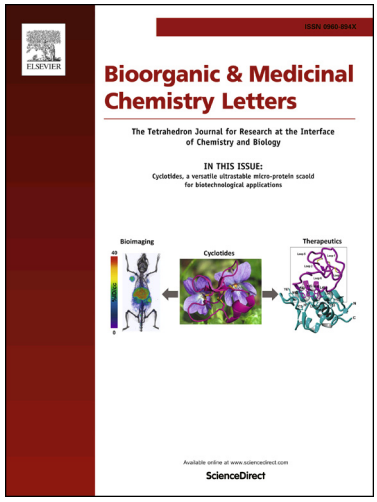
Accepted Manuscript



New 4-aryl-1,3,2-oxathiazolylium-5-olates: chemical synthesis and photochemical stability of a novel series of *S*-nitrosothiols

Monica Eilertsen, Steve M. Allin, Russell J. Pearson

PII: S0960-894X(18)30070-2
DOI: https://doi.org/10.1016/j.bmcl.2018.01.059
Reference: BMCL 25585

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 3 November 2017
Revised Date: 25 January 2018
Accepted Date: 27 January 2018

Please cite this article as: Eilertsen, M., Allin, S.M., Pearson, R.J., New 4-aryl-1,3,2-oxathiazolylium-5-olates: chemical synthesis and photochemical stability of a novel series of *S*-nitrosothiols, *Bioorganic & Medicinal Chemistry Letters* (2018), doi: https://doi.org/10.1016/j.bmcl.2018.01.059

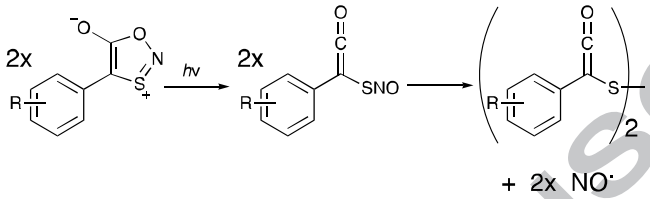
Graphical Abstract

To create your abstract, type over the instructions in the template box below.
Fonts or abstract dimensions should not be changed or altered.

New 4-aryl-1,3,2-oxathiazolylium-5-olates: chemical synthesis and photochemical stability of a novel series of *S*-nitrosothiols

Leave this area blank for abstract info.

Monica Eilertsen, Steve M. Allin and Russell J. Pearson



Bioorganic & Medicinal Chemistry Letters
journal homepage: www.elsevier.com

New 4-aryl-1,3,2-oxathiazolylium-5-olates: chemical synthesis and photochemical stability of a novel series of *S*-nitrosothiols

Monica Eilertsen[a], Steve M. Allin[b] and Russell J. Pearson[a]

[a] *School of Pharmacy, Keele University, Hornbeam Building, Keele, Staffordshire ST5 5BG, UK*
[b] *Department of Chemistry and Forensics, School of Science and Technology, Nottingham Trent University, Nottingham NG11 8NS, UK*

ARTICLE INFO

Article history:
Received
Revised
Accepted
Available online

Keywords:
S-Nitrosothiols
NO-donating
Nitric oxide
Oxathiazolylium-5-olates
Photochemical stability

ABSTRACT

S-Nitrosothiols (RSNOs) remain one of the most popular classes of NO-donating compounds due to their ability to release nitric oxide (NO) under non-enzymatic means whilst producing an inert disulphide by-product. However, alligning these compounds to the different biological fields of NO research has proved to be problematic due to the inherent instability of such compounds under a variety of conditions including heat, light and the presence of copper ions. 1,3,2-Oxathiazolylium-5-olates (OZOs) represent an interesting subclass of *S*-nitrosothiols that lock the –SNO moeity into a five membered heterocyclic ring in an attempt to improve the compound's overall stability. The synthesis of a novel series of halogen-containing OZOs was comprehensively studied resulting in a seven-step route and overall yields ranging between 4% and 28%. The photochemical stability of these compounds was assessed to determine if *S*-nitrosothiols locked within these mesoionic ring systems can offer greater stability and thereby release NO in a more controllable fashion than their non-cyclic counterparts.

