Check for updates

View Article Online
View Journal

ChemComm

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: N. Velusamy, A. Binoy, K. N. bobba, . Nedungadi, N. Mishra and S. Bhuniya, *Chem. Commun.*, 2017, DOI: 10.1039/C7CC05339H.

This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the author guidelines.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the ethical guidelines, outlined in our author and reviewer resource centre, still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

rsc.li/chemcomm

Please do not adjust margins
View Article Online
DOI: 10.1039/C7CC05339H

Journal Name

COMMUNICATION

A Bioorthogonal Fluorescent Probe for Mitochondrial Hydrogen Sulfide: New Strategy for Cancer Cell Labeling

Received 00th January 20xx,
Accepted 00th January 20xx

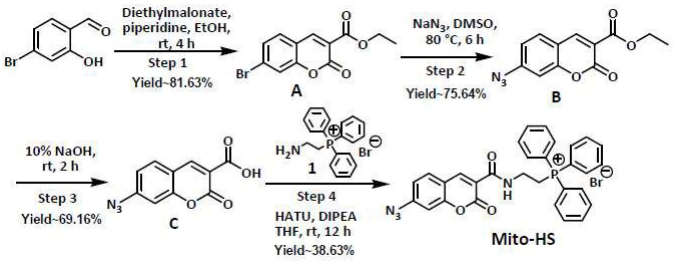
DOI: 10.1039/x0xx00000x
www.rsc.org/

Nithya Velusamy,[‡a] Anupama Binoy,[‡b] Kondapa Naidu Bobba[‡a] Divya Nedungadi,[‡b] Nandita Mishra[*b] and Sankarprasad Bhuniya,[*a,c]

We report the application of a chemodosimeter 'turn on' fluorescent probe for detecting endogenous H_2S formation in cancer cells. Mito-HS showed a bathochromic shift in UV-vis.-absorption spectrum from 355 nm to 395 nm in the presence of H_2S. Furthermore, it showed ~43-fold fluorescence enhancement at λ_{em} 450 nm in the presence of H_2S (200 µM). The cancer cell-specific fluorescence imaging reveals that Mito-HS has the ability for distinguishing cancer cells from normal cells based on the level of endogenous H_2S formation. In due course, Mito-HS would be a powerful cancer biomarker based on its ability to estimate endogenous H_2S formation in living cells.

Hydrogen sulfide (H_2S) a gaseous molecule plays an active role as a neurotransmitter in our nervous system. Hydrogen sulfide is produced endogenously by enzymes such as cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase in a controlled fashion.[1] Sulfur-containing amino acids such as cysteine, homocysteine produces H_2S by enzymatic decomposition in several organs such as heart, vasculature, brain, kidney, liver, lungs, and pancreas.[2] H_2S may be produced by non-enzymatic metabolic pathways[3] and from some bacteria in the intestine.[4] However, imbalance of endogenous H_2S production induces several diseases including, Alzheimer's disease, Parkinson's disease, and other neurodegenerative diseases.[5] Sometimes over production of H_2S in vital organs invites inception of other diseases like diabetes.[6] A study indicates that translocation of cystathionine-β-synthase (CBS) in vascular smooth-muscle cells produces the H_2S under hypoxic condition.[7] Moreover, a recent study also shows that cystathionine-β-synthase (CBS) and cystathionine-γ- lyase (CSE) are overexpressed in colon cancer and ovarian cancer cells; thus bridging its direct link with H_2S over production in cancer cells.[8]

Recently various chemical reactions based fluorogenic probes for H_2S have been developed and used for cell labeling[9] and *in vivo* imaging.[10] Even though a large number of H_2S probes have been reported in the past few years; a handful of them were for mitochondrial H_2S.[11] Unfortunately, none of them can detect H_2S formation in cellular milieu without adding any external stimulator as a source of H_2S. The detection of intra-mitochondrial H_2S formation is pivotal; because excess H_2S leads to the dysfunction the activity of mitochondria, such as energy production, depolarization, and cellular respiration.[12] Moreover, a recent study indicates that CBS enzyme has localized in the mitochondrial outer membrane in cancer cells and it accelerates cancer cell proliferation and angiogenesis.[13] Developing a fluorescent probe for getting information on endogenous H_2S formation in cancer cells over normal cells without adding any external stimulator is remains a challenging task. The quantitative information on H_2S formation *in situ* living cells can be a key hallmark for labeling of cancer cells. In this context, a new probe has synthesized (scheme-1) utilizing the basic concept of fluorescence off-on mechanism to discriminate cancer cells from normal cells.



