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A new class of NO-donor pro-drugs triggered by γ-glutamyl transpeptidase with potential for reno-selective vasodilatation†

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There are a wide range of NO-donor drugs in existence,11 including conventional organic nitrates and nitrites, S-nitrosothiols, NONOates and N-hydroxyguanidines (NHGs).12-16 The NHGs 1 are analogues of N⁶-hydroxy-L-arginine (NOHA), a biosynthetic intermediate involved in the generation of NO from L-arginine.11 Several enzymatically activated NHG pro-drugs have been reported such as peptidylglycine α-amidating mono-oxygenase (PAM)-active O-carboxymethyl N-hydroxyguanidines17 and Nβ-galactosidases-active (β-D-galactopyranos-1-yl)oxyguanidine.18 Our approach aimed to mask the NO generating ability of NHG pro-drugs with a γ-glutamyl residue to facilitate activation by the enzyme, γ-glutamyl transpeptidase (γ-GT). Given that γ-GT is primarily expressed in the kidney (5-10 fold higher than in the liver and pancreas),19 it was envisaged that this enzyme could be used to trigger reno-selective release of an NHG and subsequent in vivo generation of NO (Scheme 1). A similar strategy has been described for reno-selective 1,3,4-dihydroxyphenylalanine (DOPA), the Glu-DOPA20,21

However, the direct coupling of NHGs with a γ-glutamyl residue was hampered by intramolecular cyclization and dehydration leading to a 1,2,4-oxadiazole ring; or alternatively lactamization and release of a pyroglutamic acid (Scheme 2, data not included).

In an effort to prevent these modes of cyclization, we investigated the use of a bridge between the NHG and the γ-glutamyl group. Both γ-glutamyl itself and γ-aminobutanoyl (GABA)22 were explored as linkers. Thus 2a and 2b became synthesis targets (Scheme 3) and they were prepared via appropriately protected dipeptide intermediates (ESI† Scheme S1). Unfortunately 2a gradually decomposed presumably due to the carboxylic acid moieties promoting autodegradation.

† ‡ N. P. Botting died on 4th June 2011.

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Communication describes the synthesis of a new class of N-hydroxyguanidine (NHG) pro-drugs which release nitric oxide (NO), triggered by the action of γ-glutamyl transpeptidase (γ-GT), and have potential for the treatment of acute renal injury/failure (ARI/ARF).

Acute renal injury (AKI), or failure (ARF), is a common complication that affects millions of people worldwide, particularly in intensive care units, where it is associated with a mortality rate of between 50% and 80%.1 There is no effective pharmaceutical therapy to date. One of the major causes of AKI is ischemia-reperfusion injury,2,3 following aortic ring cross-clamping during by-pass surgery, which can lead to renal ischemia.4 Reperfusion of ischemic renal tissue causes the generation of reactive oxygen species which induce renal cell injury5 and promote impairment of renal perfusion at least in part via inactivation of the vasodilator, nitric oxide (NO).6-9 Thus, a kidney selective vasodilator with antioxidant properties is attractive to maintain blood flow to offset AKI and scavenge the reactive oxygen species. Localisation of activity to the kidney would avoid a systemic reduction in blood pressure. Dopamine and fenoldopam, specific agonists of the dopamine-1 receptor, have been used clinically in an effort to reduce the risk of perioperative renal dysfunction, but the effectiveness of these agents is not clear.9,10 We hypothesised that an effective exogenous NO-donor, which selectively increases renal vasodilatation, would offer an alternative.

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The aminobenzyl linker was also used for the $\gamma$-glutamylation of $N$-hydroxyformamidines (NHFs) (Scheme 5). $N^\prime$-Hydroxy-$N$-{(4-butyl-2-methylphenyl)formamidine} and $N^\prime$-hydroxy-$N$-(3-chloro-4-morpholin-4-ylphenyl)formamidine have been documented as 20-hydroxyeicosatetraenoic acid (20-HETE) inhibitors. 20-HETE is a major metabolite of arachidonic acid and is a potent vasoconstrictor; localisation of an NHF would counter the effect of 20-HETE and induce a synergic vasodilation effect mediated by NO. Thus $N^\prime$-hydroxyphenylethylformamidine 12 was prepared in this study and converted to pro-drug 14.

Pro-drugs 4a–c and 14 were rapidly cleaved by $\gamma$-GT and they were completely deacylated after 1 h, as judged by LC-MS. Fig. 1(a) and (b) illustrates the LCMS trace of 4b and the conversion of 4b to deacylated intermediate 15 [M-Glu$^+$] by $\gamma$-GT. This was in clear contrast to the GABA-linked candidates 2b and 3, which proved to be resistant to the action of $\gamma$-GT. 1,6-Elimination and loss of the linker from 15 to generate the parent NHG 1b is significantly slower (trace amount of parent 1b was detected by selective ion monitoring at $m/z$ 180) than the cleavage of the $\gamma$-glutamyl moiety. In preliminary experiments with animal tissue, LC-MS analysis revealed $\sim$90% conversion of 4b (100 $\mu$M) to 1b in a rat renal homogenate (37 °C; 45 min). In addition, 4b was found to induce substantial vasodilatation in rat isolated perfused kidney preparations (50% of maximum vasodilatation induced by $\sim$40 $\mu$M 4b). Details of the bioactivity of these pro-drugs will be reported elsewhere.

