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THERAPEUTICALLY-USEFUL SELENIUM HETEROCYCLES

SELENIUM IS THE LEAST ABUNDANT ELEMENT ON EARTH WITH A DEFINED BIOLOGICAL ROLE. IT IS INVOLVED IN NUMEROUS ENZYMES AND USUALLY FUNCTIONS THROUGH EFFICIENT REDOX RECYCLING. IN THIS ARTICLE WE DESCRIBE OUR WORK TOWARD THE SYNTHESIS OF BIOACTIVE, THERAPEUTICALLY-USEFUL SELENIUM HETEROCYCLES

There was a time when free radicals were scorned by organic chemists and when "practically every organic text book written" contained a statement that free radicals were "incapable of an independent existence" [1]. Except for polymer chemistry, these reactive species were mostly regarded as poorly-understood curiosities, often scapegoats for unwanted outcomes during synthesis, or when the practitioner required that elusive explanation for his or her unwanted observation. Those were the *Dark Ages* of free radical chemistry, the lengthy period between the "discovery" of organic free radicals by Gomberg in 1900 and their resurgence some seventy or so years later [2, 3]. Our interests have focused on intramolecular homolytic substitution chemistry for the construction of selenium-containing molecules. Over the past two decades we have contributed to the development of synthetically-useful free radical chemistry at selenium by exploring the mechanisms of these reactions and determining rate data for these kinetically controlled processes [4-6]. As a consequence of these pioneering efforts, intramolecular homolytic substitution chemistry has developed into a robust synthetic method that now offers the practitioner efficient and convenient methods for the preparation of selenium heterocycles that complement existing ionic processes, and can be used for the preparation of biologically active molecules [7].

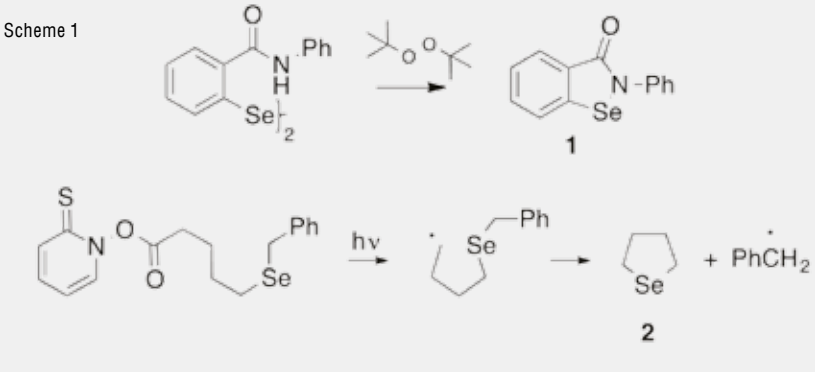
Selenium is the least abundant element on earth that has a defined biological role [8]. It is involved in numerous enzymes including glutathione peroxidase and thyroid hormone deiodinase, and usually functions through efficient redox recycling [8]. Understanding the role that selenium plays in biology has led to the development of molecules that mimic the biological role of selenoenzymes. An early important contribution to this area was ebselen (**1**), that has been shown to be a glutathione peroxidase mimic and was developed as a non-steroidal antiinflammatory therapeutic [9]. Ebselen is currently under clinical investigation for the treatment of chemotherapy induced ototoxicity in cancer parients [10].

Our seminal contribution to the synthesis of selenium-containing organic molecules was the development of S_H2 chemistry by carbon-centred radicals at selenium [11].

Our initial foray into this field saw the first free-radical syntheses of simple heterocycles such as tetrahydroselenophene (**2**) [11]. We also reported the highest-yielding synthesis of ebselen (Scheme 1) [12].