Scheme 1 Synthesis of **Mito-HS**

The probe **Mito-HS** was synthesized in four successive steps (scheme 1). The details of synthetic methods and including the spectroscopic evidence, such as mass, ^{1}H-NMR, ^{13}C-NMR, HRMS, HPLC are available in supplementary information (Fig. S1-Fig. S14).

To check whether the probe **Mito-HS** meets our expectation, we recorded UV-Vis and fluorescence changes of **Mito-HS** in aqueous buffered solutions at physiological pH (pH 7.4) (Fig. 1) in the presence of variable concentrations of H_2S. We observed in Fig. 1a, the UV-vis absorption peak at λ_{ab} 370 nm gradually decreases with the appearance of a new absorption band at λ_{ab} 395 nm in the presence of variable concentrations of H_2S. Also, in Fig. 1b, a gradual increase in fluorescence intensity at λ_{max} 450 nm upon addition of H_2S (0-200 µM) to the **Mito-HS** was observed; it finally reached a saturation point in the presence of 200 µM of H_2S. It was

[a] *Amrita Centre for Industrial Research & Innovation, Amrita School of Engineering, Amrita Vishwa Vidyapeetham, Ettimadai, Coimbatore 641112, India. E-mail: b_sankarprasad@cb.amrita.edu (SB)*
[b] *School of Biotechnology, Amrita University, Kollam, Kerala, India 690525; E-mail: nanditamishra@am.amrita.edu*
[c] *Department of Chemical Engineering and Materials Science, Amrita School of Engineering, Amrita Vishwa Vidyapeetham, Ettimadai, Coimbatore 641112, India*
‡These authors contributed equally to this work.
†Electronic Supplementary Information (ESI) available: [Synthesis procedure, NMR, MS, fluorescence, and cell imaging data]. See DOI: 10.1039/x0xx00000x

Published on 17 July 2017. Downloaded by Cornell University Library on 17/07/2017 14:30:37.
ChemComm Accepted Manuscript

View Article Online
DOI: 10.1039/C7CC05339H

Published on 17 July 2017. Downloaded by Cornell University Library on 17/07/2017 14:30:37.

observed that the fluorescence intensity of **Mito-HS** was ~ 43 fold higher in the presence of H_2S (200 μM). These results imply that **Mito-HS** is capable of detecting H_2S in physiological (10–600 μM) condition.[14] We have calculated lower detection limit by applying regression equation; which was found to be 24.3 nM (Fig. S15).

