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

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There are a wide range of NO-donor drugs in existence, including conventional organic nitrates and nitrates, S-nitrosothiols, NONOates and N-hydroxyguanidines (NHGs). The NHGs 1 are analogues of N\(^\text{\text{n}}\)-hydroxy-\(\gamma\)-arginine (NOHA), a biosynthetic intermediate involved in the generation of NO from \(\gamma\)-arginine. Several enzymatically activated NHG pro-drugs have been reported such as peptidylglycine \(\varepsilon\)-amidating monooxygenase (PAM)-active O-carboxymethyl N-hydroxyguanidines and N\(^\text{\text{\beta}}\)-galactosidasises-active (\(\beta\)-n-galactopyranos-1-yl)oxyguanidine. Our approach aimed to mask the NO generating group with a \(\gamma\)-glutamyl residue to facilitate activation by the enzyme, \(\gamma\)-glutamyl transpeptidase (\(\gamma\)-GT). Given that \(\gamma\)-GT is primarily expressed in the kidney (5–10 fold higher than in the liver and pancreas), it was envisaged that this enzyme could be used to trigger reno-selective release of an NHG and subsequent in situ generation of NO (Scheme 1). A similar strategy has been described for reno-selective 1,3,4-dihydroxyphenylalanine (1-DOPA), the Glu-DOPA.

However, the direct coupling of NHGs with a \(\gamma\)-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 \(\gamma\)-glutamyl group. Both \(\gamma\)-glutamyl itself and \(\gamma\)-aminobutanyol (GABA) 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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Scheme 1 Approach to \(\gamma\)-GT triggered release of NHG 1 and the reno-selective release of nitric oxide.
The GABA-Glu peptide linker is not suitable for generation of NO-donor pro-drugs of NHG: (i) Me₂NCH(OMe₂), reflux, 2 h, quantitative; (ii) NH₂OH.HCl, MeOH, 63%; (iii) CF₃CO₂H, DCM, 37–89%; (iv) [Pd(PPh₃)₄], PhSiH₃, DCM, rt, 6 h, 53%.

The same aminobenzyl linker was also used for the γ-glutamylamination of N-hydroxyformamidines (NHFs) (Scheme 5). N'-hydroxy-N-(4-butyl-2-methylphenyl)formamidine and N'-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 vasodilatation effect mediated by NO. Thus N'-hydroxyphenylethylformamidime 12 was prepared in this study and converted to pro-drug 14.

Pro-drugs 4a–c and 14 were rapidly cleaved by γ-GT and they were completely decoupled 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 γ-GT. This was in clear contrast to the GABA-linked candidates 2b and 3, which proved to be resistant to the action of γ-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 γ-glutamyl moiety. In preliminary experiments with animal tissue, LC-MS analysis revealed ~90% conversion of 4b (100 μM) 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 ~40 μM 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 γ-GT. The pro-drugs...
comprise the parent NO-donor, a linker and a \( \gamma \)-glutamyl moiety. GABA-linked prodrugs 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 focused on tuning the spacer to encourage a more rapid release of the parent NHG drug.

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References