At the time we assumed that the critical free radical reaction in this process, namely the attack of the carbon-centred radical at the selenium atom with the expultion of the benzyl radical as the leaving group, would be rapid. Only recently we determined the rate constants for these processes by laser flash

Scheme 1



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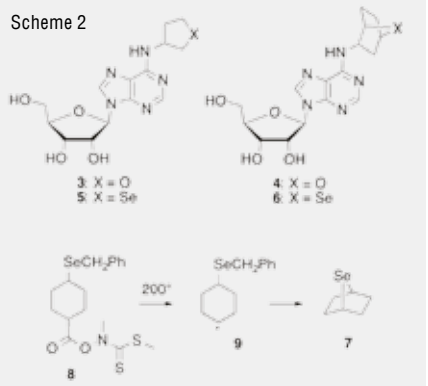
Scheme 2



photolysis, competition kinetics and computational chemistry; **2** is formed with a rate constant of 3.8×10^6 s^{-1} (22 °C) [13].

Having established that this chemistry could be used to construct selenium-containing heterocycles, we focused our attention on the development of therapeutically-useful compounds. In particular, we were interested in adding antioxidant capacity to drugs that were known to be involved in conditions that were exacerbated by oxidative stress and inflammation. As such, we focussed our attention toward conditions such as cardiovascular disease and hypertension.

Adenosine is an important endogenous cardioprotective compound released during ischaemia or hypoxia that interacts with extracellular receptors coupled to secondary messenger systems, including the enzyme adenylate cyclase as well as potassium and calcium ion channels. Exploitation of the negative dromotrophic effect of adenosine has seen its utilisation in the treatment of paroxysmal supraventricular tachycardia (PSVT) [14], however, its short half-life has seen the development of alternative therapeutics for PSVT [15].

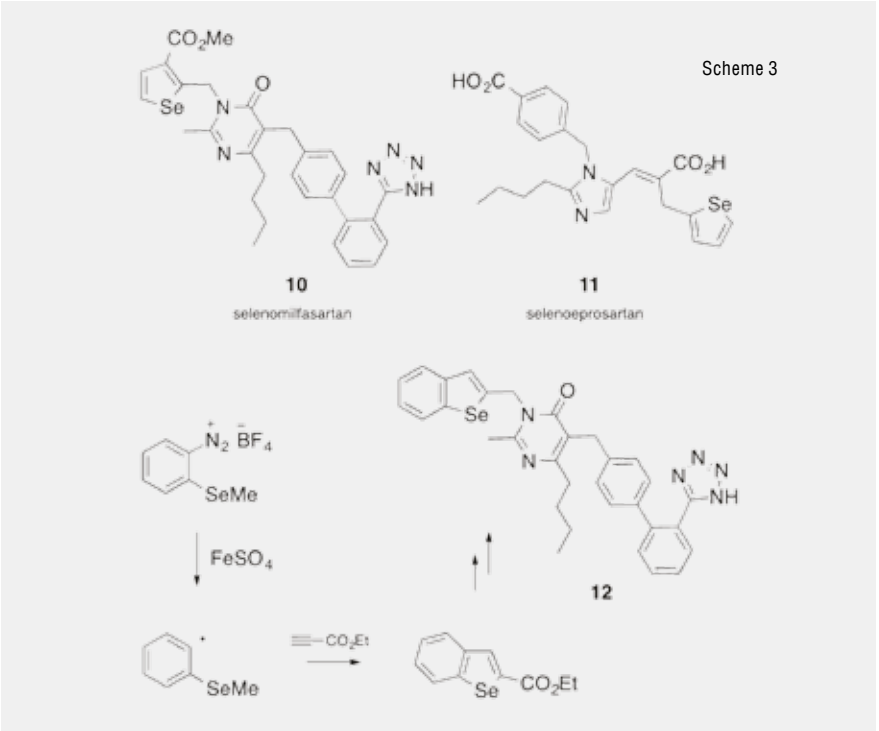
Our contribution to this field has been the synthesis of selenium analogues of compounds such as Tecadenoson (**3**) and 7-oxabicyclo[2.2.1]heptan-2-yl adenosine (**4**) that are more potent agonists of the A_1 adenosine receptor (A_1AR) subtype than adenosine itself [16]. We reported that analogue **5** has an IC_{50} for the A_1AR of 1.9 nM compared to 8.4 nM for **3**. This makes the selenium analogue **5** among the most potent A_1AR agonists [16]. The synthesis of the selenium analogue **6** required the development of a strategy for preparation of the 7-selenabicyclo[2.2.1]heptane skeleton (**7**), as this was an unknown heterocycle. We chose to use intramolecular homolytic substitution chemistry to achieve this goal and showed that when the radical precursor (**8**) was heated to 200 °C in benzene (in a sealed vessel), **7** could be isolated in 20% yield (Scheme 2) [17]. We also determined the rate constant for the ring-closure of the intermediate radical (**9**) to be 5×10^4 s^{-1} (80 °C) [17].

