Pergamon

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Bioorganic & Medicinal Chemistry Letters 13 (2003) 1695–1698

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

# Synthesis of Water-Soluble Prodrugs of BMS-191011: A Maxi-K Channel Opener Targeted for Post-stroke Neuroprotection

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Received 6 November 2002; accepted 6 March 2003

**Abstract**—A variety of water-soluble prodrugs of BMS-191011 was synthesized and evaluated for solution state stability and rate of conversion to BMS-191011 in rat and human plasma. The deoxycarnitine ester prodrug (**11c**) was selected for clinical evaluation based on its superior chemical stability, crystallinity and cleavage to BMS-191011 in human plasma.


The incidence of stroke remains a major health concern for an increasingly aging worldwide population.[1] Strokes are classified into two types: hemorrhagic stroke resulting from blood vessel rupture, and ischemic stroke, which results from vessel occlusion accounts for nearly 80% of all strokes. Proposed new post-stroke therapies have focused on removal of the occluding thrombus in ischemic stroke and neuroprotective agents that protect neurons at risk. Thrombolytic agent, tissue plasminogen activator (tPA) which restore blood flow to ischemic neuronal tissue, is the only US FDA approved therapy for acute stroke.[2] Despite numerous attempts to develop neuroprotective agents thus far none have been approved due to lack of demonstrated efficacy or because of poor side-effect profiles.[3] We have approached the problem of post-stroke neuroprotection by developing potent and specific openers of large-conductance calcium-activated (maxi-K) potassium channels. This concept has been validated preclinically in animal models of stroke with BMS-204352 (Maxi-Post™)[4] and BMS-191011.[5]

The maxi-K channel opener, BMS-191011 (**1**) has been identified as a potent neuroprotectant in two distinct animal models of stroke including permanent MCAO in the SHR rat and a normotensive model of focal stroke.[5] However, clinical evaluation of BMS-191011 as an intravenous neuroprotectant agent for the treatment of acute ischemic stroke was hampered by its extremely low aqueous solubility ($<1\,\mu g/mL$ of water).

Formation of water-soluble prodrugs has long been recognized as an effective means of increasing the aqueous solubility of drugs containing a hydroxyl group. In general, sodium salts of phosphate ester prodrugs are freely soluble in water and readily hydrolyzed by alkaline phosphatases in vivo.[6] Despite their good aqueous solubility and solution stability, sulfate esters are highly resistant to enzymatic hydrolysis in vivo.[7,8] Dialkylamino acid esters are readily cleaved by plasma esterases but exhibit poor stability in aqueous solution.[9,10] For clinical evaluation the desired prodrug of BMS-191011 was required to have sufficient aqueous solubility (at least 1 mg/mL), long duration of solution stability (up to 24 h), and the ability to generate BMS-191011 upon in-vivo administration.

With the aim of improving aqueous solubility of BMS-191011 (**1**), a series of potential prodrugs were synthesized as shown in Scheme 1. Our approach was to attach a water-solubilizing group directly or via a suitable

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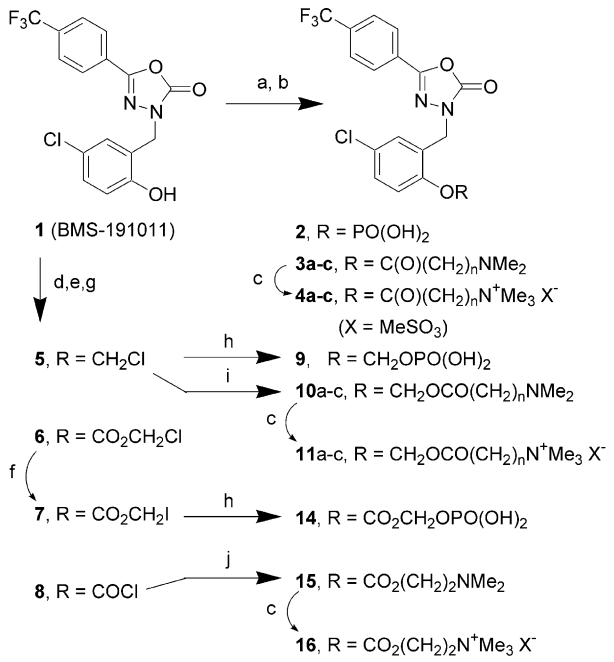
doi:10.1016/S0960-894X(03)00296-8




**Scheme 1.** (a) R = $PO(OH)_2$; (1) NaH, $((BnO)_2P(O))_2O$, THF; (2) $PtO_2$, EtOAc, $H_2$ (40 psi); (b) R = $C(O)(CH_2)_nNR_2$; NaH, $ClC(O)(CH_2)_nNMe_2{\cdot}HCl$, ether; (c) $MeSO_3Me$, EtOAc, 23 °C; (d) R = $CH_2Cl$; (1) NaH, dry HMPA, $ClCH_2SMe$; (2) $SO_2Cl_2$, DCM; (e) R = $CO_2CH_2Cl$; (1) $ClCO_2CH_2Cl$, pyridine, DCM; (f) R = $CO_2CH_2I$; NaI, acetone, reflux; (g) R = COCl; $COCl_2$, cat. $BnPh_3PCl$, $PhCH_3$, 110–120 °C, sealed tube; (h) (1) $AgOP(O)(OBn)_2$, PhH, reflux; (2) $PtO_2$, EtOAc, $H_2$ (40 psi); (i) $HO_2C(CH_2)_nNMe_2{\cdot}HCl$, $Cs_2CO_3$, acetone, 23 °C; (j) $HO(CH_2)_nNMe_2$, DCM, 0–23 °C.

