Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 1023–1026

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

The Synthesis and Characterization of BMS-204352 (MaxiPost™) and Related 3-Fluorooxindoles as Openers of Maxi-K Potassium Channels

Piyasena Hewawasam,[a] Valentin K. Gribkoff,[b] Yadagiri Pendri,[c] Steven I. Dworetzky,[b] Nicholas A. Meanwell,[a] Eduardo Martinez,[c] Christopher G. Boissard,[b] Debra J. Post-Munson,[b] Joanne T. Trojnacki,[b] Krishnaswamy Yeleswaram,[d] Lorraine M. Pajor,[d] Jay Knipe,[d] Qi Gao,[e] Robert Perrone[f] and John E. Starrett, Jr.[a,*]

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

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

[c]*Department of Process Research & Development, Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492, USA*

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

[e]*Department of Analytical Research and Development, Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492, USA*

[f]*Department of Pharmaceutics, Bristol-Myers Squibb Pharmaceutical Research Institute, 1 Squibb Drive, New Bruswick, NJ 08903, USA*

Received 8 November 2001; accepted 1 February 2002

Abstract—3-Aryl-3-fluorooxindoles can be efficiently synthesized in two steps by the addition of an aryl Grignard to an isatin, followed by treatment with DAST. Oxindole **1** (BMS-204352; MaxiPost™) can be isolated using chiral HPLC or prepared by employing chiral resolution. Cloned maxi-K channels are opened by **1**, which demonstrates a brain/plasma ratio >9 in rats.

Annually, there are more than 700,000 new cases of stroke in the US, representing a major unmet medical need.[1] Numerous neuroprotective therapies (approaches that protect neurons 'at risk' following a stroke) have been, or are currently undergoing clinical testing, but thus far none has been approved due to lack of demonstrated efficacy or because of poor side effect profiles.

We have approached the problem of neuroprotective therapy by developing potent and specific openers of large-conductance, calcium-activated (maxi-K) potassium channels. Potassium channels are a structurally diverse family of transmembrane proteins that serve a variety of functions, including controlling cell excitability and setting the resting membrane potential in excitable cells.[2] Calcium-activated potassium (K_{Ca}) channels are a family of these channels that share a dependence on intracellular calcium ion concentration for activity but may also be regulated by membrane potential and phosphorylation state.[3] On the basis of their single-channel conductance in symmetrical K^+ solutions, K_{Ca} channels have been broadly classified into three subfamilies: the large conductance (BK or maxi-K) channels, which exhibit a single channel conductance of >100 picosiemens (pS), intermediate conductance channels (50–100 pS) and small conductance channels (<50 pS).[4] Maxi-K channels are present in many excitable cell types including neurons and various

*Corresponding author. Tel.: +1-203-677-6306; fax: +1-203-677-7702; e-mail: john.starrett@bms.com

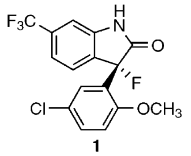
0960-894X/02/$ - see front matter
PII: S0960-894X(02)00101-4

types of smooth muscle cells. Because maxi-K channels are thought to be important regulators of cellular excitability and function, modulators of these channels have emerged as potentially useful agents in the therapy of various disease states associated with both the central nervous system and smooth muscle.[5]

In brain, pharmacological opening of maxi-K channels protects neurons by enhancing an endogenous neuroprotective mechanism.[6] Openers of these channels appear to synergize with calcium, making the modulation of channel function largely dependent on the presence of high levels of intracellular calcium, such as is encountered during neuronal ischemia. Maxi-K channel opening stimulated by Ca^{2+} and the pharmacological opener would function to limit further Ca^{2+} entry by hyperpolarizing neurons, thereby reducing voltage-dependent calcium entry points via some Ca^{2+} channels and possibly NMDA receptors. The cascade of events leading to ischemic neuronal death is initiated by abnormally high Ca^{2+} entry and therefore limiting intracellular access to Ca^{2+} via opening of maxi-K channels should interfere with this neurotoxic cascade. The opener would not be expected to significantly affect maxi-K channels in non-ischemic tissue, where the intracellular Ca^{2+} levels are low, reducing the potential for undesirable side effects.

