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COMMUNICATION

Novel paramagnetic AT_1 receptor antagonists†

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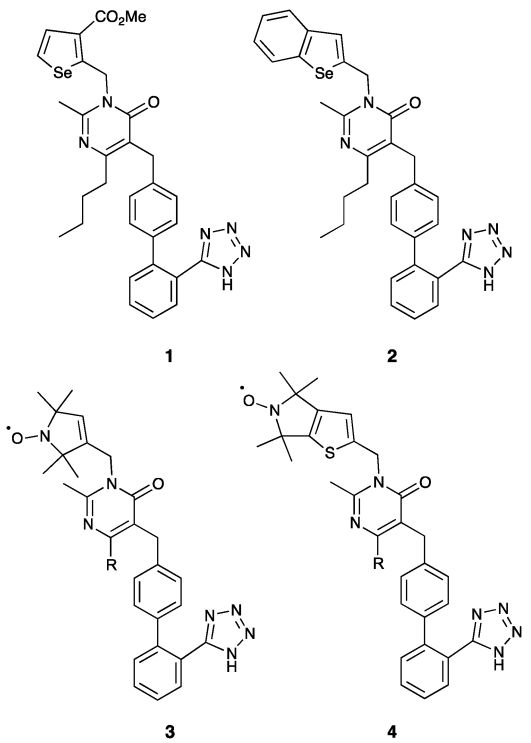
Novel paramagnetic selective angiotensin AT_1 receptor antagonists (*sartans*) bearing nitroxides (3, 4) have been prepared and their pharmacology evaluated *in vitro* as well as *in vivo*. Compounds 3, 4 proved to be effective sartans with pK_B estimates in the range 6.2–9.1. In addition, the sodium salt (11) of 4 (R = Bu) is able to protect against vascular injury in hypertensive rats as determined by its ability to attenuate the development of intimal thickening caused by balloon injury of the carotid artery.

Dual action drugs describe agents with different pharmacophore groups combined within the one molecule. Such drugs have been designed to improve the pharmacological profile of individual pharmacophores, particularly in the treatment of multifactorial diseases in which complex pathologies arise.[1,2] One such disease is hypertension which involves a range of mediators and co-morbidities.[3] Angiotensin II acting on AT_1 receptors is a key mediator in the pathogenesis of hypertension.[4] AT_1 receptor antagonists (sartans) possess antihypertensive actions by antagonising angiotensin II-induced vasoconstriction as well as anti-inflammatory actions by inhibiting angiotensin II-derived free radicals which are also AT_1 receptor-dependent.[5] Free radicals have also been strongly implicated in the maladaptive changes in vascular function that has been observed in hypertensive patients.[3] Consequently, a dual action drug combining a free radical antioxidant moiety (nitroxide) and a sartan may target the angiotensin II and the oxidative stress component of hypertension using one compound. The nitroxide moiety was chosen as it is a free radical antioxidant that can mimic the enzyme superoxide dismutase to reduce superoxide. Moreover, nitroxides in addition to directly scavenging free radicals may also enter into reductive cycles to form hydroxylamines or oxoammonium ions that can interrupt biological redox processes.[6] The high antagonist affinity and slow dissociation of the sartan pharmacophore would be expected to target and retain these hybrid nitroxide antioxidants to AT_1 receptors. AT_1 receptors are upregulated at sites of oxidative stress in cardiovascular diseases exacerbating free radical production.[7] In atherosclerotic plaques, cholesterol laden macrophages produce an excess of extracellular superoxide that cause further oxidative damage.[8] Thus combining a sartan and an antioxidant moiety may enable a targeted decrease in radical-mediated damage and inflammation by reducing AT_1 receptor-dependent and -independent free radicals.