The exogenous supply of nitric oxide (NO) can offer therapeutic benefit to a number of different medical conditions including those associated with blood flow, cancer and wound healing, in addition to many others.[1] Since the discovery in the 1980s[2] of NO as an important signalling molecule in cells and tissues there have been many studies focusing on the design and synthesis of NO-donating compounds, leading to an extensive array of reviews on the topic.[3] Of the dozen or so different classes of NO-donors that exist, the nitrates, furoxans, diazeniumdiolates and *S*-nitrosothiols are amongst those most commonly cited.[4]

The *S*-nitrosothiols, also known as the thionitrites or RSNOs, are a particularly interesting class of NO donor as there is clear evidence that these also exist endogenously, as NO reservoirs, by the *in vivo* nitrosation, of cysteine, glutathione, albumin and haemoglobin.[5] These same thiols, can take part in NO transfer (transnitrosation) reactions when in the presence of RSNOs, thus allowing NO to effectively leap-frog from one thiol to another.[6] The homolytic cleavage of the S-N bond in such compounds generates nitric oxide as a stable free radical and the corresponding disulphide, which is formed in a termination step involving two thiyl radicals. This process, leading to the generation of one mole of NO for every mole of RSNO is particularly attractive and intriguing to chemists due to the colour changes associated with NO release. Whilst primary and secondary *S*-nitrosothiols, such as *S*-nitrosocysteine (SNO-Cys) **1** and *S*-nitrosoglutathione (GSNO) **2** are characteristically pink or red in colour, tertiary derivatives, such as *S*-nitroso-*N*-acetyl-

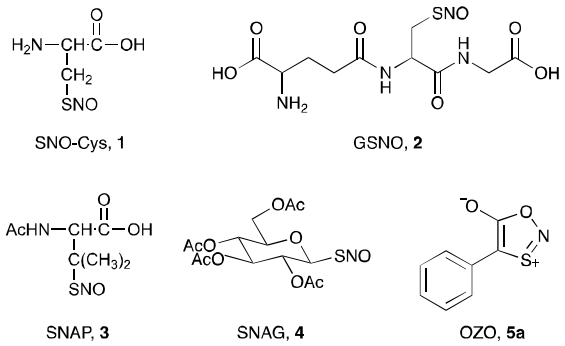


Figure 1 Previously reported *S*-nitrosothiols (**1**-**5**)

penicillamine (SNAP) **3** give green compounds (fig.1).[3] The release of NO from these RSNOs can be easily viewed by their

loss of colour and more specifically, can be monitored by the degradation of characteristic λ_{max} at 330-350nm and at 550-600nm when using UV/Vis spectrophotometry.[3] The conditions that bring about NO release from RSNOs include light, heat and the presence of copper ions (equation 1), with the latter being of particular interest since extensive work 20 years ago showed that Cu(I) rather than Cu(II) was responsible for weakening the S-N bond and catalysing this decomposition step.[7] In a twist of added complexity the disulphide by-product (e.g. GSSG), formed following NO release from certain RSNOs (e.g. GSNO **2**) can behave as a metal ion chelator and therefore remove copper from the solution resulting in the prolonged the existence of the RSNO whilst significantly reducing the levels of free NO.[8]

$$2\ \text{RSNO} \xrightarrow{\Delta,\ h\nu \text{ or } Cu^{+}} \text{RSSR} + 2\text{NO} \qquad (1)$$

Subtle structural differences, including even those between SNO-Cys **1** and SNAP **3**, have been shown to dramatically alter the stability of these compounds and thus the rate at which they release NO.[9] In the case of compound **3**, the greater steric bulk (*cf*. to compound **1**) offered by two methyl groups adjacent to the –SNO moiety to form a tertiary *S*-nitrosothiol can aid stability by reducing both the ease of copper complexation and thiyl radical dimerization.[10] Such subtleties that influence NO release have fascinated the authors and co-workers over many years, which initially led to the synthesis of a large number of carbohydrate based *S*-nitrosothiols (including SNAG **4**) to help explore the key chemical features effecting the stability of the –SNO moiety (fig.1).[11] The key result of the carbohydrate-based work, which looked at varying the lipophilicity, sugar type and even the chiral form, was to confirm that such compounds are intrinsically unstable, with many decomposing upon the removal of solvent without the need for any of the aforementioned decomposition conditions. Rather than fine-tuning the stability, this emphasised the need for a much coarser handle on controlling RSNO stability, which led to interest in 4-aryl-1,3,2-oxathiazolylium-5-olates (OZOs) **5a** (fig. 1), where the -SNO group is locked into a five-membered heterocyclic ring in an attempt to gain greater control over NO release.[12]

Previous work involving carbohydrate based RSNOs, such as SNAG **4**, showed by laser Doppler imaging to bring about a significant dose dependent vasodilator effect (P = 0.001) to forearm microvessels following transdermal delivery. A five-fold enhancement in peripheral blood flow over baseline was seen using the highest SNAG **4** concentration (0.75%, w/w) with intra- and inter-subject variability of 19% and 16%, respectively.[11] Such applications, where the NO-donor is supplied at or close to the site of action circumvents, to some degree, the need for highly stable *S*-nitrosothiols since degradation is ultimately essential for the quick and local supply of NO. However, in an attempt to synthesise and test longer acting RSNOs with improved storage capabilities, the 4-aryl-1,3,2-oxathiazolylium-5-olates were studied with particular interest in the required ring-opening step prior to RSNO degradation and NO release.