We have identified the novel fluorooxindole **1** (BMS-204352; MaxiPost™) as a potent opener of maxi-K channels (Fig. 1). The electrophysiological characterization of **1** and activity in models of stroke have recently been reported.[7] It is of interest to note that in addition to maxi-K activation, **1** has also been reported to activate KCNQ4 potassium channels.[8] However, in the current study, cloned maxi-K channels were expressed in cells with minimal native background potassium current, and therefore it is reasonable to assume that the observed effects are due to modulation of maxi-K channels. Herein, we describe the synthesis and maxi-K channel activity of analogues related to **1**, as well as the physical characterization, pharmacokinetic profile and stereospecific synthesis of **1**.

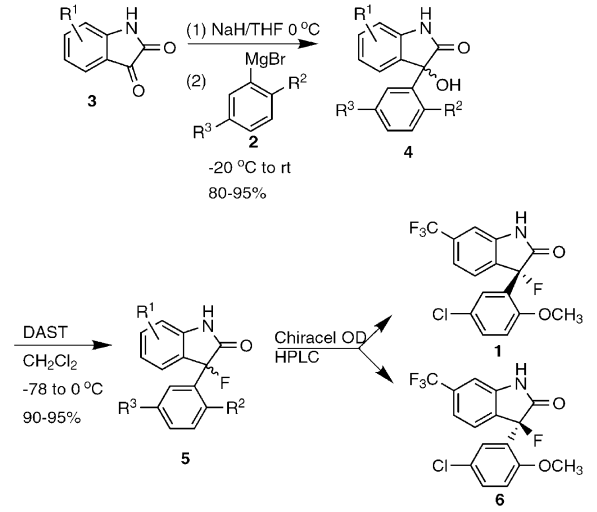
The fluorooxindoles were prepared in a straightforward manner as depicted in Scheme 1.[9] Addition of an aryl Grignard reagent **2** to the sodium salt of isatin **3** in THF gave the corresponding 3-aryl-3-hydroxyindol-2-ones **4** in 80–95% yield. Fluorination of the tertiary hydroxyl moiety of **4** with DAST in dichloromethane provided the desired 3-aryl-3-fluorooxyindol-2-ones **5** in 90–95% yield. The presence of fluorine was confirmed by mass spectroscopy and by fluorine NMR as depicted in Table 1. The racemate **5c** was resolved by preparative HPLC using a Chiracel-OD column, eluting with 9:1 hexanes/isopropanol to afford (+)-**1** and (−)-**6**.



1

Figure 1. Structure of **1** (BMS-204352; MaxiPost™).

For electrophysiological evaluation of maxi-K opening properties, compounds were tested using two-electrode voltage clamp recording from *Xenopus laevis* oocytes injected with *hSlo1* mRNA, as previously described.[10] Voltage-clamp protocols ranged from a holding potential of −60 mV to a maximal potential of +140 mV with +20 mV increments. The maxi-K current, defined as the iberiotoxin-sensitive component of total outward current, was measured in the absence or in the presence of 20 μM drug. Compounds were prepared as 10 mM stock solutions in dimethyl sulfoxide (DMSO) and diluted in recording medium to the desired study concentration. When insolubility precluded evaluation at 20 μM, current was measured at 10 μM (**5c**). The increase in outward current in the presence of drug is reported as percent of drug free control, over a voltage step to +140 mV, and the data are an average of experiments conducted in at least five different oocytes.



Scheme 1. Synthesis of 3-fluorooxindoles.