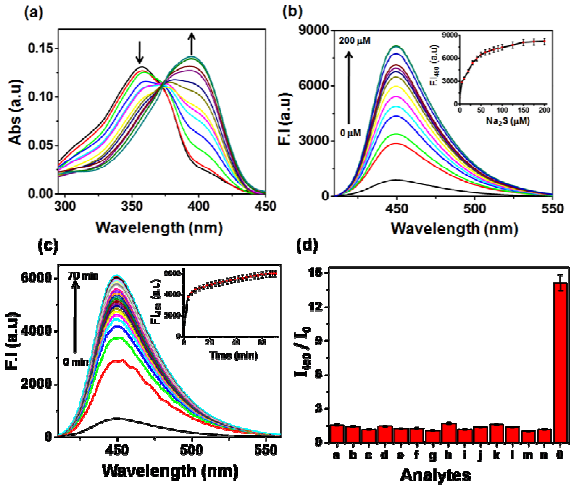


Fig. 1 (a) UV-Vis absorption and (b) fluorescence spectra of **Mito-HS** (5 μM) were recorded in the presence of variable concentrations of Na_2S (0-200 μM) Inset: average fluorescence intensity (λ_{em} = 450 nm) changes with Na_2S. (c) Fluorescence change of **Mito-HS** (5 μM) with Na_2S (100 μM) at different time intervals (0-70 min.). (d) Fluorescence responses of **Mito-HS** (5 μM) in the presence of various biologically important analytes: (a) cysteine (Cys), (b) H_2O_2, (c) $NaNO_2$, (d) $Cu(OAc)_2$, (e) $Zn(OAc)_2$, (f) $FeSO_4$, (g) $FeCl_3$, (h) Na_2CO_3, (i) GSH, (j) NO, (k) $Na_2S_2O_3$, (l) K_2S_5, (m) N_2S_4, (n) ascorbic acid (AA) and (o) Na_2S. (GSH: 5 mM). Experiment was carried out in PBS (0.2% DMSO) at 37 °C.

The response time of probe for a particular analyte is crucial; especially biological complex environment as chemical entities in cellular milieu is temporal. Thus, time-dependent fluorescence recoveries at λ_{max} 450 nm were recorded in the presence of H_2S (20.0 eq.). The results presented in Fig. 1c indicate that the fluorescence intensity gradually increased with time and plateaued at 60 min. It was found that **Mito-HS** reacts with H_2S at a rate of 5.4 X 10^{-3} S^{-1} (Fig. S16). It suggests that **Mito-HS** may be able to provide real-time information on H_2S formation in the cellular microenvironment.

To justify the selectivity of **Mito-HS** toward H_2S over other competitive ions, we recorded fluorescence signal changes of **Mito-HS** in the presence of various chemical entities such as H_2S, cysteine (Cys), H_2O_2, $NaNO_2$, $Cu(OAc)_2$, $Zn(OAc)_2$, $FeSO_4$, $FeCl_3$, Na_2CO_3, GSH, (AA), NO, $Na_2S_2O_3$, Na_2S_4, K_2S_5 and ascorbic acid. Spectroscopic data in Fig. 1d indicated that the **Mito-HS** didn't show any considerable fluorescence change as noticed for H_2S. Further, this finding strongly supports our proposed concept to use **Mito-HS** for tracking of H_2S formation in cellular milieu.

Next, the stability and reactivity of **Mito-HS** toward H_2S at variable physiological pH ranges were estimated. The result in Fig. S17 indicates that **Mito-HS** was stable within biological pH scale as its fluorescence intensity remained unaltered. Whereas, in the presence of H_2S; the fluorescence intensity of **Mito-HS** was high with marginal depreciation in the lower pH range. This finding suggests that **Mito-HS** is very stable; thus, it is most appropriate to detect H_2S formation in cellular milieu.

Further, we estimated the ability of **Mito-HS** to detect H_2S in blood serum. The result in Fig. S18 indicates that the fluorescence intensity of **Mito-HS** was increased ~ 30-fold in blood serum (0.45 mM albumin protein) within 25 min.

Before proceeding to apply it to validate *in vitro* hydrogen sulfide (H_2S) in living cells, we have established the cause of fluorescence increment of **Mito-HS** in the presence of hydrogen sulfide (H_2S). The probe **Mito-HS** pretreated with H_2S (200.0 μM) for an hour, was subjected to ^{1}H-NMR and liquid chromatography-mass spectroscopy (LC-MS). ^{1}H-NMR data in Fig. S19 indicates that it corresponds to the amino-coumarin derivative as proposed in Scheme-S2. Further, in LC-MS (Fig. S20), the mass of the major component (> 93.01%) had matched the amino- coumarin derivative (**FL-1**; MS = 492.6). These findings altogether suggest that H_2S had reduced $-N_3$ moiety to $-NH_2$[15] and concomitantly a new fluorophore, 7-amino- coumarin derivative had been generated. Thus, the quantum yield of **Mito-HS** had increased to 0.479 from 0.045 due to formation **FL-1** in the presence of H_2S (Na_2S).

