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Water soluble prodrug of a COX-2 selective inhibitor suitable for intravenous administration in models of cerebral ischemia

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Abstract—A water soluble choline prodrug (**17**) of a COX-2 selective inhibitor (**16**) suitable for *intravenous* dosing in models of cerebral ischemia has been developed. Constant infusion studies using **17** demonstrate that extrapolated brain levels of **16** may be maintained for over 24 h in rats.

Stroke, resulting from an interruption of cerebral blood flow, is one of the leading causes of death and neurological disability worldwide.[1] A multitude of mechanisms are involved in the brain damage that accompanies stroke[2] and previous work has implicated the cyclooxygenase-2 (COX-2) enzyme as a contributor to this damage.[3–5] However, previous work aimed at inhibiting the COX mechanism in stroke models using small molecule inhibitors has led to conflicting results.[3,6–10] Further, those inhibitors reported to be effective were dosed via *intra-peritoneal* (ip) or *per oral* (po) routes. As stroke patients are often unconscious, *intravenous* (iv) administration is the preferred dosing route and so we sought to identify an effective prodrug of a COX-2 inhibitor suitable for *intravenous* administration.

We initially selected COX-2 inhibitor **1** to test in our stroke model due to its good potency against COX-2 in Human Whole Blood (HWB) $IC_{50} = 0.9\ \mu M$ (Fig. 1).[11]

As **1** is insoluble in water and unsuitable for iv dosing, we sought to develop a water soluble prodrug. Our approach was to open the lactone moiety of **1** and to append water solubilizing groups X and/or Y on the resulting acid and alcohol moieties, respectively, to give prodrug **2**. Ideally, X and Y would be endogenous to the body (minimizing any possible adverse side effects) and result in a *water soluble and stable* prodrug **2** that upon iv dosing would rapidly release the COX-2 inhibitor **1**.

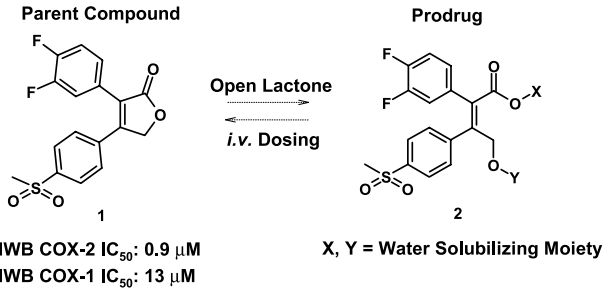

Figure 1. General prodrug strategy.

An example of the synthesis of prodrugs of general structure **2** is exemplified below for the synthesis of **14** (Scheme 1).

Briefly, the parent COX-2 inhibitor **1** was prepared by reacting bromoketone **3** with the appropriately substituted phenyl acetic acid in the presence of base. Lactone

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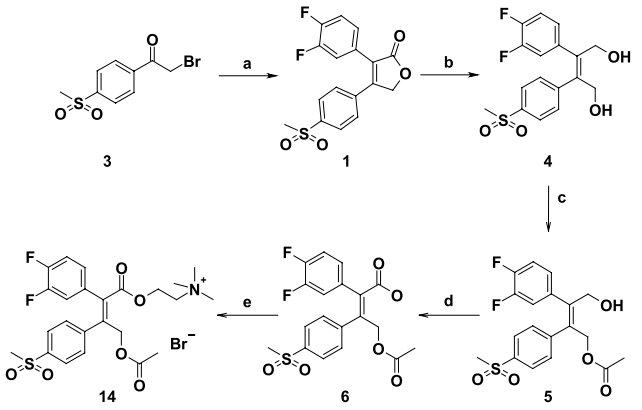


Scheme 1. Synthesis of prodrug **14**. Reagents and conditions: (a) 3,4-difluorophenylacetic acid, DIEA, DMF, 70 °C. (b) DIBALH, CH_2Cl_2, −78 °C to RT. (c) Acetyl chloride, Et_3N, DMAP, 0 °C. (d) (i) Dess–Martin Periodinane (ii) $NaClO_2$. (e) (2-bromoethyl)trimethylammonium bromide, K_2CO_3, DMF.