Persistently high blood pressure (hypertension) is a major risk factor for many serious illnesses including stroke, myocardial infarction, heart failure and renal failure. Hypertension is considered to be the world's third leading cause of death and, as such, there is some urgency in developing new and improved treatments for this condition.

Among drug therapies developed for the treatment of hypertension, selective AT_1 (angiotensin type 1) receptor antagonists (sartans) appear to show the most promise. Activation of the AT_1 receptor also produces free radical species that have been shown to cause inflammation [18]. As such, there is a need to develop antioxidant sartans, and our contribution to this field includes the synthesis of selenium-containing analogues (**10**, **11**) of milfasartan and eprosartan,[19] as well as benzoselenophene analogues such as **12** [20]. The key step in the sythesis of the selenium-containing ring in each case was our "signature" intramolecular homolytic substitution chemistry (Scheme 3). Each of these analogues (**10**-**12**) was shown to bind to the AT_1 receptor with similar binding constants (pK_B) to drugs such as eprosartan (pK_B values, **10**: 9.8; **11**: 8.1; **12**: 9.5; eprosartan: 8.4) [19, 20]. However, none of these compounds proved to be effective antioxidants, presumable due to the aromaticity of their selenophene rings.

Myeloperoxidase (MPO) is an enzyme released by neutrophils and other immune cells, and is responsible for the production of strong oxidants. MPO catalyses the reaction between hydrogen peroxide and halide ions (Cl^-, Br^-) to form hypohalous acids that include HOCl and HOBr [21, 22]. Under normal physiological conditions, HOCl is considered to be the major oxidant produced. *In vivo*, HOCl plays a major role in the killing of invading pathogens as part of the innate immune response [23]. However, there is also significant evidence that links MPO and its oxidants, particularly HOCl, to the promotion of cellular damage particularly in association with chronic inflammation leading to diseases such as atherosclerosis, kidney disease, neurodegenerative disease and some cancers [21, 22]. This is particularly serious in individuals with overactive immune systems. There is a need therefore for new drugs that can intercept the harmful oxidants produced

Scheme 3



by MPO before they can do damage, especially to proteins containing vulnerable amino acids such as cysteine and methionine [24]. It is also important to create compounds capable of stopping the development of atheromas that develop as part of the athersclerotic condition. Recognising that atheroma growth is associated with smooth muscle cell replication and the overexpression of AT_1 receptors and the production of free radicals [25], we reasoned that an antihypertensive molecule that is also a powerful antioxidant should be capable of stopping the development of atheromas because it would be delivered directly to the site of inflammation [26]. To that end we synthesised *nitrasartan* (**13**), a stable nitroxide-containing free radical molecule based on the milfasartan structure [26]. Nitroxides are well known to have antioxidant properties [27].