Next we checked the biocompatibility of a probe before applying to the cells for imaging; thus we assessed the cytotoxicity of **Mito-HS** in HeLa, MDA-MB-231 and DU145 cells. The cytotoxicity of **Mito-HS** was estimated by the conventional MTT assay. The dose-dependent MTT assay data at 48 h as given in Fig. S21 indicates that **Mito-HS** didn't show any cytotoxicity against all the three cancer cells. This result encourages that **Mito-HS** can be used as a probe for tracking of endogenous H_2S formation in living cells.

The fluorescence 'turn-on' chemoselectivity, stability in physiological pH range and biocompatibility of probe **Mito-HS** were checked. Next, we evaluated the ability of **Mito-HS** for distinguishing cancer cells from normal cells based on endogenous H_2S mediated fluorescence labeling of cells. The results in Fig. 2 (b, f, j) indicated that **Mito-HS** (5.0 μM) pretreated HeLa, MDA-MB-231 and DU145 cells, were strongly fluorescent. The merged fluorescence images with their corresponding bright-field images in Fig. 2 (d, h, l), suggest that large percentage of each type of cells were fluorescence-labeled. In contrast, **Mito-HS** pretreated normal cells (3T3-L1 cells) remained non-fluorescent under similar conditions.[16] Even after an hour, **Mito-HS** pretreated 3T3-L1 cells

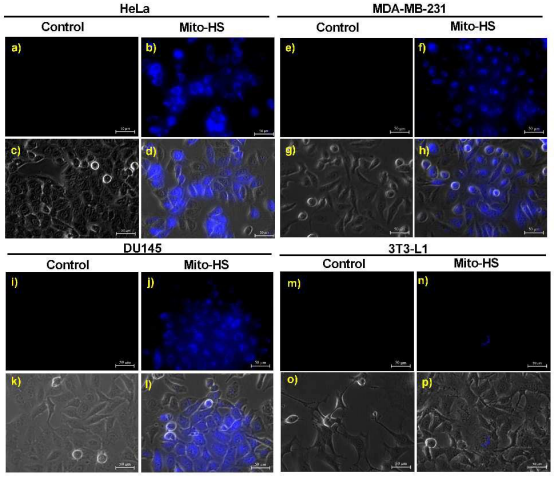


Fig. 2 Fluorescence microscopic images of untreated HeLa (a), MDA-MB-231(e), DU145 (i) 3T3-L1 (m) cells and (b, f, j, n) are treated with **Mito-HS** (5 μM). (c - d), (g - h), (k - l) and (o - p) are overlay DIC Bright field images with corresponding fluorescence images. Scale bar, 50 μm. Images were obtained using excitation wavelengths of 390 nm with the emission being monitored over the 440–500 nm.

ChemComm Accepted Manuscript

This journal is © The Royal Society of Chemistry 20xx

Please do not adjust margins
View Article Online
DOI: 10.1039/C7CC05339H

Published on 17 July 2017. Downloaded by Cornell University Library on 17/07/2017 14:30:37.

were non-fluorescent. Fluorescence labeling of **Mito-HS**- pretreated 3T3-L1 cells in the presence of external H_2S (Na_2S) (Fig. S22) indicates that due to insufficient endogenous H_2S in 3T3-L1 cells, **Mito-HS** was non-fluorescent. These findings suggest that **Mito-HS** enables to discriminate cancer cells from normal cells depending on the production of endogenous H_2S within a very short time (15 min.).

Next, we checked whether this fluorescence is only due to endogenous H_2S in cancer cells and not because of other endogenous thiol-entities like GSH, cysteine, and homocysteine. For this, HeLa, MDA-MB-231 and DU145 cells were pretreated with *N*-ethyl maleimide (NEM) for masking S- containing thiol entities. *N*-Ethyl maleimide (NEM) pretreated cells were equally fluorescent-labeled with **Mito-HS** like the NEM-untreated cells (Fig. S23, S24). These results suggest that **Mito-HS** can detect endogenous H_2S production in HeLa, MDA-MB-231 and DU145 cells without interference from other thiol containing entities in the cellular milieu.

The cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CSE) are the endogenous H_2S producing enzymes in the cancer cells.[13] To justify the H_2S producing activity of cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CSE), HeLa, MDA-MB-231 and DU145 cells were separately pre-treated with corresponding inhibitors, aminooxyacetic acid (AOAA) and *N*-propargylglycine (PAG) respectively.[13] The results in Fig. S23 b-d indicate that **Mito-HS** showed reduced fluorescence in inhibitor-treated HeLa cells. The suppression of cystathionine-β- synthase (CBS) and cystathionine-γ-yase (CSE) by corresponding inhibitors had reduced endogenous H_2S formation. By using ImageJ software,[17] we calculated the quantitative decrease in fluorescence intensity of AOAA and PAG pretreated HeLa cells in comparison to untreated cells. There was ~ 7-8-fold fluorescence intensity decreased in the

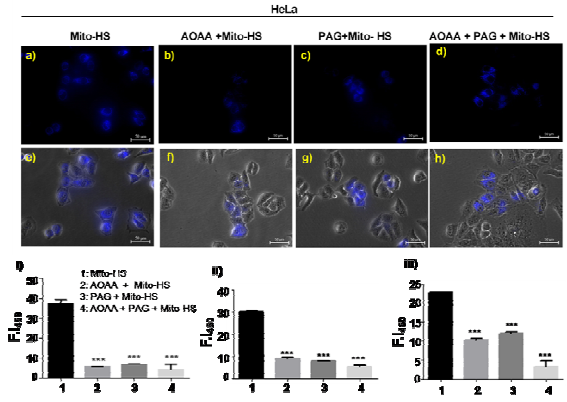


Fig. 3 Cellular fluorescence images of HeLa cells. (a) **Mito-HS** (5 μM) incubated for 15 min; (b) cells were pretreated with AOAA (2 mM), (c) PAG (2 mM) separately and (d) with both (2 mM each) for 1h and then incubated with **Mito-HS** (5 μM) for 15 min; (e-h) are overlay DIC images of corresponding fluorescence images. Scale bar, 50 μm. Images were obtained using excitation wavelengths of 390 nm with the emission being monitored over the 440–500 nm. Quantification of fluorescence intensity of **Mito-HS**, **Mito-HS** with inhibitors, AOAA (2 mM), **Mito-HS** with PAG (2 mM) and both AOAA & PAG (2 mM each) treated HeLa (i), MDA-MB-231 (ii), and DU145 (iii) cells using ImageJ software. The representative data is provided from 3 independent set of experiments. Scale bar 50 μm

presence of individual inhibitors (AOAA and PAG) or a combination of both (Fig. 3i). Whereas, individually AOAA and PAG partially inhibited endogenous H_2S formation in both MDA-MB-231 and DU145 cells respectively. Thus, the extent of fluorescence labeling of **Mito-HS**-pretreated MDA-MB-231 and DU145 cells were only ~2-3-fold reduced (Fig. 3ii-iii and Fig. S25). Interestingly, a combination of AOAA and PAG (2.0 mM each) substantially inhibited H_2S production by ~ 7.5- fold in these cells (Fig. 3ii-iii). These findings suggest that H_2S producing enzymes *i.e.* cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CSE) are more abundant in cancer cells e.g. HeLa, MDA-MB-231, and DU145 cells when compared to the normal 3T3-L1 cells. Furthermore, a detailed investigation is required for understanding the pathway of H_2S biosynthesis in cancer cells; surely it will pave the way to a new guideline in cancer therapy and targeted drug discovery research.

Finally, we evaluated whether triphenyl phosphonium moiety in **Mito-HS** guides it to localize in mitochondria or not.[18] For this, we carried out a co-localization experiment with the mitochondrial targeting dye, **MitoSOX Red** From Fig. 4c, we observed that fluorescence image of **Mito-HS** treated HeLa cells co-localized with the mitochondrial targeting dye, **MitoSOX Red.** Furthermore, the 3-D