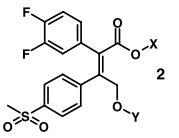
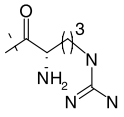
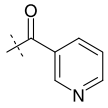
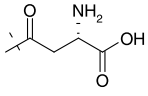
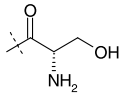
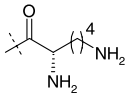
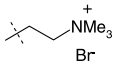
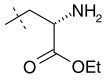
1 was then reduced using DIBALH to give the diol **4**, which was acylated with 1 equivalent of acetyl chloride to give a mixture of products. The desired regio-isomer **5** was readily isolated by silica chromatography from this mixture and then oxidized to acid **6**. Alkylation of acid **6** with a choline equivalent gave the desired prodrug **14**.

Table 1 shows a selection of compounds that were synthesized in an analogous manor to this route and that were evaluated as potential prodrugs of COX-2 inhibitor **1**.

Thus, ring opened lactone **7** with a free acid and alcohol (X = H, Y = H) could not be isolated presumably due to immediate ring closure to the parent lactone **1**. To prevent spontaneous ring closure, the alcohol of **7** was capped while maintaining the free acid (X = H) as a potential water-solubilizing group. Thus, capping the alcohol with acetate (**8**), arginine (**9**) or nicotinic acid (**10**) led, in each case, to a compound that could be isolated. Unfortunately, nicotinate **10** was found to be insoluble in water (2 mg/mL) as either the hydrochloride or sodium salt. Conversely, although **8** and **9** were soluble in water at 4 mg/mL, the solutions formed were found to decompose over 24 h making them unsuitable as prodrugs. It was hypothesized that the aqueous instability of **8** and **9** might be due to the presence of a free acid in these prodrugs and so the acid was capped as the ethyl ester as in **11**, **12**, and **13** (X = Et). Unfortunately, **11** (Y = aspartic acid) and **12** (Y = serine), although both soluble, were also found to decompose over 24 h as an aqueous solution. However, **13**, where Y = lysine, was found to be stable as a solution in water. To test whether **13** would function as a prodrug of **1**, a 2 mg/kg iv dose was administered to rats and the plasma was monitored for the formation of **1**. Gratifyingly at the 30 min time point, 0.4 μM of **1** was detected, indicating the conversion of **13** to **1** in vivo.

In **11**, **12**, and **13**, the water-solubilizing moiety is appended to the alcohol of **2** (i.e., group Y). Alternatively, the solubilizing group can be appended to the acid of **2** (i.e., group X). Thus, while keeping the alcohol capped with an acetate group, **14** (X = choline) and **15** (X = ethyl-serine) were synthesized. Both **14** and **15** were stable as solutions in water, and more importantly, generated **1** when dosed in the rat iv screen. Both **14** and **15** displayed an increase in 'prodrug efficiency' over **13**, with

Table 1. Prodrugs of **1**

Compound	 X	Y	Water stable[a]	Plasma concentration of **1**[b]
7	H	H	NA[c]	NA[c]
8	H	 Ac	No	NA[c]
9	H	 Arg	No	NA[c]
10	H	 Nicotinate	NA[c,d]	NA[c]
11	Et	 Asp	No	NA[c]
12	Et	 Ser	No	NA[c]
13	Et	 Lys	Yes	0.4 μM
14	 Choline	 Ac	Yes	0.9 μM
15	 Et -Ser	 Ac	Yes	1.4 μM

[a] 4 mg/mL solution in water. Stability judged at 24 h by LCMS.
[b] Concentration of **1** following a 2 mg/kg iv dose of prodrug in Sprague–Dawley rats ($n = 2$); measuring at 5, 15, 30, and 60 min. Value quoted is at 30 min.
[c] Not applicable.
[d] Compound not soluble in water at 2 mg/mL as HCl salt.

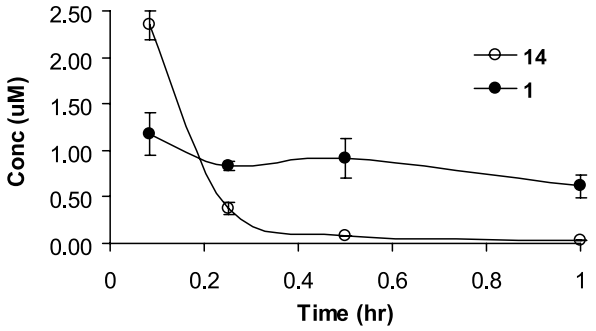


Figure 2. Plasma levels of **14** and **1** following a 2 mg/kg iv dose of **14**.