Table 1. Effects of 3-fluorooxindoles on maxi-K current in oocytes

Compd[a]	R^1	R^2	R^3	Maxi-K current[b]	^{19}F NMR (ppm)
(*S*)-(+)-**1**	6-CF_3	Cl	OCH_3	I	−159.87
5a	H	H	H	100	−154.05
5b	6-CF_3	H	H	107	−155.44
5c	6-CF_3	Cl	OCH_3	170[c]	−159.87
5d	5-CF_3	Cl	OCH_3	106	−158.67
5e	6-CN	Cl	OCH_3	106	−160.18
5f	6-I	Cl	OCH_3	95	−158.26
5g	5-F	Cl	OCH_3	110	−158.81
5h	5-Br	Cl	OCH_3	104	−158.55
5i	5-Me	Cl	OCH_3	112	−157.38
(*R*)-(−)-**6**	6-CF_3	Cl	OCH_3	I	−159.87

[a]All new compounds exhibited spectroscopic and elemental analyses consistent with the assigned structures (I = insoluble at 1 μM).
[b]% of control of outward potassium current in oocytes injected with *hSlo*. Unless otherwise noted, compounds were evaluated at 20 μM.
[c]Due to insolubility at 20 μM, analysis was conducted at 10 μM.

The maxi-K activity in Table 1 is shown as a percent of control opening; 100% denotes a compound which had no effect on the channel. Previous studies from our laboratories of maxi-K activity of related 3-aryloxindoles have revealed optimal activity with a 2-oxo-5-chloro substitution pattern.[11,12] This information was utilized in the selection of synthetic targets in the current study. The structure–activity relationship in this series of oxindoles is exquisitely sensitive to the position and properties of the substituents. Significant channel opening was observed with **5c**, which contains a 6-substitued trifluoromethyl oxindole and a 5-chloro-2-anisole moiety at C-3. The unsubstituted phenyl analogues **5a** and **5b** had no significant effect. Moving the trifluoromethyl group to the 5-position (**5d**) resulted in a loss of activity, as does replacement with other electron-withdrawing functionalities (**5e–h**). Interestingly, the 5-methyl derivative **5i**, demonstrated modest opening activity. The enantiomers of **5c**, compounds **1** and **6**, could not be evaluated in the oocyte preparation due to a lack of solubility in the assay medium.

In order to further characterize the effects of enantiomers **1** and **6** on outward K+ currents, they were evaluated using whole-cell patch clamp recordings in HEK cells stably-transfected with *hSlo1*. Utilizing a cell holding potential of −60 mV, a series of three voltage steps (to +20, +40 and +60 mV) was applied to the cell. The final 25 data points of the aggregate current elicited by the +60 mV step were averaged to determine the current amplitude. In this preparation, both enantiomers were very effective in increasing maxi-K current (Table 2). Although not significantly different, **1** consistently produced a more robust increase in outward current than either racemate **5c** or the opposite enantiomer **6**.

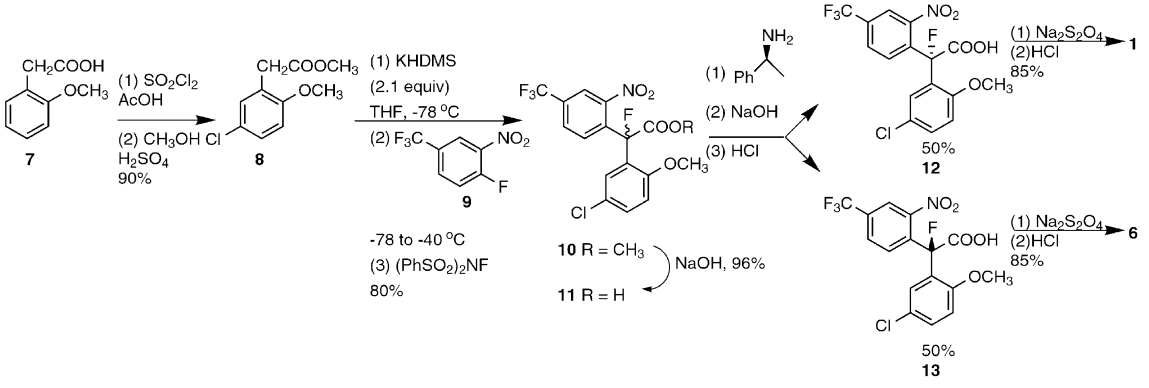
Table 2. Effects of **1** and isomers on whole cell maxi-K current in HEK 293 cells

Compd[a]	10 nM	100 nM	1000 nM
(±)-**5c**	Not tested	131.9	169.6
(*S*)-(+)-**1**	127.8	185.3	247.4
(*R*)-(−)-**6**	105.0	164.4	205.3

[a]The current amplitude in the presence of compound is presented as the percent of pre-drug control current elicited at +60 mV. A minimum of four cells was recorded at each drug concentration.