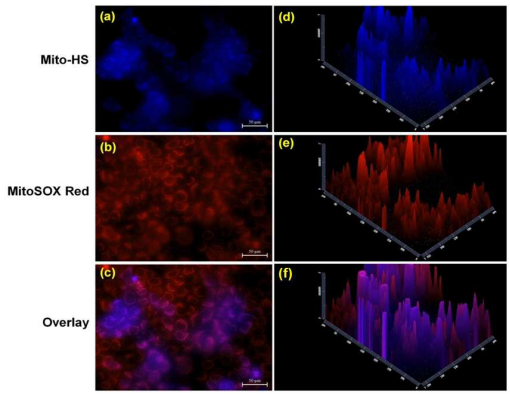


Fig. 4 Fluorescence and co-localization images of **Mito-HS** with **MitoSOX Red**. The HeLa cells were co-treated with **Mito-HS** (5 μM) and **MitoSOX Red** (250 nM) for 15 min. The images were collected at (a) ex. 390 nm/ em. 440-500 nm, (b) ex.510 nm/ em.580 nm (c) overlay image of (a) and (b); (d-f) are 3D images of the cells.

co-localized images in Fig. 4f indicates that **Mito-HS** has predominantly localized in mitochondria. Similar results were obtained in MDA-MB-231 and DU145 cells (Fig. S26). These findings strongly recommend that **Mito-HS** is a first such kind of probe which aids us for tracking mitochondrial H_2S formation in cancer cells.

In conclusion, we describe the synthesis, characterization of probe **Mito-HS** and its optical modulated validation of mitochondrial H_2S formation in living cells. The probe **Mito-HS** was synthesized in four successive steps and characterized by various spectroscopic techniques. **Mito-HS** showed a remarkable UV-vis. absorption increment at λ_{ab} 395 nm in the presence of H_2S. Moreover, the fluorescence intensity (λ_{em} 450 nm) of **Mito-HS** was ~43-fold enhanced in the presence of H_2S (200 μM). The sensitivity of **Mito-HS** toward H_2S was found to be as low as 24.3 nM. The probe **Mito-HS** showed high chemoselectivity toward H_2S over other competitive ubiquitous entities in the living system. The comparative fluorescence images in living cells implied that **Mito-HS** is able to detect H_2S formation in cancer cells (HeLa, MDA-MB-231 and DU145 cells). **Mito-HS** is the first probe, which can rapidly (15 min.) sense endogenous H_2S formation in cancer cells without adding any external stimulators. By inhibition of cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CSE), the extent of fluorescence labeling of HeLa, MDA-MB-231, and DU145 cells by

ChemComm Accepted Manuscript

View Article Online
DOI: 10.1039/C7CC05339H

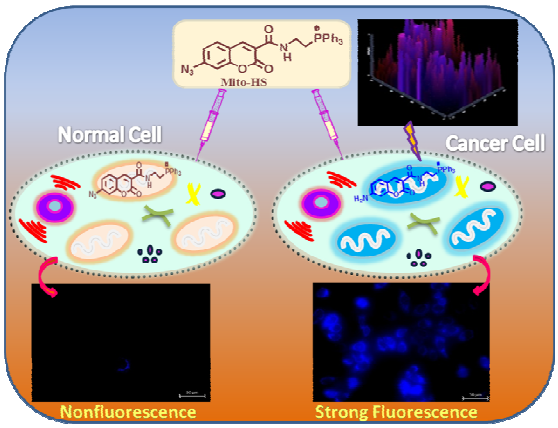
Mito-HS decreased ~7-8-fold; which indicates that cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CSE) are the key H_2S forming enzymes in these cancer cells. The co-localization of **Mito-HS** with **MitoSOX- Red** indicates that **Mito-HS** predominantly monitored H_2S formation in mitochondria. Based on the endogenous H_2S tracking ability of **Mito-HS**, it would be a promising biomarker for distinguishing cancer cells from normal cells. Furthermore, the study encourages for developing H_2S producing enzyme inhibitors as targeted cancer therapeutics.

SB thankful to DST-SERB, India for a research grants (ECR/2015/00035). N.M. is thankful to DST (SB/FT/LS-144/2012). A.B. is supported by UGC-JRF/SRF fellowship (F.2/2012(SA-I) D.N. is supported by ICMR- JRF/SRF fellowship (20097).