As a consequence, we chose to prepare and examine nitroxide-containing sartans. As part of on-going work aimed at the development of novel compounds for the treatment of hypertension, we prepared analogues of existing sartans with the aim of exploring the effect of heteroatom substitution and substituent



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† Electronic supplementary information (ESI) available: Experimental details for the preparation of new compounds; HPLC traces for compounds **3**, **4**; EPR spectrum of **4** (R = Bu); ^{1}H and ^{13}C NMR spectra for compound **9**; CIF for **4** (R = Me). CCDC 833015. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c1cc14920b

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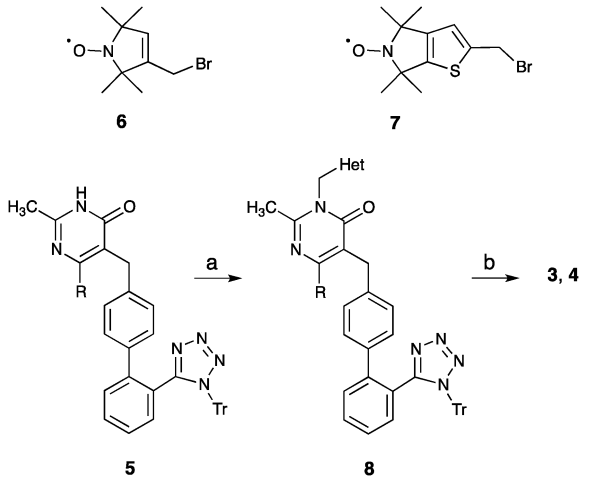
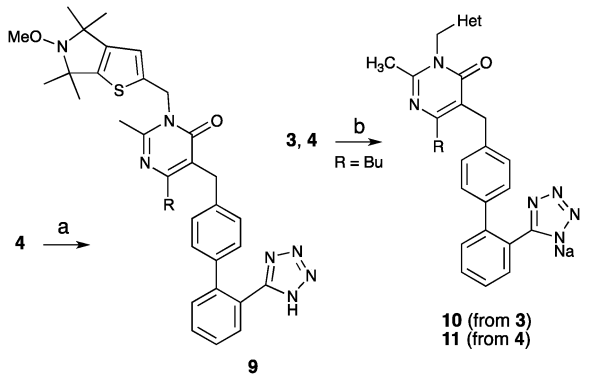


Scheme 1 *Reagents and conditions*: (a) NaH, LiBr, DMF, **6** or **7**, 0 °C → rt; (b) MeOH, reflux, 18–56% (2 steps).



Scheme 2 *Reagents and conditions*: (a) $FeSO_4$, DMSO, H_2O_2, 89%; (b) NaH, THF, 85%.

size on AT_1 receptor activity of sartans. We reported recently that a selenium substitution has a minimal negative effect; for example selenomilfasartan (**1**) proved to have a pK_B value within one order of magnitude of the parent compound;[9] while benzo analogues (*e.g.* **2**) also proved to be effective sartans.[10] As a consequence, we examined whether nitroxide-containing compounds that include **3** and **4** would be effective AT_1 receptor antagonists with added therapeutic benefits.

Compounds **3**, **4** were readily prepared by the coupling of the "biphenyl core" (**5**)[11] with the appropriate halide (**6**, **7**), themselves prepared following literature procedures,[12] to give the

Table 1 pK_B Estimates of the sartans (**3**, **4**) prepared in this study as compared with *milfasartan* and *eprosartan*

Entry	Sartan	pK_B estimate[a]
1	Milfasartan	9.5 ± 0.3
2	Eprosartan	8.4 ± 0.5
3	**3** (R = Me)	6.6 ± 0.3
4	(R = Et)	6.2 ± 0.3
5	(R = Pr)	8.2 ± 0.2
6	(R = Bu)	9.1 ± 0.4
7	**4** (R = Me)	7.1 ± 0.3
8	(R = Et)	7.2 ± 0.3
9	(R = Pr)	8.4 ± 0.4
10	(R = Bu)	8.4 ± 0.5

[a] See the text.

