi-K channels, as described previously.[36] All compounds were tested in at least five different oocytes to evaluate the effect of a single drug concentration on channel current sensitive to iberiotoxin (IbTx). The average percentage change in *mSlo* (or *hSlo*) current relative to drug-free control (100%) was determined for each compound tested. The results obtained are listed in Table 1 along with data for NS-004, which allows the efficacy comparison of the 4-aryl-3-aminoquinolin-2-one derivatives to a prototypical maxi-K channel opener.

The structure–activity relationships presented in Table 1 provide a rudimentary understanding of the maxi-K channel opening pharmacophore of 4-aryl-3-aminoquinolin-2-ones. For this preliminary study, the optimal substitution pattern of the quinoline nucleus was probed while restricting the 4-aryl moiety to the *p*-chlorophenol, an element present in prototype maxi-K openers **1** and **3**–**5**. Compound **12a**, which is unsubstituted on the quinoline nucleus, was only poorly active. From the results presented in Table 1, it appears that introduction of an electron withdrawing substituent such as CF_3 (except at C-8) markedly enhances channel opening efficacy. Both 5-CF_3 (**12e**) and 6-CF_3 (**12d**) isomers were superior to the 7-CF_3 (**12c**) isomer. The 6-chloro analogue, **12f** was less efficacious when compared to the 6-CF_3 analogue, **12d**. Removal of either the chlorine atom or both chloro and hydroxyl groups from the 4-aryl moiety of **12d** results in a substantial loss of activity. Thus, the presence of both a *p*-chlorophenol element and an electron-withdrawing group is critical for maintaining the maxi-K channel opening activity. Derivatization of the 3-amino group of **12d** and **12c** as the (trifluoromethyl)sulfonamides, **14a**–**b** resulted in dramatic increase in maxi-K channel opening activity. However, both the methylsulfonamide (**14c**) and the acetamide (**14d**) derivatives were shown to be relatively less active compared to the parent 3-aminoquinolone, **12d**. This result when taken together with the effect of CF_3 moiety indicates an important relationship between increasing acidity of the 3-amino moiety and enhanced channel opening activity.

As a prelude to evaluating compounds in animal models of stroke, the ability of the selected maxi-K openers, **12c**, **12d**, and **14a**, to enter rat brain following intravenous administration was determined. Table 2 shows the plasma and whole brain concentrations, as well as the brain/plasma (B/P) ratios of each compound at 15 min and 2 h after administration of an IV bolus dose (5 mg/kg). The whole brain concentrations of **12c** and **12d**

Table 2. Whole brain and plasma concentrations of **12c**, **12d**, and **14a** after iv administration (5 mg/kg) to male rats

Compd	Plasma level[a]		Brain level[b]		B/P ratio	
	0.25 h	2 h	0.25 h	2 h	0.25 h	2 h
12c	1166±145	339±14	1467±88	1480±75	1.3	4.4
12d	802±92	281±10	1309±52	1493±110	1.6	5.3
14a	1230±125	125±10	<50[c]	<50[c]	ND	ND

[a]Concentration as ng/mL; mean of three animals±standard deviation.
[b]Concentration as ng/g wet weight; mean of three animals±standard deviation.
[c]Values below the detection limit of the assay (50 ng/g).

Table 1. Structure and physical properties of 3-amino-4-arylquinolin-2-one derivatives, effect on maxi-K-mediated outward current in *Xenopus laevis* oocytes expressing the cloned maxi-K channels *mSlo*