Notes and references

1 H. Kimura, *Amino Acids,* 2011, **41**, 113.
2 S. Singh and R. Banerjee, *Biochim. Biophys. Acta,* 2011, **1814**, 1518.
3 (*a*) M. Ishigami, K. Hiraki, K. Umemura, Y. Ogasawara, K. Ishii and H. Kimura, *Antioxid. Redox. Signal,* 2009, **11**, 205; (*b*) G. A. Benavides, G. L. Squadrito, R. W. Mills, H. D. Patel, T. S. Isbell, R. P. Patel, V. M. Darley-Usmar, J. E. Doeller and D. W. Kraus, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 17977.
4 M. C. Pitcher and J. H. Cummings, *Gut*, 1996, **39**, 1.
5 K. Eto, T. Asada, K. Arima, T. Makifuchi and H. Kimura, *Biophys. Res. Commun.*, 2002, **293**, 1485.
6 (*a*) Y. Kaneko, Y. Kimura, H. Kimura and I. Niki, *Diabetes* 2006, **55**, 1391; (*b*) X. Feng, Y. Chen, J. Zhao, C. Tang, Z. Jiang and B. Geng, *Biochem. Biophys. Res. Commun.*, 2009, **380**,153.
7 J. A. Madden, S. B. Ahlf, M. W. Dantuma, K. Olson and D. L. Roerig, *J. Appl. Physiol.*, 2012, **112**, 411.
8 C. Szabo and M. R. Hellmich, *Cell Cycle*, 2013, **12**, 2915.
9 (*a*) A. R. Lippert, E. J. New and C. J. Chang, *J. Am. Chem. Soc.*, 2011, **133**, 10078; (*b*) H. Peng, Y. Cheng, C. Dai, A. L. King, B. L. Predmore, D. J. Lefer and B. A. Wang, *Angew. Chem. Int. Ed.*, 2011, **50**, 9672; (*c*) Y. Qian, J. Karpus, O. Kabil, S. Y. Zhang, H. L. Zhu, R. Banerjee, J. Zhao and C. He, *Nat. Commun.*, 2011, **2**, 495; (*d*) C. Liu, J. Pan, S. Li, Y. Zhao, L. Y. Wu, C. E. Berkman, A. R. Whorton and M. Xian, *Angew. Chem. Int. Ed.*, 2011, **50**, 10327; (*e*) F. Yu, P. Li, P. Song, B. Wang, J. Zhao and K. Han, *Chem. Commun.*, 2012, **48**, 2852; (*f*) L. A. Montoya and M. D. Pluth, *Chem. Commun.*, 2012, **48**, 4767; (*g*) S. Chen, Z. J. Chen, W. Ren and H. W. Ai, *J. Am. Chem. Soc.*, 2012, **134**, 9589; (*h*) S. K. Das, C. S. Lim, S. Y. Yang, J. H. Han and B. R. Cho, *Chem. Commun.*, 2012, **48**, 8395; (*i*) C. Liu, B. Peng, S. Li, C. M. Park, A. R. Whorton and M. Xian, *Org. Lett.*, 2012, **14**, 2184; (*j*) F. Hou, L. Huang, P. Xi, J. Cheng, X. Zhao, G. Xie, Y. Shi, F. Cheng, X. Yao, D. Bai and Z. Zeng, *Inorg. Chem.* 2012, **51**, 2454; (k) Z. Guodong, H. Wang, Y. Ma and X. Chen, *Tetrahedron*, 2013, **69**, 867; (*l*) V. S. Lin, A. R. Lippert and C. J. Chang, *Proc. Natl. Acad. Sci. U. S. A.,* 2013, **110**, 7131; (*m*) X. Wu, J. Shi, L. Yang, J. Han and S. Han, *Bioorg. Medchem. Lett.*, 2014, **24**, 314; (*n*) T. S. Bailey and M. D. Pluth, *J. Am. Chem. Soc.*, 2013, **135**, 16697; (*o*) M. D. Hammers, M. J. Taormina, M. M. Cerda, L. A. Montoya, D. T. Seidenkranz, R. Parthasarathy and M. D. Pluth, *J. Am. Chem. Soc.*, 2015, **137**, 10216.