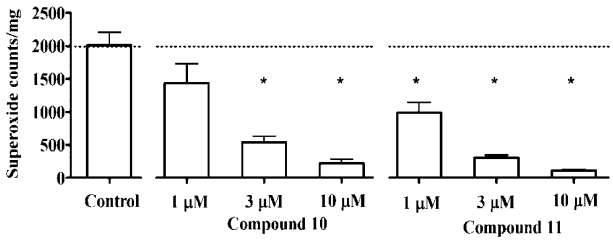


Fig. 1 Effects of NADPH (100 μM; Control) on luminescent superoxide counts detected using lucigenin (5 μM) in the presence of 1–10 μM compound **10** or compound **11** in rat isolated aorta. Values are mean (±SEM) superoxide luminescent counts per milligram of dry tissue weight (counts mg^{-1}). Error bars not visible are contained within bars. *$P < 0.05$ *vs.* Control using one-way ANOVA with Dunnett's post-test. n = 5–7 vessel segments, taken from separate rats.

trityl-protected drugs **8** in moderate yield (Scheme 1). Subsequent reflux in methanol afforded the deprotected compounds **3** and **4** in 18–56% yield following flash chromatography.

As these novel compounds are paramagnetic, it was not possible to obtain ^1H or ^{13}C NMR spectra, although each compound exhibited the "classic" triplet expected for nitroxides (g factor 2.005; a_N 13.875 G);[13] an example is provided in the ESI.† The purity of compounds **3**, **4** was assessed by HPLC analysis (see ESI†). In addition, **3** and **4** were characterised by ^1H and ^{13}C NMR spectroscopy of their (diamagnetic) methoxyamine derivatives **9**, prepared as per the example shown in Scheme 2.

Fortunately nitroxide **4** (R = Me) proved to be a crystalline solid and its structure was established by X-ray crystallography and is displayed in Fig. S1 (ESI†).

Chinese hamster ovary (CHO) cells stably expressing the rat AT_{1A} receptor (CHO-AT_{1A} cells) were used in a preliminary study to assess AT_1 receptor antagonist potency of the *sartan* analogues prepared in this study.[14] In these experiments, the ability of 30 nM of compounds **3**, **4** to inhibit angiotensin II-induced calcium release was determined. The pK_B estimates of antagonist potency are summarized in Table 1 together with those of *milfasartan* and *eprosartan* (standards). Clearly compounds **3** and **4** are effective AT_1 receptor antagonists in this assay, with the propyl and butyl substituted systems (entries 5, 6, 9, and 10) being more potent in this assay.

On the basis of the pK_B data provided in Table 1, we chose to examine the antioxidant capacity of the most potent AT_1 receptor antagonist, namely **3** and **4** (R = Bu); these were first converted to their sodium salts **10** and **11** (for solubility reasons) by treatment with sodium hydride in THF (Scheme 2).

Table 2 Effects of 7 day treatment with compounds on mean arterial pressure (MAP) in conscious unrestrained SHR using telemetry. Values are mean ± SEM MAP (mmHg) on day 0 prior to treatment and on day 7 of treatment. Treatment was delivered over 7 days *via* an osmotic mini-pump implanted s.c. *$P < 0.05$ on ΔMAP over 7 days compared to rats treated with DMSO. There were 4–6 rats in each treatment group

Treatment	Day 0 (mmHg)	Day 7 (mmHg)	ΔMAP/ mmHg
50% DMSO (240 μl per day)	132 ± 3	138 ± 3	6
Compound **11** (30 mg kg^{-1} per day)	135 ± 4	112 ± 4	−23*
Eprosartan (30 mg kg^{-1} per day)	128 ± 3	113 ± 4	−15*

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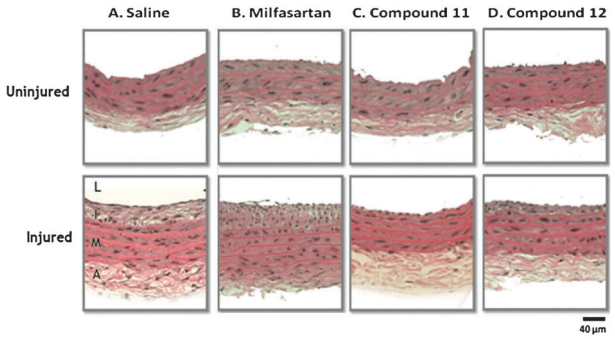