linker to the hydroxyl group of **1**. Reaction of sodium salt of **1** with tetrabenzyl pyrophosphate in THF gave the corresponding dibenzylphosphate ester, which was subjected to catalytic hydrogenation to provide the phosphate ester **2**. Acylation of the hydroxyl group of **1** with $ClCO(CH_2)_nNMe_2{\cdot}HCl$[11a,b] provided the desired dimethylamino esters **3a–c**. Methylation of **3a–c** with methyl methanesulfonate in EtOAc afforded the trimethylammonium salts **4a–c**. The chloromethyl ether **5** was prepared by chlorinating[12] the corresponding methylthiomethyl ether as indicated in Scheme 1. Reaction of **5** with silver dibenzyl phosphate in refluxing benzene gave the corresponding dibenzyl phosphate ester which was debenzylated by catalytic hydrogenation using $PtO_2$ to afford the phosphate ester **9**. Acyloxylation of **5** with cesium salt of the dimethylamino acids in acetone gave the esters **10a–c**, which upon methylation afforded the quaternary ammonium salts **11a–c**. The chloromethyl carbonate **6**, prepared by acylation of **1** with chloromethyl chloroformate was converted to iodomethyl carbonate **7** under Finkelstein conditions. The iodomethyl carbonate **7** was converted to the phosphate ester **14** under identical conditions used for the preparation of **9**. Chloroformylation of **1** with excess phosgene in the presence of catalytic amount of $BnPh_3PCl$ at 110–120 °C in a sealed tube gave the chloroformate **8**. Condensation of **8** with $HO(CH_2)_2NMe_2$ afforded the corresponding dimethylaminoethyl carbonate **15**, which upon methylation provided the trimethylammonium salt **16**. The prodrugs prepared by these methods were evaluated for their aqueous solubility, solution state stability and enzymatic cleavage in rat and human plasma.

While phosphate esters **9** and **14** displayed insufficient solution state stability, the completely stable phosphate prodrug **2** did not convert to the parent in rat or human plasma. The dialkylamino ester derivatives **3a–c**, **10a–c**, and **15** showed very poor solution state stability. In order to overcome the instability of dialkylamino ester prodrugs quaternization of the dialkylamino moiety was attempted. Unlike dialkylamino ester prodrugs, the corresponding quaternary ammonium salts were found to be crystallizable solids with excellent aqueous solubility and exceptional solution state stability. In addition, majority of the quaternary ammonium salts was immediately cleaved to BMS-191011 in rat plasma (Table 1). Based on its superior chemical stability, crystallinity and formation of BMS-191011 in rat and human plasma, the prodrug **11c** ($n = 3$) was selected for further evaluation. As a prelude to evaluating compound **11c** in animal models of stroke, more detailed in vitro conversion studies (rat and human whole blood), as well as a rat pharmacokinetic study, were performed.

The pharmacokinetics of **11c** and its conversion to BMS-191011 were studied following intravenous infusion administration of **11c** (1.7 mg/kg, equivalent to 1 mg/kg of BMS-191011) to male SD rats ($N = 3$). To prevent ex-vivo hydrolysis of **11c**, blood samples immediately after collection were added to an equal volume of water and quenched with four volumes of acetonitrile and stored at −20 °C until analysis. Concentrations of **11c** and BMS-191011 in the quenched blood samples were determined by two separate LC/MS/MS assays. The results are shown in Figure 1 and the pharmacokinetic parameters are summarized in Table 2.

To determine the in vitro rate of conversion of **11c** to BMS-191011, incubations at 37 °C were conducted in

**Table 1.** Chemical stability, aqueous solubility and rat plasma cleavage data of selected prodrugs of BMS-191011

| Compd | % conversion to BMS-191011[a] | Solubility (@ pH) in water mg/mL | % of prodrug remains @ 24 h in PEG400/water, 1:1 |
|---|---|---|---|
| **2** | 0 | (5.5) 4.9<br>(7.2) 14.6 | ND |
| **9** | NT | (7.0) 6.1<br>(8.4) > 16 | Not stable |
| **14** | NT | (2.4) 0.055<br>(7.5) 0.004 | Not stable |
| **4a** ($n = 1$) | 100 | > 4 mg[b] | > 90 |
| **4b** ($n = 2$) | 100 | > 4 mg[b] | > 90 |
| **4c** ($n = 3$) | 95 | > 4 mg[b] | > 90 |
| **11a** ($n = 1$) | Converted to an unknown compound | (4.2) 4.5<br>(3.3) 4.5<br>(4.8) 3.7 | ND |
| **11c** ($n = 3$) | > 80 | > 25 mg[b] | > 97 |
| **16** | 100 | (3.0) 3.9<br>(4.1) 4.4<br>(5.5) 3.6 | 72 |

[a]After 30-min incubation in rat plasma at 37 °C.
[b]Solubility of prodrug in deionized water.