Decomposition of 4-aryl-1,3,2-oxathiazolylium-5-olates by photochemical unimolecular reactions were reported over 30 years ago[13] whilst thermal degradation has also been described at temperatures of 80-140°C.[14] More recently this same family of compounds were shown to decompose when exposed to pH values close to 5, which would match quite nicely to the skin pH range.[15] In this latter work, the ring-opening step required prior to NO release was shown to depend on the type of substituent attached to the aryl ring. Based on these findings this work attempted to expand this family of compounds with a focus on chloro and fluoro derivatives, since previous findings reported that a 4'-chloro substituent added stability.[15] The incorporation of halogens onto the aromatic ring may also serve to improve the overall lipophilicity of the OZO and thus make them attractive candidates to study as peripheral vasodilators via the transdermal delivery route. To further explore the role of the substituents, placement on the ring at the 2', 3' and 4' positions was explored as well as di-substituted ring derivatives. As a control, the aryl ring with no substituents, which has been made previously,[13,15-17] was re-synthesised in this work and subjected to the same photochemical stability testing. All halogen-containing target compounds (**5b**-**5g**) are summarised in figure 2.

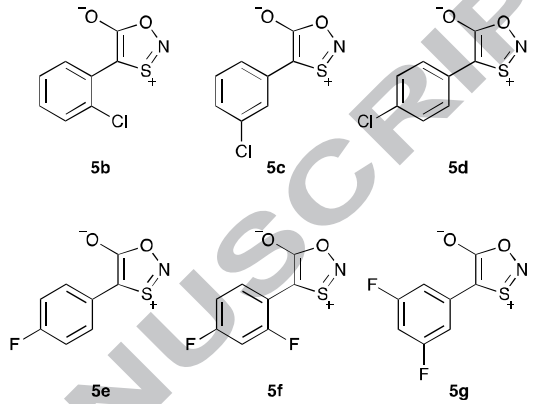
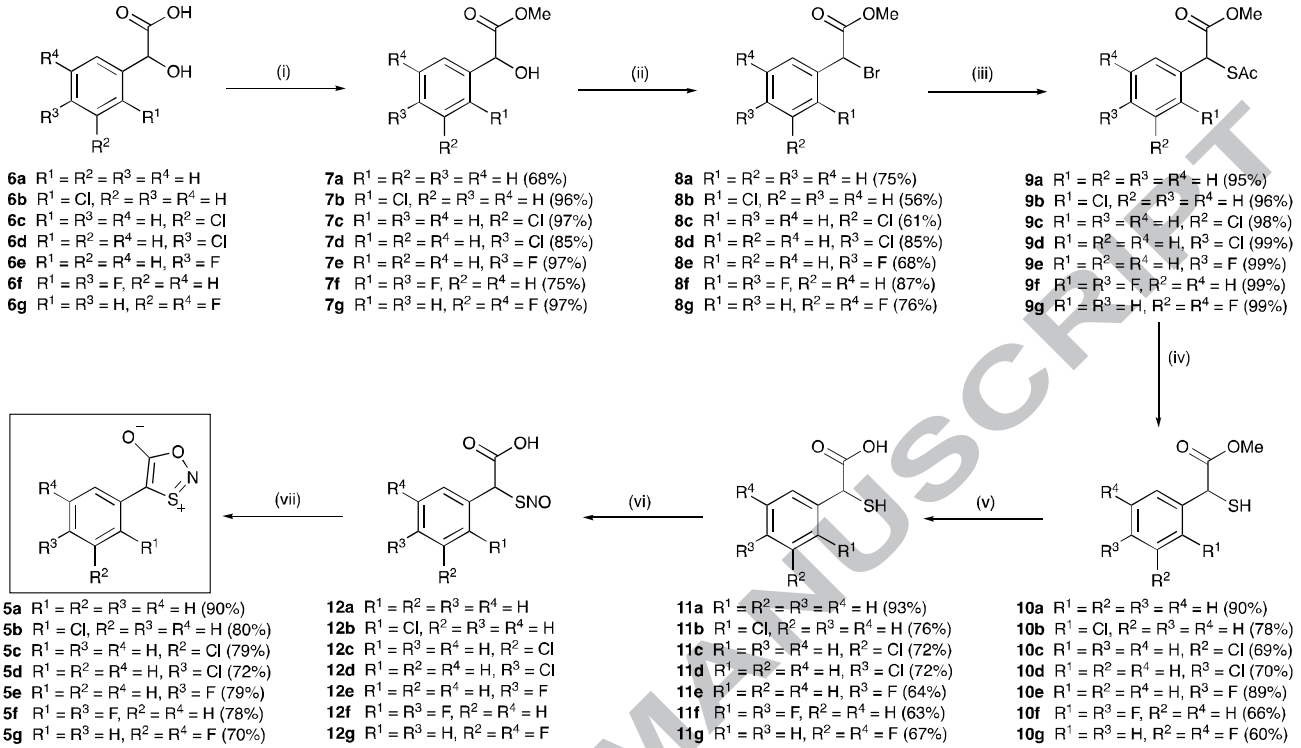