Fig. 2 Representative pictures of uninjured (top panels) and injured (bottom panels) carotid artery cross-sections (5 μm thick; H & E staining) taken from SHR allocated to different treatment groups: (A) saline (120 μl per day), (B) milfasartan, (C) compound **11** or (D) compound **12** (30 mg kg^{-1} per day, each) in saline s.c. *via* an osmotic mini-pump for 14 days. Lumen (L), intima (I), media (M) and adventitia (A) are marked on the bottom left panel. Injury was caused by passing an inflated balloon catheter (Fogarty 2F) through the left common carotid artery 3 times, with the right, uninjured vessel serving as a control.

NADPH was used to stimulate superoxide production in rat isolated aorta segments in the presence or absence of compounds (**10**, **11**) which was detected using lucigenin-enhanced chemiluminescence as described previously.[15,16] Fig. 1 represents NADPH-derived superoxide per mg of dry tissue weight (counts mg^{-1}; details provided in the ESI†) which for control tissues was 2009 ± 197 counts mg^{-1}. Compound **11** (3 or 10 μM only) significantly reduced superoxide counts to 535 ± 98 or 221 ± 65 counts mg^{-1}, respectively. All three concentrations of **10** concentration-dependently decreased superoxide counts to 991 ± 162, 310 ± 39 or 115 ± 14 counts mg^{-1}, respectively, indicating that both of these compounds act as antioxidants, with **11** (R = Bu) performing slightly better in this assay. For this reason, we chose to progress **11** (R = Bu) to preliminary *in vivo* assays.

Mean arterial pressure (MAP) was measured in conscious, unrestrained male spontaneously hypertensive rats (SHR) using telemetry.[17] As shown in Table 2, subcutaneous 50% DMSO had no significant effect on MAP. Importantly, SHR receiving eprosartan or **11** had significant decreases in MAP over 7 days, confirming that **11** is an effective antihypertensive.

Finally, we examined the ability of **11** to attenuate the development of intimal thickening caused by balloon injury of the carotid artery, a model for restenosis and atherosclerosis.[18] Carotid artery rubbing in SHR results in a thickening of the intimal layer due to local production of free radicals, growth factors and cytokines at the site of damage.[18,19] There is also an upregulation of AT_1 receptors at the site of injury, which contributes to smooth muscle cell hypertrophy and hyperplasia.[20]

Fig. 2 shows representative histological sections from injured vessels and their respective contralateral uninjured controls. In the uninjured vessels from rats treated with saline for 2 weeks the average intimal thickness was 1.8 ± 0.2 μm (n = 6). This was 27-fold thicker in the injured vessels from the same rats with a change in the intimal thickness of 46 ± 10 μm (Fig. 2A). Treatment with milfasartan (30 mg kg^{-1} per day) had no effect on intimal thickening (Fig. 2B). However, treatment with **11** (30 mg kg^{-1} per day) significantly attenuated intimal thickening in injured vessels observed as a change in intimal thickness of only 12 ± 4 μm. Indeed in 2 of the 6 rats treated with **11**, intimal thickness was completely prevented (Fig. 2C). This was associated with a reduction in superoxide counts detected using lucigenin-enhanced chemiluminescence in abdominal aorta of rats treated with **11**. It is interesting to note that treatment with sodium 2,2,5,5-tetramethyl-2,5-dihydropyrrole-1-oxyl-3-carboxylate (**12**), a representative nitroxide, also decreased superoxide production, however this was insufficient to significantly attenuate intimal thickening (Fig. 2D).

Nitroxide **11** is a water soluble dual action drug. Its dual actions have been determined in a number of different assays both *in vitro* and *in vivo*. *In vivo*, **11** possessed anti-hypertensive actions and decreased free radical production and vascular injury in SHR. This may result in a novel therapeutic agent that targets both the hypertension and oxidative stress components of cardiovascular diseases using one drug.

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