10 (*a*) Y. Qian, L. Zhang, S. Ding, X. Deng, C. He, X. E. Zheng, H. L. Zhu and J. A. Zhao, *Chem. Sci.*, 2012, **3**, 2920; (*b*) X. Feng, T. Zhang, J. T. Liu, J. Y. Miao and B. X. Zhao, *Chem. Commun.*, 2016, **52,** 3131; (*c*) K. Zhang, J. Zhang, Z. Xi, L.-Y. Li, X. Gu, Q.-Z. Zhang and L. Yi, *Chem. Sci.*, 2017, **8**, 2776.
11 (*a*) X. Chen, S. Wu, J. Han and S. Han, *Bioorg. Medchem. Lett.*, 2013, **23**, 5295-5299; (*b*) S. K. Bae, C. H. Heo, D. J. Choi, D. Sen, E. H. Joe, B. R. Cho and H. M. Kim, *J. Am. Chem. Soc.*, 2013, **135**, 9915; (*c*) L. Yuan and Q. P. Zuo, *Chem. Asian. J.*, 2014, **9**, 1544; (*d*) X. Wang, J. Sun, W. Zhang, X. Ma, J. Lv and B. Tang, *Chem. Sci.*, 2013, **4**, 2551; (*e*) Y. Chen, C. Zhu, Z. Yang, J. Chen, Y. He, Y. Jiao, W. He, L. Qiu, J. Cen and Z. Guo, *Angew. Chem. Int. Ed.*, 2013, **52**, 1688; (*f*) X. L. Liu, X. J. Du, C. G. Dai and Q. X. Song, *J. Org. Chem.*, 2014, **79**, 9481; (*g*) C. Zhang, R. Wang, L. Cheng, B. Li, Z. Xi and L. Yi, *Sci. Rep.*, 2016, **6**, 30148 (1-8).
12 T. M. Hildebrandt and M. K. Grieshaber, *FEBS. J.*, 2008, **275**, 3352-3361.
13 C. Szabo, C. Coleata, C. Chao, K. Modis, B. Szczesny, A. Papapetropoulos and M. R. Hellmich, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, 12474.
14 (*a*) G. Yang, L. Wu, B. Jiang, W. Yang, J. Qi, K. Cao, Q. Meng, A. K. Mustafa, W. Mu, S. Zhang, S. H. Snyder and R. Wang, *Science*, 2008, **322**, 587; (*b*) A. Papapetropoulos, A. Pyriochou, Z. Altaany, G. Yang, A. Marazioti, Z. Zhou, M. G. L. K. Jeschke, Branski, D. N. Herndon, R. Wang and C. Szabo, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 21972; (*c*) K. Abe and H. Kimura, *J. Neurosci.*, 1996, **16**, 1066.
15 H. A. Henthorn and M. D. Pluth, *J. Am. Chem. Soc.*, 2015, **137**, 15330.
16 (*a*) D. N. Heo, D. H. Yang, H. J. Moon, J. B. Lee, M. S. Bae, S. C. Lee, W. J. Lee, I. C. Sun and I. K. Kwon, *Biomaterials*, 2012, **33**, 856; (*b*) K. Y. Yasoda, K. N. Bobba, D. Nedungadi, D. Dutta, M. S. Kumar, N. Kothurkar, N. Mishra and S. Bhuniya, *RSC Adv.*, 2016, **6**, 62385.
17 (*a*) C. A. Schneider, W. S. Rasband and K. W. Eliceiri, *Nat. Methods,* 2012, **9**, 671; (*b*) D. Dutta, S. M. Alex, K. N. Bobba, K. K. Maiti and S. Bhuniya, *ACS. Appl. Mater. Interfaces, 2016,* **8**, 33430.
18 K. Sunwoo, K. N. Bobba, J.- Y. Lim, T. Park, A. Podder, J. S. Heo, S. G. Lee, S. Bhuniya and J. S. Kim, *Chem. Commun.*, 2017, **53**, 1723.

TOC



Published on 17 July 2017. Downloaded by Cornell University Library on 17/07/2017 14:30:37.
ChemComm Accepted Manuscript

This journal is © The Royal Society of Chemistry 20xx