Figure 2 Target halogen-containing OZO compounds (**5b**-**5g**)

The first successful OZO synthesis dates back to 1960 when Bacchetti and Alemagna chose to study such compounds with a view to taking advantage of the characteristic mesoionic ring.[17] Similar rationale led Gotthardt to propose a four-step synthesis in the 1970s and 80s,[13,18] before Schaffer and Thompson outlined a six-step synthesis in the 1990s,[16] by which time the significance of NO and the role of OZOs as NO-donors had been realised, leading to a patent on their potential use as cardiovascular agents.[16] In 2007, Wang proposed an additional route in four-steps with overall yields ranging from 39-50%, which to date, are the highest to be reported for this compound class.[15] In common with all earlier examples, the work reported here uses the mandelic acids as starting materials to outline a synthetic route, which the authors believe to be more robust and reliable than previous methodology (Scheme 1).

From all previous synthetic approaches the Mitsunobu reaction, as described by Wang,[15] was initially seen as the most attractive and efficient means of replacing the benzylic secondary alcohol in **6a** with an *S*-acetyl group. This chemistry relies on the formation of a complex between diisopropyl azodicarboylate (DIAD) and triphenyl phosphine, which then deprotonates the benzylic alcohol before thiolacetic acid is added, to provide the *S*-acetyl derivative. However, this approach could not be replicated in this work despite manipulating the order of addition and reaction times, as well as tinkering with the number of equivalents of each reagent. The lack of desired product was attributed to the carboxylic acid being by far the most acidic group and therefore being the preferred site of de-protonation. With this in mind, the carboxylic acid was converted into the methyl ester **7a**. This step, using sulphuric acid and methanol, proceeded without the need for further purification in a 90% yield, which is consistent with literature values.[19] The Mitsunobu reaction involving **7a** still proved to be problematic even when the thiol was replaced with the thiolate in an attempt to produce a better nucleophile.

Attention switched to a bromination and *S*-acetylation as two separate steps, using HBr to yield **8a**[20] and 1.1 equivalents of KSAc to give **9a** (see scheme 1). When repeated on a larger scale it was found that PBr_3 was a preferable route to **8a**, which mirrors the approach taken by Schaffer and Thompson.[16] After washing through a silica pad, brominated product **8a** was obtained in 80% yield and in pure form, whilst *S*-acetyl **9a** gave an orange oil in a yield of 95% that did not require further purification. Unlike



Scheme 1 The preferred synthetic route for OZO **5a** and the halogenated OZOs (**5b**-**5g**). (i) H_2SO_4, MeOH, 60°C, 4hr. (ii) 1.1 equiv. PBr_3, $CHCl_3$, r.t., 96hr. (iii) 1.1 equiv. KSAc, MeOH, r.t., 4hr. (iv) NaOMe / MeOH, r.t., 5hr. (v) H_2SO_4 / H_2O, 0°C, 24 hr. (vi) 2 equiv. *i*BuONO / CH_2Cl_2, 0°C, 2 hr. (vii) 1 equiv. polymer bound DCC, CH_2Cl_2, 0°C, 2 hr.

Schaffer and Thompson, the best brominations involving PBr_3 took place in dry chloroform at room temperature, rather than using refluxing conditions. Dry dichloromethane, carbon tetrachloride and tetrahydrofuran were also tested in parallel as potential solvents for the bromination step at temperatures ranging from 0°C to 60°C, but chloroform proved to be the best compromise with regards to safety, yield and reaction time.

The de-*S*-acetylation step used the same methodology as Wang[15] to yield the free thiol **10a** in 95%, without the need for purification as none of the corresponding disulphide was identified. Hydrolysis of the methyl ester to give **11a** proved more difficult due to the generation of disulphide when using basic conditions. As a result, a variety of different reagents and conditions were studied and the deprotection sequence for the *S*-acetyl and methyl ester groups was also reversed and attempted in a single step, without success. After multiple attempts involving NaOH, LiOH, NaOMe, HCl and BBr_3, the best compromise was found to be the use of dilute sulphuric acid at 0°C, which gave pure **11a** in 93% yield.