When tested in an atherosclerotic rat model, **13** proved to be capable of reducing blood pressure, protecting cardiac tissue against doxorubicin-induced free radical damage, and was effective at reducing the formation of the atherosclerotic lesion when compared to a control [26]. Neither milfasartan or a simple nitroxide (**14**) was capable of achieving this outcome (Fig. 1). As part of this research it became clear that strong, water-soluble antioxidants would be required in order to effectively deal with biologically-derived oxidants and their propensity to damage critical amino acids. Some time ago we considered this problem and concluded that incorporation of selenium into a carbohydrate

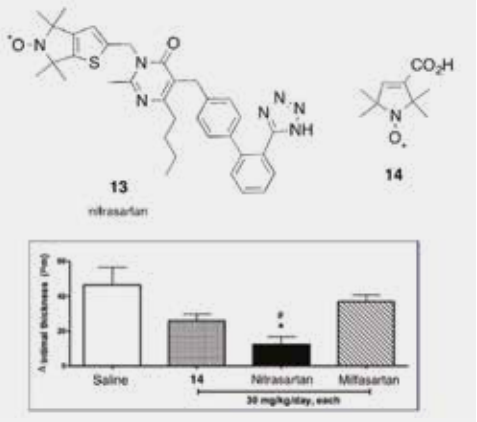
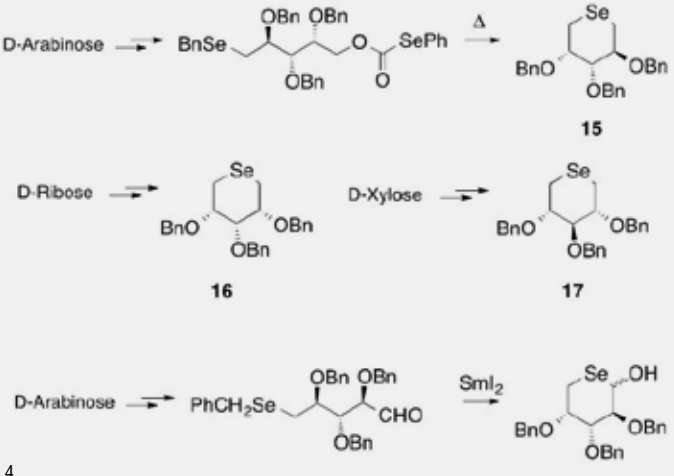


Fig. 1

Nitrasartan (**13**) effectively reduces formation of intimal thickening of corotid artery in rat atherosclerosis model when compared to control (saline), nitroxide (**14**) and miltasartan



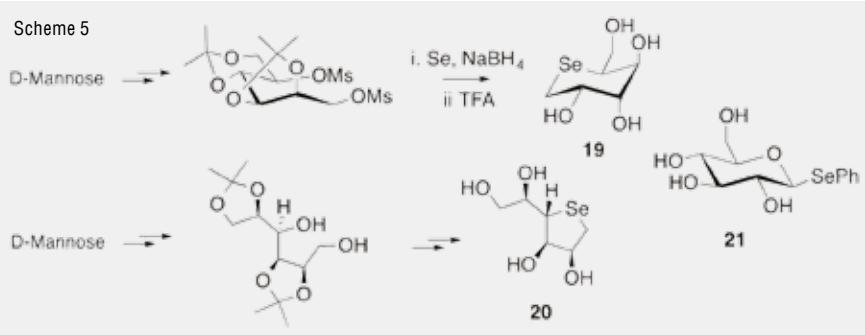
Scheme 4

should afford molecules that met the required criteria. Consequently, we prepared the protected selenosugars (**15**-**18**) using both free radical and traditional methods (Scheme 4) [24]. Unfortunately we were unable to successfully remove the protecting groups without destroying the carbohydrate, so it became clear that an alternative strategy was required.

Inspired by the work of Pinto, we successfully prepared 5-selenopyranose (eg. **19**) and 4-selenofuranose sugars (eg. **20**) and determined kinetics for their reactions with biological oxidants such as HOCl, HOBr and HOSCN (Scheme 5) [24, 29]. It is interesting to note that 1,4-dideoxy-4-seleno-L-talitol (**20**) reacts with HOCl with a rate constant of $1x10^8$ $M^{-1}s^{-1}$ (25 °C), very close to that of cysteine ($3x10^8$ $M^{-1}s^{-1}$) and therefore can provide protection through concentration control [24]. It is also interesting to note that the exocyclic selenide (**21**) is approximately one order of magnitude less active as an antioxidant.

