Multicomponent reactions for the synthesis of multifunctional agents with activity against cancer cells†

Saad Shabaan,[a] Lalla A. Ba,[a] Muhammad Abbas,[ab] Torsten Burkholz,[a] Annika Denkert,[b] André Gohr,[b] Ludger A. Wessjohann,[b] Florenz Sasse,[c] Wolfgang Weber[d] and Claus Jacob*[a]

Received (in Cambridge, UK) 24th December 2008, Accepted 4th June 2009
First published as an Advance Article on the web 22nd June 2009
DOI: 10.1039/b823149d

Multicomponent Passerini and Ugi reactions enable the fast and efficient synthesis of redox-active multifunctional selenium and tellurium compounds, of which some show considerable cytotoxicity against specific cancer cells.

Oxidative stress (OS) is a biochemical condition characterized by a disturbed, oxidizing intracellular redox environment. It is found in many diseases, among them various types of cancer, where it is associated with an increase in concentrations of reactive oxygen species (ROS). Certain redox-active agents are able to 'modulate' OS present in such cancer cells.[1,2] These compounds induce sharp *increases* in intracellular levels of ROS or catalyze the oxidation of redox sensitive cysteine proteins and enzymes. Ultimately, such processes may result in cancer cell death. Among the 'redox modulators' considered to date, agents combining two or more redox centers in one molecule have shown considerable promise.[1,2] These compounds are designed to recognize the 'biochemical signature' of OS in cancer cells—and therefore may act efficiently and selectively against those cells. They may, for instance, simultaneously increase levels of ROS and catalyze the oxidation of cysteine proteins in the presence of H_2O_2. Due to their unique redox and catalytic properties, compounds containing selenium and tellurium are of particular interest.[1,2] Unfortunately, the synthesis of such agents is far from trivial.[1–4] This chemistry is frequently marred by decomposition of products, difficult purification processes and low yields. It is often quite cumbersome to synthesize complicated organo-selenium and tellurium compounds, such as highly functionalized, biochemically 'multifunctional' agents.[1,5,6]

In theory, the most promising synthetic routes to combine functionalized selenium and tellurium agents might lead *via* isonitrile multicomponent reactions (IMCRs), such as the Passerini and Ugi reactions.[7] Here, one may use specially designed building blocks carrying just one or two functions, which could be combined to form larger, highly functionalized molecules with four or more biologically interesting sites. Ultimately, such molecules should recognize OS in cancer cells with great precision and selectivity. In reality, matters are more complicated. This is due to the need for appropriate building blocks, some of which have to be developed first. Here, we report the first selected examples of multifunctional selenium and tellurium agents which have been obtained *via* the Passerini and Ugi reactions. In the case of tellurium, these are to the best of our knowledge also the first examples where this element participates in such IMCRs. Some of the agents obtained *via* this synthetic route have been screened for biological activity and show considerable, yet selective, toxicity against certain types of cancer cells.

The Passerini reaction is a three component reaction combining an acid, aldehyde, and isonitrile to form an α-acyloxy amide, while the Ugi reaction is a four component reaction (acid, aldehyde, isonitrile, and amine) leading to amide-bonded (α-aminoacyl amide) structures.[8,9]

Fig. 1 shows the acids (**1a–d**), aldehydes (**2a–c**), isonitriles (**3a–e**), and the amine (**4**) used in this study. Most of the building blocks carry one relevant redox or metal binding site, some of them, such as **1b** and **2c**, even two (quinone, plus sulfur or selenium). Other building blocks are more difficult to 'functionalize': in the case of isonitrile, commercial *tert*-butyl isonitrile (**3a**) is frequently used.[10] To turn this apparent limitation of the isonitrile building block into an advantage, we have devised chalcogen bearing isonitriles suitable for multicomponent chemistry, including the tellurium containing isonitrile **3b** (Fig. 1, see ESI† for details regarding synthesis). This approach has two major benefits. Firstly, we are now able

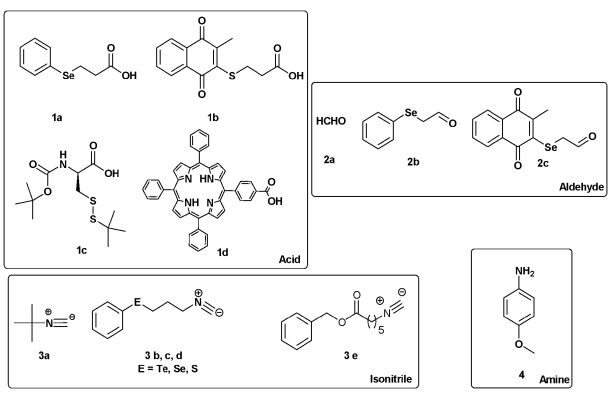

Fig. 1 Building blocks of the Passerini and Ugi reactions used to synthesize multifunctional redox agents.