To circumvent the necessity for the use of a chiral HPLC column to prepare **1**, a chiral resolution was developed as outlined in Scheme 2.[13] Starting with commercially available 2-methoxyphenyl acetic acid (**7**), chlorination with sulfuryl chloride followed by Fischer esterification of the acid afforded ester **8**. To introduce the trifluoromethyl phenyl group, ester **8** was deprotonated with two equivalents of potassium hexamethyldisilazide and then treated with commercially available 4-(trifluoromethyl)-2-nitroflurobenzene (**9**). Ipso displacement of the fluorine, followed by quenching of the benzylic anion with *N*-fluorobenzenesulfonimide (Accufluor®), an electrophilic source of fluorine, provided racemic ester **10**. Saponification of ester **10** gave racemic carboxylic acid **11**, which was resolved into its enantiomers **12** and **13** through the formation of a pair of diastereomeric salts with (*S*)-(−)-α-methyl benzyl amine in >99% ee. Reduction of the nitro group of **12** with sodium dithionite, followed by cyclization with HCl cleanly afforded **1** in 85% yield with an enantiomeric excess of >99% (as determined by chiral HPLC). In a similar manner, reduction and cyclization of **13** afforded **6**.

Recrystallization of **1** from hexane/CH_2Cl_2 afforded large plate-like crystals (mp 198–200 °C) suitable for single-crystal X-ray analysis.[14] Using the anomalous scattering from chlorine atoms, the absolute configuration at the asymmetrical carbon atom was established as *S*. The trifluoromethyl group was disordered. The molecule adopted a conformation with a dihedral angle of 99.03(9)° between the two aromatic rings. In the crystal, molecules form infinitely long chains along the crystallographic *b*-axis through N–H...O=C hydrogen bonds, and a three dimensional network of interactions involving fluorine atoms (F...CH) was observed. A similar analysis of **6** confirmed the *R*-configuration for this enantiomer. Solid-state stability studies indicated excellent stability for **1**; it retained full potency after storage in sealed vials for 4 weeks at 70 °C, 50 °C/75% relative humidity (RH), or 23 °C/1000 footcandles light. The samples stored at 70 °C or 50 °C/75% RH also showed no apparent change in crystal form based on X-ray powder diffraction analyses. To examine the potential hygroscopicity of **1**, samples were stored for 4 weeks



Scheme 2. Stereoselective synthesis of **1** and **6**.

Table 3. Pharmacokinetic parameters in plasma and brain following intravenous administration of **1** to male Sprague–Dawley rats

Parameter	Brain[a]	Plasma
Dose (mg/kg)	5.0	1.0
Plasma half-life (h)	1.9	1.8
Brain half-life (h)	1.6	Nd
Plasma AUC (μg h/mL)	3.0	0.32
Brain AUC (μg h/mL)	28.8	Nd
Brain AUC/plasma AUC (ratio)	9.6	Nd
Clearance (mL/min kg)	Nd	51.5
Volume of distribution (L/kg)	Nd	5.8

[a]Nd, not determined.

in open glass vials at 25 °C/87% RH and 50 °C/75% RH. Karl Fisher analyses indicated no significant increase in water content.

To characterize the pharmacokinetic disposition and brain uptake of **1**, two studies were conducted. To determine brain uptake, groups of rats were administered an intravenous bolus dose of **1** at 5 mg/kg and plasma and whole brain samples were collected from 2–3 rats at each of 10 time points through 8 h after dosing. Concentrations of **1** in plasma and whole blood brain homogenate samples were determined by LC/MS analysis following acetonitrile extraction. The area under the curves of plasma or brain concentration versus time was used to assess brain uptake. To determine pharmacokinetics, three rats were administered an intravenous dose of **1** at 1 mg/kg and serial blood samples were withdrawn from an indwelling jugular vein cannulae through 8 h after dosing. The results from both studies are summarized in Table 3.