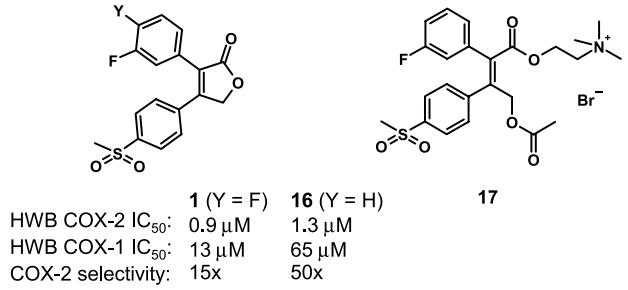
0.9 and 1.4 μM of **1** being detected at 30 min, respectively.

Figure 2 shows the rat plasma levels of the prodrug **14** and the parent COX-2 inhibitor **1** following the 2 mg/kg iv dose of **14**. As can be seen, not only are there high levels of **1** detected at the earliest time point (5 min), there is also a rapid decrease in the levels of circulating prodrug **14**.

Although higher levels of **1** were detected in vivo with **15**, compound **14** was selected for further profiling as it utilizes choline as a water-solubilizing group. This is because citicholine, a compound shown to be beneficial in human clinical trials of cerebral ischemia,[12,13] is metabolized to uridine and choline in vivo.[14] In this way, we hoped to obtain higher levels of neuroprotection due to the potential additive effect of a COX-2 inhibitor and choline.

As we wanted to inhibit COX-2 in the brain, we next determined the brain levels of **1** after dosing with **14**. Thus following a 10 mg/kg iv dose of **14** in rats, at the 3 h time point, the brain and plasma levels of **1** were found to be 0.7 and 1.4 μM, respectively—a brain penetration of 50%. For our in vivo stroke studies, we wanted levels of **1** in the rat brain of 10× its COX-2 IC_{50} (i.e., 9 μM).[15] With rat brain penetration of 50%, this would mean plasma levels of **1** of 18 μM. However, previous publications have suggested that COX-1 inhibition is detrimental to stroke outcome,[16] indicating that COX-2 inhibition in the brain is desired, while inhibition of COX-1 in the brain and periphery is not. At 18 μM, the plasma concentration of **1** would, indeed, result in undesired COX-1 inhibition in the periphery (COX-1 IC_{50} = 13 μM).

Due to this undesired COX-1 inhibition with **1**, we identified an alternative COX-2 selective inhibitor **16** (Fig. 3).[11] Although **16** has less absolute potency against COX-2 compared to **1** (1.3 μM vs 0.9 μM), it has greater selectivity for COX-2 (50-fold versus 15-fold). This would mean that at 10× the COX-2 IC_{50} concentration of **16** in the brain (i.e., 13 μM), the concentration in the plasma would be 26 μM (the brain penetration of **16** is also approximately 50%—vide supra). This plasma level is significantly less than the COX-1 IC_{50} of **16** of 65 μM, so minimizing any undesired peripheral COX-1 inhibition.



	1 (Y = F)	**16** (Y = H)	**17**
HWB COX-2 IC_{50}:	0.9 μM	1.3 μM	
HWB COX-1 IC_{50}:	13 μM	65 μM	
COX-2 selectivity:	15x	50x	

Figure 3. **17**—a choline prodrug of COX-2 inhibitor **16**.

Thus, we synthesized **17**, the choline prodrug of **16** (Fig. 3). Gratifyingly, as with **14**, **17** was stable as a solution in water, released the parent inhibitor **16** upon iv administration in rats, and had good brain penetration of approximately 50% (vide supra).