[a] *Division of Bioorganic Chemistry, School of Pharmacy, Saarland University, D-66123 Saarbruecken, Germany. E-mail: c.jacob@mx.uni-saarland.de; Fax: +49 681 302 3464; Tel: +49 681 302 3129*
[b] *Department of Bioorganic Chemistry, Leibniz Institute of Plant Biochemistry, Weinberg 3, D-06120 Halle (Saale), Germany*
[c] *Department of Chemical Biology, Helmholtz Centre for Infection Research, Inhoffenstrasse 7, D-38124 Braunschweig, Germany*
[d] *Department of Experimental Medicine, Biochemistry I, University Hospital Hamburg-Eppendorf, D-20246 Hamburg, Germany*
† Electronic supplementary information (ESI) available: Synthetic procedures, analytical data for each of the products and cancer cell screening data. See DOI: 10.1039/b823149d

to introduce phenyl telluride (and other phenyl chalcogenides) into molecules *via* IMCRs. Secondly, the synthetically more accessible acid, aldehyde, and amine building blocks remain variable and can be used to contribute further functionalities to the multicomponent reaction product.

This allows us to generate selenium and tellurium containing molecules, which combine two, three or even four redox sites in one molecule at will. A selection of products is shown in Fig. 2, with Table 1 providing an overview of building blocks used, reaction conditions, and yields obtained (details of the synthesis and analytical data are provided in the ESI†). One may note the mild reaction conditions employed (water as solvent, room temperature) and the good yields obtained (*e.g.* up to 96% for tellurium compound **10**).[11] Both aspects of the IMCR compare highly favorably to conventional methods employed in selenium and tellurium synthesis, such as the ones used previously to synthesize quinone–chalcogen redox agents.[1] The synthetic approach described here together with the availability of a large and diverse arsenal of redox-active acids, aldehydes, isonitriles and amines—many of which are suitable building blocks—enables the design and synthesis of an unprecedented range of highly functionalized redox agents. This may ultimately also allow QSAR-relationships, for instance for cancer-type selective targeting.

The comparably straightforward synthesis of such multi-functional agents does not, of course, address the question if such rather large and complex agents are at all useful in cancer therapy. We have therefore performed the thiophenol assay, which measures catalytic activity of compounds in the presence of thiols and H_2O_2. This assay has been used as a predictor of activity in cell culture.[1] The results shown in Table 1 confirm that all compounds enhance the oxidation of thiols in the presence of H_2O_2. Several compounds are even considerably more active than the benchmark compound ebselen (1.5-fold increase *vs.* DMSO), with tellurium compounds **10** and **11** being the most active. These findings are in excellent agreement with previous studies emphasizing the activity of tellurium agents in this assay. In order to rule out any major antioxidant activity (which may be counter-productive), the thiobarbituric acid (TBA) assay has also been performed (see ESI† for details). This assay measures the ability of compounds to sequester oxygen-based radicals. Most compounds were not particularly active in this assay. Only the tellurium compounds **10** and **11** showed a significant activity, probably due to the higher reducing power of tellurides compared to selenides. Interestingly, HRMS also gave signals for telluroxides, but not selenoxides, pointing towards a ready oxidation of the tellurium compounds. Most compounds complied with Lipinski's rules of five regarding hydrogen donors and acceptors, and were only slightly over a molecular mass of 500 (**9** being an exception). Importantly, partition coefficients $\log P_{OW}$ were in the range of 1.40 to 4.23, *i.e.* above −0.4 and well below 5 (see ESI†).