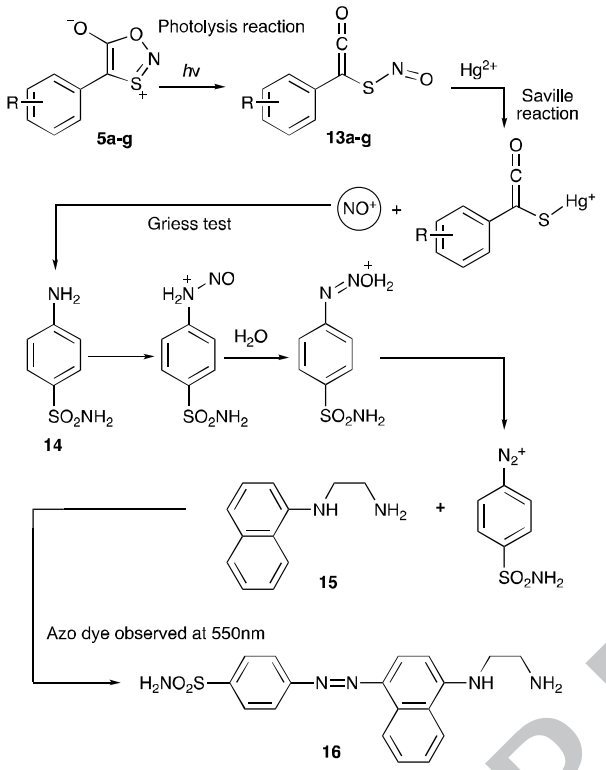
Nitrosation of the thiol to give the desired *S*-nitrosothiol has previously been attempted[20,21] using *tert*-butyl nitrite, ethyl nitrite and *iso*-butyl nitrite, with the latter proving to be the preferred reagent in this work to give **12a**, which was immediately reacted on to give the ring-closed OZO compound **5a**. Attempts were also made to nitrosate using the N_2O_4 and N_2O_3 fuming method,[11] however, nitrosation of the ethanol solvent[10] led to the requirement for an inert gas to be bubbled through the product in order to remove excess fumes, and nitrous acid was also formed as a by-product, which was seen as a major disadvantage. Hart's sodium nitrite method[22] was also trialled here using an acidified aqueous mixture that relies on precipitation when the reaction mixture is cooled, but in this work no solid product was seen using this approach.

The ring-closing dehydration step was previously described using DCC,[15] however, following filtration through a celite pad to remove excess *iso*-butyl nitrite and column chromatography, the desired product **5a** was found to co-elute with residual DCC. Due to this, EDC was substituted in placed DCC, which led to a very low yield due to solubility issues during the aqueous work-up. In light of these yield and purification problems, polymer bound DCC was instead used, since this was simply filtered off following the ring-closure reaction, to give OZO **5a** in 90% from the free thiol **11a**, after column chromatography.

This modified seven-step synthesis, which is clearly a longer route than those previously described,[13,15-17] was seen as a reliable and robust method to give OZO 5a in a yield of 13%. Despite this pathway having a yield that was 38% lower than that reported elsewhere[15] this method consistently converted starting materials **6a**-**6g** into OZOs **5a**-**5g** in overall yields ranging between 4 and 28%, with five of these products being novel OZO examples.

The instability of *S*-nitrosothiols under a variety of different conditions has been well documented over the last twenty-five years with reported half-lifes ranging from 2 seconds to in excess of 9.5 months (see equation 1).[23] This work does not wish to address the relative stability of *S*-nitrosothiols **12a**-**12g**, rather, the

focus here was to ascertain if any improvements in photochemical stability can be achieved with ring-locked RSNOs; thus the ring opening of OZOs **5a**-**5g** was monitored. With this in mind, a decomposition study was designed to establish the time and conditions required to convert **5a**-**5g** into **13a**-**13g**. Identification of ring-opened derivatives (**13a**-**13g**) was best achieved in one-pot using the Saville reaction in combination with the Griess test as presented in Scheme 2.[24]



Scheme 2 Monitoring the ring opening step using UV/Vis spectrophotometry in a one-pot method utilising the Saville reaction in combination with the Griess test.

Previous work has shown that OZOs will photochemically ring-open via one of two different pathways, which is dependent on the polarity of the solvent used.[25] The photochemical reaction in benzene results in a phenylthiazirine intermediate on route to a phenylnitrile derivative, whilst in ethanol the free *S*-nitrosothiols derivatives **13** are formed, which subsequently results in NO release; thus the latter solvent model was adopted in this work. Photochemical decomposition at room temperature was studied over a 40-minute period at an absorbance of 496nm to monitor the formation of the purple azo dye **16** following the Saville and Griess reactions (Scheme 2).[24] This model NO releasing system, which was calibrated using GSNO **2**, allowed the rate of ring-opening to be monitored and compared for **5a**-**5g**. All OZO compounds were dissolved in EtOH:H_2O (1:1) at an initial concentration of 75mM and an equimolar concentration of $HgCl_2$, sulphanilamide **14** and *N*-(1-naphthyl)ethylenediamine **15** was included in each sample.