The disposition of **1** in rats is characterized by a short half-life, high clearance and a moderate-to-high volume of distribution. Elimination half-lives were similar in both plasma and brain, suggesting rapid distribution between brain and the systemic circulation. The observed brain/plasma AUC ratio (>9) indicates excellent brain uptake of this compound.

In conclusion, we have developed generalized methodology for the synthesis of 3-fluorooxindoles and have characterized oxindole **1** as a brain penetrant maxi-K opener with good solid state stability. A stereospecific synthesis of **1** was developed employing chiral resolution.

References and Notes

1. Williams, G. R.; Jiang, J. G.; Matchar, D. B.; Samsa, G. P. *Stroke* **1999**, *30*, 2523.
2. Hille, B. In *Ionic Channels of Excitable Membranes*, 2nd ed.; Sinaur; Sunderland, 1992.
3. Hinrichsen, R. D. In *Calcium-Dependent Potassium Channels*; R. G. Landes: Austin, TX, 1993.
4. Latorre, R.; Oberhauser, A.; Labarca, P.; Alvarez, O. *Annu. Rev. Physiol*. **1989**, *51*, 385.
5. Starrett, J. E., Jr.; Dworetzky, S. I.; Gribkoff, V. K. *Curr. Pharm. Des*. **1996**, *2*, 413.
6. Gribkoff, V. K.; Starrett, J. E., Jr.; Dworetzky, S. I. *Neuroscientist* **2001**, *7*, 166.
7. Gribkoff, V. K.; Starrett, J. E., Jr.; Dworetzky, S. I.; Hewawasam, P.; Boissard, C. G.; Cook, D. A.; Frantz, S. W.; Heman, K.; Hibbard, J. R.; Huston, K.; Johnson, G.; Krishnan, B. S.; Kinney, G. G.; Lombardo, L. A.; Meanwell, N. A.; Molinoff, P.; Myers, R. A.; Moon, S. L.; Ortiz, A.; Pajor, L.; Pieschl, R. L.; Post-Munson, D. J.; Signor, L. J.; Srinivas, N.; Taber, M. T.; Thalody, G.; Trojnacki, J. T.; Wiener, H.; Yeleswaram, K.; Yeola, S. W. *Nature Med*. **2001**, *7*, 471.
8. Rikke, L. A.; Jespersen, T.; Christpheresen, P.; Strøbæk, D.; Jensen, B. S.; Olesen, S.-P. *Neuropharmacology* **2001**, *40*, 888.
9. Hewawasam, P.; Meanwell, N. A.; Gribkoff, V. K. US. Patent 5,602,169, 1997; *Chem. Abstr*. **1997**, *126*, 181369.
10. Gribkoff, V. K.; Lum-Ragan, J. T.; Boissard, C. G.; Post-Munson, D. J.; Meanwell, N. A.; Starrett, J. E., Jr.; Kozlowski, E. S.; Romine, J. L.; Trojnacki, J. T.; McKay, M. C.; Zhong, J.; Dworetzky, S. I. *Mol. Pharm*. **1996**, *50*, 206.
11. Hewawasam, P.; Meanwell, N. A.; Gribkoff, V. K.; Dworetkzy, S. I.; Boissard, C. G. *Bioorg. Med. Chem. Lett*. **1997**, *7*, 1255.
12. Hewawasam, P.; Erway, M.; Moon, S. L.; Knipe, J.; Weiner, H.; Boissard, C. G.; Plst-Munson, D. J.; Gao, Q.; Huang, S.; Gribkoff, V. K.; Meanwell, N. A. *J. Med. Chem*. In press.
13. Pendri, Y. R.; Martinez, E. J.; Thottathil, J. K.; Hewasasam, P. US Patent 5,892,106, 1998: *Chem. Abstr*. **1998**, *129*, 230636.
14. X-ray crystallographic data has been deposited at the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK; Deposit number CCDC 169402.