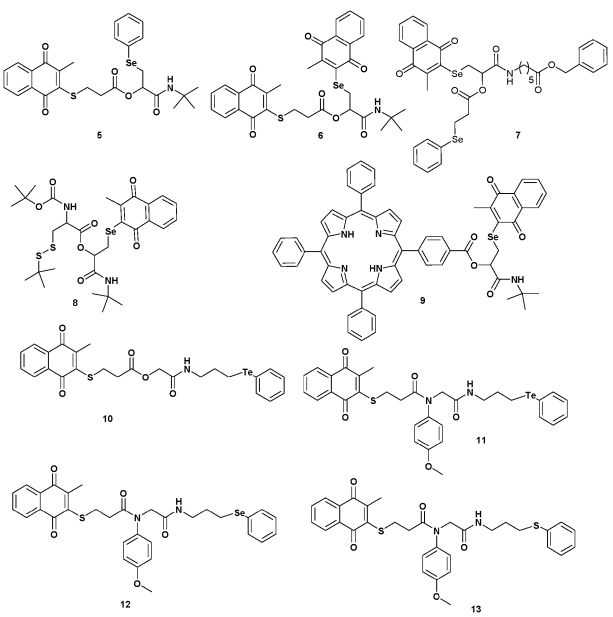

Fig. 2 Multifunctional redox agents synthesized employing the Passerini and Ugi reactions. Experimental details are provided in the text, in Table 1 and as part of the ESI†.

Based on the rather promising estimates obtained *in vitro*, some of the compounds were studied in cell culture. Here, we briefly present and discuss the results for compounds **5**, **6** and **8** obtained in three rather distinct assays which in combination provide some information regarding cytotoxicity and selectivity: firstly, an IC_{50} determination in cell culture employing cancer-like, permanently growing L-929 murine fibroblast cells and A-431 human epidermal tumor cells—both of which are commonly used to screen for cytotoxicity against mammalian cells. Secondly, a toxicity screen in cultured PC-3 human prostate cancer cells. Thirdly, a single- and five-dose screen in 58 tumor cell lines clustered in cells representing leukemia, non-small cell lung cancer, colon cancer, cancer of the central nervous system, melanoma, ovarian cancer, renal cancer, prostate cancer and breast cancer, performed independently by the National Cancer Institute (NCI) of the National Institute of Health (NIH) in Bethesda, MD (US), to estimate selectivity and to identify possible cancer targets. The results obtained in L-929 cells are presented as example in Fig. 3; the other cell culture results are provided in the ESI†.

Interestingly, compounds **6** and **8** showed promising results in the L-929, A-431 and PC-3 assays as well as in the 58 cell line screen whereas **5** was less active. In the L-929 cell assay, **8** killed these cells rather efficiently with an IC_{50} value of 0.7 μM (Fig. 3). A comparable IC_{50} value was obtained for the Passerini diquinone **6** (1.1 μM), while **5** was considerably less active (see Fig. 3). **8** was also rather active in the A-431 cell line, with an IC_{50} of 5 μM. **5** and **6** were less active in this cell line (IC_{50} of 135 μM and 55 μM, respectively). In PC-3 cells, concentrations of 10 nM of **5**, **6** and **8** reduced cell survival to approximately 80%, while a concentration of 10 μM was quite toxic, *e.g.* reducing survival to 10% for compound **8** (see ESI†). These numbers are promising but do not indicate if this is based on a general cytotoxicity, or if these compounds possess selectivity against certain types of cancer cells which may be useful for future therapy. These questions were addressed by the 58 cell line screen. Compounds **6** and **8** were most active in the 10 μM one-dose screen and were selected for five-dose testing (for results see ESI†). In contrast, quinone-sulfide **5** was less active. LC_{50} values for **6** and **8** in the 58 cell lines were generally around 1–10 μM, which is in good agreement with

Table 1 Building blocks and solvents used for the synthesis of **5–13**. Yields, number of redox centers and activity in the thiophenol (PhSH) assay (normalized for DMSO) are given (including porphyrin in **9** designed to complex iron or copper ions inside the cell). Experimental details and analytical data are provided in the ESI†. (n.d.: not determined)

Product	Starting materials	Solvent	Yield (%)	No. of redox centers	PhSH-assay
5	**1b**, **2b**, **3a**	H_2O	68	3	1.32
6	**1b**, **2c**, **3a**	H_2O	76	4	1.81
7	**1a**, **2c**, **3e**	H_2O	79	3	n.d.
8	**1c**, **2c**, **3a**	H_2O	54	3	2.09
9	**1d**, **2c**, **3a**	$CHCl_3$	86	3	1.95
10	**1b**, **2a**, **3b**	$CHCl_3$	96	3	5.09
11	**1b**, **2a**, **3b**, **4**	$CHCl_3$	81	3	4.73
12	**1b**, **2a**, **3c**, **4**	$CHCl_3$	92	3	1.23
13	**1b**, **2a**, **3d**, **4**	$CHCl_3$	88	3	1.14