The first series of experiments examined NO release at room temperature under dark conditions. This highlighted **5a**, **5c**, **5e** and **5g** as being the least stable with all four showing between 40% and 80% ring opening without any light exposure. Since compounds **5b**, **5d** and **5f** showed greater stability these were further explored in the presence of ambient daylight conditions, alongside **5e**, which served as a comparison from the less stable derivatives. The results of this work are displayed in figure 3. From this photochemical stability study three key observations were identified: (1) the fluoro OZOs were generally less stable than the chloro OZOs (*cf.* para fluoro **5e** showed 76% decomposition versus 42% for para chloro **5d**); (2) in the chloro series the stability order was ortho, **5b** > para, **5d** > meta, **5c**; (3) in the fluoro series the ortho and para di-substituted derivative **5f** was more stable than the mono-substituted para fluoro derivative **5e**. This latter observation is consistent with the ortho position providing greater stability as explained by observation (2).

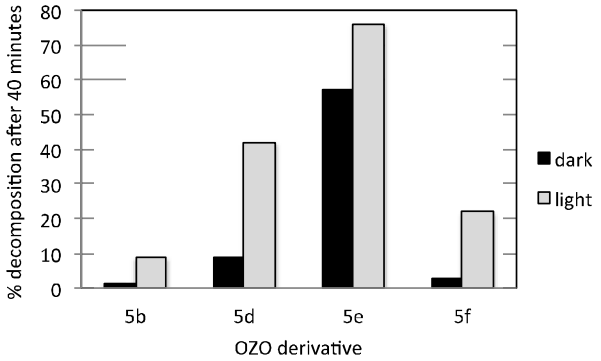


Figure 3 The percentage ring-opening of OZOs (**5b**, **5d**, **5e** & **5f**) after 40 minutes at 25°C when either left in the dark (black shading) or exposed to ambient daylight levels (grey shading).

Whilst it is fascinating to observe these stability differences, it is challenging to rationalise how such subtle substituent changes can afford such large variability in the rate of the photochemical ring-opening step. As outlined in previous work,[15] there is clearly a link between OZO stability and the level of electron-donation or electron-withdrawal provided by the substituent on the benzene ring, with the latter reducing stability and the former prolonging the existence of the OZO compound when in solution.

For OZO **5a** previous work found 7% overall decomposition and NO release when exposed to light over a 3-hour period,[15] however, in this work 48% decomposition was observed in the dark over a 40-minute period. Although this would tend to suggest a large discrepancy between studies, it is very important to note that this study is designed to only focus on the ring-opening event as beyond this step all RSNO is instantly degraded by $HgCl_2$, as was desired by the authors, thus the two sets of data should not be directly compared. The OZOs in this series clearly release NO over a greater time period than suggested by the data in figure 3 as these results are based on the complete breakage of the S-N bond upon generation of the free *S*-nitrosothiol due to the presence of $HgCl_2$ which enables the Saville reaction to yield the nitrosonium ion that is needed for the generation of the azo-dye from the reaction with **14** and **15**. Whilst the purple dye acts as marker for OZO decomposition in this stability model, in reality, the free *S*-nitrosothiol will exist in solution and therefore release NO over a greater time period. As such, it is important to reiterate that this study was only concerned with monitoring the added stability, if any, that the mesoionic rings provide to such compounds.

In summary, when dissolved in a dilute ethanolic solution the OZO compounds described in this work provide only a minimal advantage over their ring-opened counterparts. It is fair to conclude that whilst OZOs do not fully address the issues surrounding *S*-nitrosothiol instability in solution, they do afford analogues that can be isolated and stored more easily in the solid form than some acyclic –SNO moeities (e.g. SNAG, **4**), which

spontaneously decompose in the solid state.[25] This particular issue explains the one-pot method used for steps 6 and 7 of the OZO synthesis outlined in this work (Scheme 1) where the free RSNO form was not isolated on route to the final ring-closed OZO products. Furthermore, in solution, it has been shown here that OZOs do provide extra stability, due to the added necessity of the ring-opening step, albeit not the kind of stability that is optimal for the slow, controlled delivery of NO over many hours. OZOs should therefore be viewed as a partial rather than the complete answer to enhancing RSNO stability. In addition to expanding the number of OZO derivatives for applications tailored towards NO related studies, the improved synthetic methodology outlined in this work will undoubtedly interest those wishing to further explore cycloaddition reactions involving OZO compounds.[25]

The modified synthetic sequence described in this work successfully yielded five novel OZO derivatives (**5b**, **5c**, **5e**-**g**), which in solution were shown to ring-open within minutes when exposed to ambient light conditions and thus produce the naked, acyclic form of the *S*-nitrosothiol. As such the improved stability, in solution, gained by these ring-locked *S*-nitrosothiols was only considered to be a partial success. The added synthetic challenge in forming these mesoionic rings was rewarded with RSNOs of greater stability in the solid form when compared to acyclic RSNOs (e.g. SNAG, **4**), however, in solution these compounds did not provide the level of tailored stability that is desired by those wishing to further investigate the role, if any, of exogenous NO when delivered to a variety of different biological models. So, whilst *S*-nitrosothiols remain one of the most attractive categories of NO-donor available, the focus on improving the overall stability should consider studying the importance that other functionality has on the rate of NO release from the -SNO moiety. In short, the medicinal chemist's work is far from complete in identifying the key structural features that have the greatest influence on the rate of NO release from this intriguing class of compounds.