The animal model we intended to use for cerebral ischemia proof-of-concept experiments using prodrug **17** is the transient middle cerebral artery model (tMCAO).[17] In this model, blood flow to the middle cerebral artery (MCA) of rats is occluded by the use of a filament. After a 90-min occlusion period, the filament is withdrawn to allow blood to reperfuse into the MCA. To determine the time course of COX-2 up-regulation in this model (and hence the required time course of COX-2 inhibition), we carried out a microdialysis experiment to examine the levels of PGE_2 (a product of COX-2 enzymatic activity) in the brain (Fig. 4).[18]

Figure 4 illustrates that PGE_2 levels in the stroked hemisphere of the rat brain increase compared to those in sham operated animals starting at 6 h after occlusion, peaking at 10 h, and with high levels maintained out to 24 h and beyond.[19]

Since dosing **17** via a single iv bolus would provoke transient inhibition of the COX-2 enzyme in the brain (rat $t_{1/2}$ of **16** = 2 h), we wished to establish a protocol to enable continuous COX-2 inhibition over the course of the experiment. For this reason, we next carried out infusion studies with **17** aimed at maintaining steady-

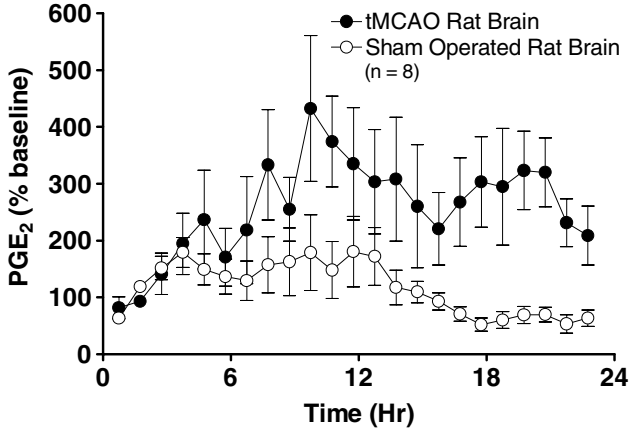


Figure 4. Microdialysis levels of PGE_2.

Table 2. Infusion studies with **17**[a]

Dose of prodrug **17**	Concentration of **16** (μM)[b]			Brain/plasma 24 h (%)
	Plasma 6 h	Plasma 24 h	Brain 24 h	
10 mg/kg Bolus + 5 mg/kg/h infusion	16	20	11	55
5 mg/kg Bolus + 3 mg/kg/h infusion	6.0	9.2	5.5	59

[a] Sprague–Dawley rats ($n = 3$).
[b] Plasma concentration of **16** measured at 0.08, 0.25, 0.5, 1, 2, 4, 6, and 24 h.

state concentrations of the parent compound **16** in the plasma (and hence brain) of rats over 24 h. We targeted brain concentrations of **16** in the rat at two levels—13 and 6.5 μM (10× and 5× the COX-2 IC_{50}, respectively, Table 2).

Table 2 shows that a 10 mg/kg bolus dose followed by a 5 mg/kg/h infusion of **17** results in levels of **16** of 11 μM in the brain and 20 μM in the plasma at 24 h (55% brain penetration). Gratifyingly, this is close to the targeted brain levels of 13 μM (10× COX-2 IC_{50}) and importantly at 20 μM, the plasma levels of **16** are significantly below the COX-1 IC_{50} of 65 μM. Further, plasma levels of **16** were maintained at 16–20 μM during the course of the experiment, meaning COX-1 inhibition in the periphery is minimal (COX-1 $IC_{50} = 65$ μM).

These infusion studies with prodrug **17** demonstrate that high levels of **16** may be sustained in the rat brain for the extended period of time required to inhibit the COX-2 enzyme activity indicated by the PGE_2 time-course in Figure 4. In vivo proof-of-concept experiments using prodrug **17** in animal models of cerebral ischemia are currently underway.

In summary, a water soluble and stable prodrug of a COX-2 selective inhibitor **17** has been designed using choline as a solubilizing group. Microdialysis experiments measuring PGE_2 levels in the stroked hemisphere of rat brain indicate COX-2 activity over a prolonged period from 6 to 24 h following occlusion. Infusion studies in rats with **17** demonstrate that rapid conversion to the parent inhibitor **16** occurs and that steady state levels of **16** may be achieved in the plasma (and by extrapolation the brain) over the desired time period of COX-2 up-regulation indicated by the microdialysis experiment. In vivo proof-of-concept experiments using **17** are currently underway.

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19. A similar time-course in the up-regulation of COX-2 mRNA has been reported: See Ref. 4.