Compd	R^1	R^2	R^3	R^4	R^5	R^6	R	Mp (°C)[a]	% Increase in *mSlo* current @ 20 μM
11a	H	H	H	H	OMe	Cl	H	248–250	108±3
11b	H	H	CF_3	H	H	H	H	200–202	111±2
12a	H	H	H	H	OH	Cl	H	265–268	114±5
12b	CF_3	H	H	H	OH	Cl	H	209–212	112±5
12c	H	CF_3	H	H	OH	Cl	H	225–227	159±11
12d	H	H	CF_3	H	OH	Cl	H	233–235	186±12
12e	H	H	H	CF_3	OH	Cl	H	267–268	191±8
12f	H	H	Cl	H	OH	Cl	H	184–185	157±12
12g	H	H	CF_3	H	OH	H	H	244–245	137±10
14a	H	H	CF_3	H	OH	Cl	SO_2CF_3	267–270	343±23
14b	H	CF_3	H	H	OH	Cl	SO_2CF_3	267–270	213±13
14c	H	H	CF_3	H	OH	Cl	SO_2Me	263–265	164±12
14d	H	H	CF_3	H	OH	Cl	$COCH_3$	222–224	120±6
1 (NS-004)									132±13[b]

[a]All new compounds exhibited spectroscopic and combustion data in accord with the designated structure.
[b]Reference compound **1** (NS-004) shown to have identical effects on *mSlo* and *hSlo*-mediated maxi-K currents.[36]

Table 3. Mean whole brain and plasma pharmacokinetic parameters of **12d** after iv administration (4.7 mg/kg) to male rats ($N=3$ at each time point)

Parameter	Plasma	Brain	B/P ratio[b]
C_{max} (ng/g)	—	1990 ± 324	—
T_{max} (h)	—	0.08	—
AUC[a]	2704	19,306	7.1
$T_{1/2}$ (h)	7.6	8.1	—

[a]Expressed as ng-h/mL for plasma and ng-h/g for brain calculated by the trapezoidal rule over 0–24 h after dosing.
[b]Based on ratio of AUC values.

were comparable at both time points and greatly exceeded those of **14a**. The increase in absolute brain levels and B/P ratio over 0.25–2 h suggested the accumulation and possibly prolonged elimination of **12c** and **12d** from brain tissue.

In order to fully explore the relationship between brain and plasma levels of **12d** over time, a larger group of rats was intravenously dosed (iv bolus; 4.7 mg/kg) and whole brain and plasma concentrations were determined through 24 h after dosing. The results from this expanded study (Table 3) verified that **12d** rapidly entered rat brain and attained concentrations, which exceeded those of plasma over a prolonged interval (total brain/plasma AUC ratio of 7.1, evaluated over 24 h). Furthermore **12d** was eliminated slightly more slowly from rat brain than from plasma (elimination half-lives of 8.1 and 7.6 h, respectively).

To determine the ability of **12d** to reduce cell loss resulting from neuronal ischemia, a standard rodent model of permanent focal ischemia, involving occlusion of the middle cerebral artery in the spontaneously hypertensive rat (MCAO model) was employed.[37] This procedure results in a reliably large neocortical infarct volume that is measured by means of vital dye exclusion in serial slices through the brain 24 h after middle cerebral artery occlusion (MCAO). In this model, effects of dose response and time course exposure of **12d** on neocortical infarct volume were examined. In the dose response study, highest reduction (9%) in neocortical infarct volume was observed with 0.001 mg/kg dose when administered iv 2-h post-MCAO as compared to vehicle-treated (2% DMSO, 98% propylene glycol) control. In the time course exposure study with 0.001 mg/kg dose a significant reduction (14%, $p < 0.05$) in neocortical infarct volume was observed when administered as a single IV bolus 30 min after MCAO as compared to vehicle-treated control.

In summary, we have identified a novel class of brain penetrable maxi-K channel openers and demonstrated that channel opening activity is sensitive to both the nature and pattern of substitution of both aromatic elements. The preliminary structure–activity data for this series indicates the importance of both an electron-withdrawing substituent on the quinolone nucleus and the presence of a phenolic hydroxyl for effective expression of maxi-K channel opening properties. Based on in vitro and in vivo profiling it has been demonstrated that 4-aryl-3-aminoquinolones are brain penetrable maxi-K channel openers with significant neuroprotective properties in a rat MCAO model of stroke.

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