Acknowledgments

The authors would like to acknowledge the EPSRC UK National Mass Spectroscopy Facility at Swansea University. Keele University's School of Pharmacy and EPSAM research institute provided the funding for this work.

References and notes

1. (a) G. R. J. Thatcher, A. C. Nicolescu, B. M. Bennett and V. Toader, *Free Radical Biology & Medicine*, 2004, **37**, 8, 1122; (b) G. R. J. Thatcher, *Curr. Topics Med. Chem*., 2005, **5**, 597; (c) S. Huerta, S. Chilka and B. Bonavida, *Int. J. Oncol*., 2008, **33**, 909.
2. (a) R. F. Furchgott and J. V. Zawadzi, *Nature*, 1980, **288**, 373; (b) C. R. Molina, J. W. Andresen, R. M. Rapoport, S. Waldman and F. Murad, *J. Cardiovasc. Pharmacol*., 1987, **10**, 371; (c) R. M. J. Palmer, A. G. Ferrige and S. Moncada, *Nature*, 1987, **327**, 524; (d) L. J. Ignarro, G. M. Buga, K. S. Wood, R. E. Byrns and G. Chaudhuri, *Proc. Natl. Acad. Sci. USA*, 1988, **84**, 9265.
3. (a) I. L. Megson, *Drugs of the Future*, 2000, **25**, 7, 701; (b) P. G. Wang, M. Xian, X. Tang, X. Wu, Z. Wen, T. Cai and A. J. Janczuk, *Chem. Rev*., 2002, **102**, 1091; (c) M. R. Miller and I. L. Megson, *Brit. J. Pharmacol*., 2007, **151**, 305; (d) K. Sharma and H. Chakrapani, *Nitric Oxide*, 2014, **43**, 8.
4. (a) A. B. Seabra, A. Fitzpatrick, J. Paul, M. G. De Oliveira and R. Weller, *Brit. J. Dermatol.*, 2004, **151**, 5, 977; (b) R. Scatena, P. Bottoni, G. E. Martorana and B. Giardina, *Expert Opin. Investig. Drugs*, 2005, **14**, 7, 835; (c) R. Scatena, P. Bottoni, A. Pontoglio and B. Giardina, *Curr. Med. Chem*., 2010, **17**, 61; (d) Y. Li and P. I. Lee, *Mol. Pharmaceut*., 2010, **7**, 1, 254; (e) S. Priya, R. Nithya and S. Berchmans, *J. Mater. Sci.: Mater. Med.*, 2014, **25**, 1;
5. (a) W. N. Kuo, J. M. Kocis and J. Nibbs, *Frontiers in Bioscience*, 2003, **8**, A62; (b) A. J. Gow, D. G. Buerk and H. Ischiropoulos, *J. Biol. Chem*., 1997, **272**, 5, 2841; (c) A. J. Gow, B. P. Luchsinger, J. R. Pawloski, D. J. Singel and J. S. Stamler, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 9027; (d) S. S. Gross and P. Lane, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 9967; (e) G. Richardson and N. Benjamin, *Clin. Sci.*, 2002, **102**, 1, 99; (f) D. Giustarini, A. Milzani, R. Colombo, I. Dalle-Donne and R. Rossi, *Clin. Chim. Acta*, 2003, **330**, 85; (g) D. Jourd'heuil, F. L. Jourd'heuil and M. Feelisch, *J. Biol. Chem.*, 2003, **278**, 18, 15720; (h) J. L. Alencar, I. Lobysheva, M. Geffard, M. Sarr, C. Schott, V. B. Schini-Kerth, F. Nepveu, J.-C. Stoclet and B. Muller, *Mol. Pharmacol*., 2003, **63**, 1148.
6. D. J. Barnett, A. Rios and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1995, 1279.
7. A. P. Dicks, H. R. Swift, D. L. H. Williams, A. R. Butler, H. H. Al-Sa'doni and B. G. Cox, *J. Chem. Soc., Perkin Trans. 2*, 1996, 481.
8. (a) D. R. Noble, H. R. Swift and D. L. H. Williams, *Chem. Commun*., 1999, 2317; (b) D. R. Noble and D. L. H. Williams, *Nitric Oxide: Biol. Chem*., 2000, **4**, 4, 392.
9. H. A. Moynihan and S. M. Roberts, *J. Chem. Soc., Perkin Trans. 1*, 1994, 797.
10. C.-E. Lin, S. K. Richardson, W. Wang, T. Wang and D. S. Garvey, *Tetrahedron*, 2006, **62**, 8410.