"Wound healing" is a complex temporally and spatially coordinated series of cellular, molecular, physiologic and biochemical events regulated by a delicately balanced cascade of mediators that include free radicals.

Within a few minutes of an injury to skin, platelets adhere to the injury site and become activated and aggregate. A coagulation cascade results, forming a clot of aggregated platelets enmeshed in cross-linked fibrin to halt bleeding. During the inflammation phase, neutrophils and macrophages phagocytose bacteria and cell debris removing them from the wound. During this phase platelet derived growth factor is released into the wound and cause the migration and division of cells. During the proliferation phase, angiogenesis occurs and vascular endothelial cells form new blood vessels. Collagen deposition also occurs along with granular tissue formation, fibroblasts grow and form a new, provisional extracellular matrix. Concurrently, re-epithelialisation occurs, in which epithelial cells proliferate under the scab to close the wound. The wound contracts as myofibroblasts grip the wound edges. During maturation, collagen is remodelled and realigned along tension lines and redundant cells undergo apoptosis.



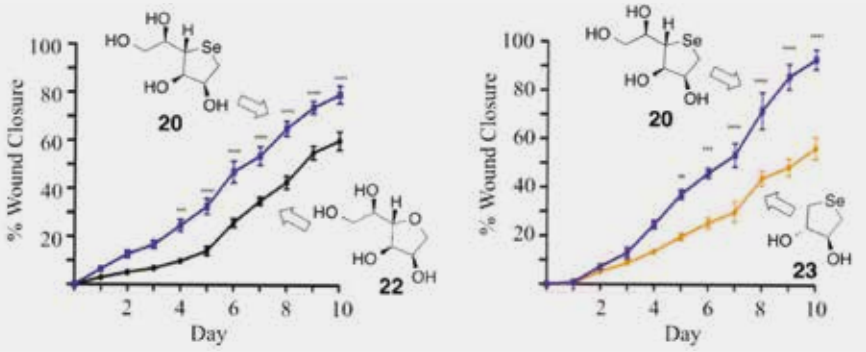


Fig. 2
Performance of compounds **20**, **22**, **23** (1 mM) in wound healing studies in wild-type (C57/BL6) mice

Skin tissue repair is a process susceptible to disruption by factors that include metabolic syndrome, old age and infection, and this often involves the formation of a bacterial biofilm in the wound. In a veterinary context, horses are particulary susceptible to lower-limb wounds that are difficult to heal, a consequence of the inflammatory response to the injury, together with poor blood flow, low oxygen tension and an imbalance of free radical mediators [30, 32]. To our surprise, the talitol **20** accelerates wound healing in a mouse model (Fig. 2). It is particularly noteworthy that **20** appears to increase neutrophil levels in the wound, while concomitantly increasing apoptosis and elastin levels, and decreasing levels of MPO. It is not surprising therefore that **20** is also antiinflammatory [32]. These observations are exciting because they have the potential to provide new treatment options for wound healing. It is clear that neither the carbohydrate structure of **20**, nor its antioxidant capacity are completely responsible for its wound healing properties because neither the parent talitol **22**, nor the similar DHS_{red} **23** are as effective (Fig. 2).

Conclusions

New chemical methodology developed over the past few decades has led to improved methods for the synthesis of selenium-containing heterocyclic molecules. Many of these compounds have been shown to possess beneficial therapeutic properties that may lead to new and improved methods for the treatment of a variety of conditions, especially those involving oxidative stress and free radicals.

Acknowledgements

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Eterocicli a base di selenio per usi terapeutici

Il selenio è l'elemento meno abbondante sulla terra con un ruolo biologico definito. È coinvolto in numerosi enzimi e di solito funziona attraverso un efficace riciclo ossidoriduttivo. In questo articolo viene descritta la ricerca sulla sintesi di eterocicli a base di selenio bioattivi e terapeuticamente utili.

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