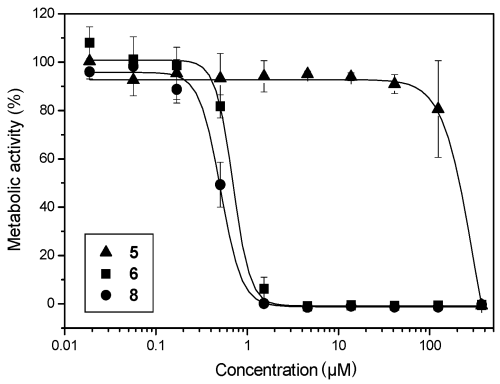

Fig. 3 Cytotoxicity of compounds **5**, **6**, and **8** in L-929 murine fibroblasts. Viable cells were measured after 5 days of incubation using an MTT assay (see ESI† for details).

the L-929, A-431 and PC-3 assay. Importantly, some cell lines were particularly responsive, such as HL-60 leukemia cells and some non-small cell lung cancer cells, while others were more resistant. A COMPARE analysis indicates that the activity profile of **6** and **8** correlates best with one of the known anthracycline-based redox cyclers, such as menogaril and daunomycin derivatives (see ESI†).[12] While these correlations are moderate and hence may point towards a class of agents with novel activity, they also support the notion that **6** and **8** may act as intracellular redox cyclers.

Compounds **6** and especially **8** are therefore among the most promising selenium compounds tested by us to date. Interestingly, both feature a quinone–selenide moiety whereby the Se atom is attached directly to the quinone ring. This constellation may possibly result in some synergistic effects between the two redox sites. It may, for instance, allow the formation of unusually highly oxidized species, such as a quinone–selenoxide, or increase the HOMO-energy and thus reducing power in the hydroquinone/semiquinone states. This quinone–selenide feature is notably absent in **5**, which may explain the differences in toxicity observed.

Further and considerably more detailed studies are required to explore these questions. Nonetheless, the studies presented here have shown that it is possible to generate a wide range of diverse and highly functionalized redox agents designed to modulate the redox environment of cells with comparable ease employing multicomponent reactions. While their precise mode(s) of biochemical action need to be investigated further, there is little doubt that some of these Passerini or Ugi products obtained are biologically active at low concentrations—and that their activity is not indiscriminate, but cell type specific. This is also likely to be the case for the new tellurium agents, such as **10** and **11**, which can now be obtained *via* this comparably simple multicomponent method, and which are of special interest as redox modulators.

The authors thank the University of Saarland, Ministry of Economics and Science of Saarland, Deutsche Forschungsgemeinschaft (DFG grant JA1741/2-1), European Union (ITN RedCat, 215009), National Cancer Institute and the government of Egypt for financial support.

Notes and references

1 G. I. Giles, N. M. Giles, C. A. Collins, K. Holt, F. H. Fry, P. A. S. Lowden, N. J. Gutowski and C. Jacob, *Chem. Commun.*, 2003, 2030–2031.
2 F. H. Fry, A. L. Holme, N. M. Giles, G. I. Giles, C. Collins, K. Holt, S. Pariagh, T. Gelbrich, M. B. Hursthouse, N. J. Gutowski and C. Jacob, *Org. Biomol. Chem.*, 2005, **3**, 2579–2587.
3 L. A. Wessjohann and A. Schneider, *Chem. Biodiversity*, 2008, **5**, 375–388.
4 A. Schneider, W. Brandt and L. A. Wessjohann, *Biol. Chem.*, 2007, **388**, 1099–1101.
5 N. Petragnani and H. Stefani, *Tellurium in Organic Synthesis*, Elsevier, London, 2nd updated and enlarged edition, 2007.
6 G. Zeni, D. S. Lüzdtke, R. B. Panatieri and A. L. Braga, *Chem. Rev.*, 2006, **106**, 1032–1076.
7 M. Abbas, J. Bethke and L. A. Wessjohann, *Chem. Commun.*, 2006, 541–543.
8 L. Banfi, A. Basso, G. Guanti and R. Riva, in *Multicomponent Reactions*, ed. J. Zhu and H. Bienaymé, Wiley-VCH, 2005, ch. 1, pp. 1–29.
9 A. Dömling and I. Ugi, *Angew. Chem., Int. Ed.*, 2000, **39**, 3168–3210.
10 I. Ugi, B. Werner and A. Dömling, *Molecules*, 2003, **8**, 53–66.
11 M. C. Pirrung and K. Das Sarma, *J. Am. Chem. Soc.*, 2004, **126**, 444–445.
12 F. M. Muggia and M. D. Green, *Crit. Rev. Oncol. Hematol.*, 1991, **11**, 43–64.