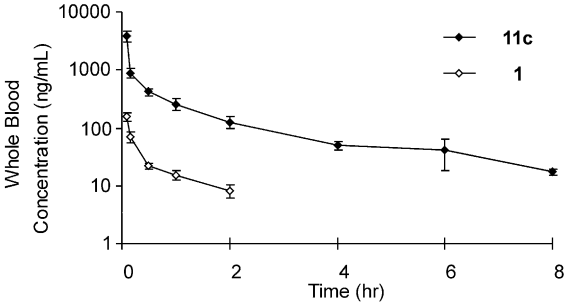



**Figure 1.** Mean (±SD) blood concentrations of **11c** and BMS-191011 (**1**) in rats following administration of an IV dose (1.7 mg/kg) of **11c** (dosing vehicle: PEG-400/water, 1:1).

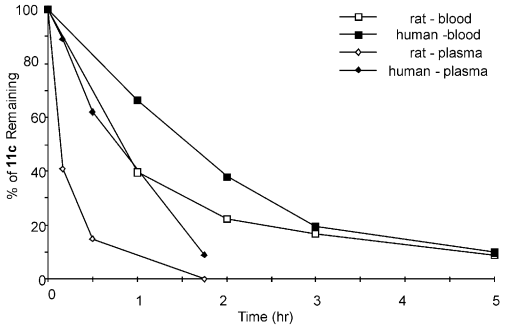



**Figure 2.** Disappearance of **11c** as a function of time after its incubation in human and rat whole blood and plasma.

**Table 2.** Summary of pharmacokinetics of **11c** and BMS-191011 in the rat following administration of **11c**

| Parameter | **11c** | BMS-191011 |
|---|---|---|
| $AUC_{0\text{-}t}$ (ng h/mL) | 1258.6±153.8 | 52.4±4.3 |
| Apparent elimination $t_{1/2}$ (h) | 2.7±0.2 | 1.2±0.5 |
| Clearance (mL/min kg) | 22.9±0.8 | — |
| Vdss (L/kg) | 2.6±0.3 | — |

**Table 3.** Percent reduction of neocortical infarct volume in rats after IV administration of **11c** (2 h post-MCA)[a]

| | Dose (μg/kg) | | | | |
|---|---|---|---|---|---|
| | 0.00001 | 0.0001 | 0.001 | 0.1 | 1000 |
| % Reduction in infarct volume | −13 | −16[b] | −14[b] | −12[b] | −17[b] |
| Number of animals | 28 | 29 | 28 | 28 | 28 |

[a]Compared to vehicle-treated (2%DMSO/98% propylene glycol) controls in the same study.
[b]$p < 0.05$, ANOVA and Dunnett's *t*-test.

rat and human plasma and whole blood. The results are shown in Figure 2. Cleavage of **11c** was not immediate in either plasma or whole blood from rat or human; this may, at least in part, be the cause of the apparent long half-life of **11c** observed in the rat. The identity of the esterases responsible for the hydrolysis of **11c**, the quantitative expression and the inter-individual variability of those enzymes have not been determined. In the absence of such information, quantitative extrapolations of human pharmacokinetics cannot be made from the human in-vitro data.

It was found that prodrug **11C** crosses the rat blood brain barrier but not as efficiently as BMS-191011, resulting in brain/blood concentration ratios of 0.1 and 5.7 for **11c** and BMS-191011, respectively. By using whole-cell voltage clamp recordings and patch clamp analysis it has been confirmed that prodrug **11c** is not a maxi-K opener prior to the cleavage to BMS-191011.

## Effect of 11c on Infarct Volume in a Normotensive rat Model of Focal Stroke

The prodrug **11c** was evaluated in a stringent normotensive model that combines permanent occlusion of the left middle cerebral artery (MCA) and left common carotid artery (CCA) with a 1-h occlusion of the right common carotid artery in the Wistar rat. In this model, the compound was administered as a single intravenous bolus 2 h after the occlusion of the MCA. A single-dose study showed that 0.1 μg/kg of **11c** significantly reduced cortical infarct size compared to vehicle-treated control rats. A dose–response study confirmed the potency and efficacy of this prodrug. The prodrug **11c** reduced infarct volume when administered at doses between 100 pg/kg and 1 mg/kg (Table 3) compared to a therapeutic window of 1.0 ng/kg–10 μg/kg for BMS-191011.

In summary, we have successfully prepared a variety of prodrug derivatives of BMS-191011 with significantly enhanced aqueous solubility. Furthermore, we demonstrated that several of these water-soluble prodrugs possessed long duration of solution state stability and undergo extensive conversion to the parent drug, BMS-191011. Deoxycarnitine ester prodrug (**11c**) was selected for clinical evaluation based on its superior chemical stability, crystallinity and ability to generate BMS-191011, in vitro and in vivo.

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