11. (a) A. R. Butler, R. A. Field, I. R. Greig, F. W. Flitney, S. K. Bisland, F. Khan and J. J. F. Belch, *Nitric Oxide: Biol. Chem*., 1997, **1**, 3, 211; (b) F. Khan, I. R. Greig, D. J. Newton, A. R. Butler and J. J. F. Belch, *The Lancet*, 1997, **350**, 410; (c) R. Weller, *Clin. Sci*., 2003, **105**, 533; (d) F. Khan, R. J. Pearson, D. J. Newton, J. J. F. Belch and A. R. Butler, *Clin. Sci*., 2003, **105**, 577.
12. (a) M. P. Sammes, *Comprehensive Heterocyclic Chemistry I*, 1984, A.R. Katritzky and C. W. Rees, (Eds), Elsevier, Oxford, **4.34**, 897; (b) L. I. Khmelnitski and O. A. Rakitin, *Comprehensive Heterocyclic Chemistry II*, 1996, A.R. Katritzky, C. W. Rees and E. F. V. Scriven (Eds), Elsevier, Oxford, **4.12**, 433.
13. (a) H. Gotthardt, *Chem. Ber.*, 1972, **105**, 188; (b) A. Holm, N. Harrit, K. Bechgaard, O. Buchardt and S. E. Harnung, *J.C.S. Chem. Comm*., 1972, 1125; (c) A. Holm, N. Harrit and N. H. Toubro, *J. Am. Chem. Soc*., 1975, **97**, 21, 6197; (d) A. Holm, N. Harrit and N. H. Toubro, *Tetrahedron.*, 1976, **32**, 2559; (e) I. R. Dunkin, M. Poliakoff, J. J. Turner, N. Harrit and A. Holm, *Tet. Lett*., 1976, **11**, 873; (f) A. Holm, N. Harrit and I. Trabjerg, *J. Chem. Soc. Perkin I*, 1978, 746; (g) H. Gotthardt, F. Reiter and C. Kromer, *Liebigs Ann. Chem.*, 1981, 1025; (h) N. Harrit and A. Holm, *J. Chem. Soc. Perkin Trans. II*, 1987, 1227.
14. M. Tono, H. Aoto, Y. Matsudaira, T. Sugiyama, M. Kajitani, T. Akiyama and A. Sugimori, *Tet. Lett.*, 1991, **32**, 32, 4023.
15. D. Lu, J. Nadas, G. Zhang, W. Johnson, J. L. Zweier, A. J. Cardounel, F. A. Villamena and P. G. Wang, *J. Am. Chem. Soc.*, 2007, **129**, 5503.
16. J. E. Shaffer and S. A. Thomson, *US Pat.*, US005087631A, 1992.
17. T. Bacchetti and A. Alemagna, *Atti accad. nazl. Lincei, Rend., Classe ci. fis., mat. e nat*., 1960, **28**, 646.
18. H. Gotthardt, *Tet. Lett*., 1971, **17**, 1277.
19. D. Basavaiah and P. R. Krishna, *Tetrahedron*, 1995, **51**, 8, 2403.
20. W. S. Sheldrick, N. Weferling and R. Schmutzler, *Liebigs Ann. Chem.*, 1981, 1035.
21. (a) H. M. S. Patel and D. L. H. Williams, *J. Chem. Soc. Perkin Trans. 2*, 1990, 37; (b) L. Soulère, J.-C. Sturm, L. J. Núñez-Vergara, P. Hoffman and J. Périé, *Tetrahedron*, 2001, **57**, 7173.
22. T. W. Hart, *Tet. Lett*., 1985, **26**, 16, 2013.
23. (a) M. D. Bartberger, J. D. Mannion, S. C. Powell, J. S. Stamler, K. N. Houk and E. J. Toone, *J. Am. Chem. Soc*., 2001, **123**, 8868; (b) L. Field, R. V. Dilts, R. Ravichandran, P. G. Lenhert and G. E. Carnahan, *J. Chem. Soc. Chem. Comm.*, 1978, 249.
24. J. A. Cook, S. Y. Kim, D. Teague, M. C. Krishna, R. Pacelli, J. B. Mitchell, Y. Vodovotz, R. W. Nims, D. Christodoulou, A. M. Miles, M. B. Grisham and D. A. Wink, *Anal. Biochem.*, 1996, **238**, 150.
25. (a) H. Gotthardt, *Tet. Lett.*, 1971, **17**, 1281; (b) H. Gotthardt, *Chem. Ber*., 1972, **105**, 196; (c) H. Gotthardt and F. Reiter, *Tet. Lett*., 1976, **25**, 2163; (d) R. K. Howe, T. A. Gruner, L. G. Carter, L. L. Black and J. E. Franz, *J. Org. Chem*., 1978, **43**, 19, 3736; (e) H. Gotthardt, F. Reiter, R. Gleiter and R. Bartetzko, *Chem. Ber*., 1979, **112**, 260.