In summary, several candidate NO-donor pro-drugs have been prepared, designed for activation by $\gamma$-GT. The pro-drugs

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### Scheme 2

Cyclization of direct coupling of NHGs with $\gamma$-glutamyl residue(s).

### Scheme 3

Design of Glu/Gaba linked $\gamma$-glutamyl NO-donor pro-drugs of NHG and hydroxamic acid.

### Scheme 4

Design and synthesis of aminobenzyl linked $\gamma$-glutamyl NO-donor pro-drugs of NHG. (i) 4-aminobenzylalcohol, EEDQ, DCM, rt, 12 h, 85%; (ii) $\text{Pd}((\text{PPh})_3)_4$, PhSiH$_3$, DCM, reflux, 2 h, quantitative; (iii) $\text{NH}_2\text{OHCl}$, MeOH, 63%; (iv) $\text{CF}_3\text{COOH}–\text{DCM}$, gave the key intermediate 9a–c, THF, 0 °C, 2 h, 87%; (iii) BocNHOH, NaH, THF, 0 °C, 4 h, 83%; (iv) $\text{CF}_3\text{C}_2\text{H}_4\cdot\text{DCM}$, 92%; (v) $\text{CF}_3\text{CO}_2\text{H}\cdot\text{DCM}$, 92%; (vi) $\text{CF}_3\text{CO}_2\text{H}\cdot\text{DCM}$, 38–53%; (vii) $\text{PhSiH}_3\cdot\text{DCM}$, 37–89%.

### Scheme 5

Synthesis of $N$-hydroxyformamidines and its glutamyl pro-drug: (i) $\text{Me}_{2}\text{NCH(OMe)_2}$, reflux, 2 h, quantitative; (ii) $\text{NH}_2\text{OHCl}$, MeOH, 63%; (iii) $\text{THF}$, reflux, 29%; (iv) $\text{CF}_3\text{COOH}–\text{DCM}$, gave the key intermediate 9a–c, THF, 0 °C, 4 h, 83%; (v) $\text{CF}_3\text{CO}_2\text{H}\cdot\text{DCM}$, 92%; (vi) $\text{CF}_3\text{CO}_2\text{H}\cdot\text{DCM}$, 38–53%. In the event, the synthesis of 4a–c was successfully accomplished through a six-step reaction sequence (Scheme 4).

Firstly, $\gamma$-glutamylation of 4-aminobenzylalcohol with Alloc-$\gamma$-glutamyl acid 1-allyl ester (Alloc-Glu-OAll) (ESI† Scheme S1) gave benzyl alcohol 5. Conversion of the benzylalcohol moiety to the corresponding bromide 6 followed by nucleophilic displacement with BocNHOH generated aminoxide 7, and then treatment with $\text{CF}_3\text{COOH}–\text{DCM}$, gave the key intermediate 8 which was coupled with the required amino(alkyl/aryliminio) methanesulfonate 9a–c to generate 10a–c. Finally the All/Alloc groups were removed under neutral conditions with $\text{CF}_3\text{COOH}–\text{DCM}$, gave the key intermediate 9a–c, THF, 0 °C, 2 h, quantitative; (iii) BocNHOH, NaH, THF, 0 °C, 4 h, 83%; (iv) $\text{CF}_3\text{COOH}–\text{DCM}$, 92%; (v) $\text{CF}_3\text{CO}_2\text{H}\cdot\text{DCM}$, 92%; (vi) $\text{CF}_3\text{CO}_2\text{H}\cdot\text{DCM}$, 38–53%; (vii) $\text{PhSiH}_3\cdot\text{DCM}$, 37–89%.

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$\gamma$-Glutamyl anilines are known substrates for $\gamma$-GT and presented an alternative linker option. The success of such an approach would involve a 1,6-elimination following the action of $\gamma$-GT on $N^\gamma$-glutamylanobenzyloxy-guanidine 4a–c, as illustrated in Scheme 4. Similar spacers have been employed previously in anticancer pro-drug design.

On the other hand, 2b could be purified by preparative HPLC but was found to be resistant to $\gamma$-GT-mediated cleavage in vitro and was considered not to be a useful pro-drug. This prompted the preparation of 3 (Scheme 3), involving the conjugation of only one GABA-Glu dipeptide onto a hydroxamic acid, an alternative NO-donor. Compound 3 too, unfortunately, was found to be resistant to $\gamma$-GT mediated deacylation, suggesting that the GABA-Glu peptide linker is not suitable for $\gamma$-GT cleavage in this setting.
comprise the parent NO-donor, a linker and a \( \gamma \)-glutamyl moiety. GABA-linked pro-drugs are not suitable substrates for \( \gamma \)-GT, but those linked by the aminobenzyl moiety proved to be good substrates for the enzyme. The \( \gamma \)-glutamyl group is cleaved rapidly, with a slower decomposition of the aminobenzyl linker. Improved design is now focussed on tuning the spacer to encourage a more rapid release of the parent NHG drug.

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References