Exploring synthetic avenues for the effective synthesis of selenium- and tellurium-containing multifunctional redox agents†

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Various human illnesses, including several types of cancer and infectious diseases, are related to changes in the cellular redox homeostasis. During the last decade, several approaches have been explored which employ such disturbed redox balances for the benefit of therapy. Compounds able to modulate the intracellular redox state of cells have been developed, which effectively, yet also selectively, appear to kill cancer cells and a range of pathogenic microorganisms. Among the various agents employed, certain redox catalysts have shown considerable promise since they are non-toxic on their own yet develop an effective, often selective cytotoxicity in the presence of the 'correct' intracellular redox partners. Aminoalkylation, amide coupling and multicomponent reactions are suitable synthetic methods to generate a vast number of such multifunctional catalysts, which are chemically diverse and, depending on their structure, exhibit various interesting biological activities.

1. Introduction

All living organisms need to maintain a healthy intracellular redox balance in order to survive and to proliferate.[1,2] Not surprisingly, the maintenance of a healthy redox balance has been a matter of intense investigation during the last fifty years, and there have been numerous attempts to interfere with the redox state of the cell, for instance by administering antioxidants to counteract Oxidative Stress (OS). More recently, it has become apparent that certain imbalances in the intracellular redox state are not just detrimental, but may actually be exploited for therapeutic purposes. Similar to cancer-cell-specific receptors or protein expression profiles, unusual intracellular redox states may be used to 'single out' and kill target cells.[3]

A range of studies conducted during the last ten to fifteen years have demonstrated that many different types of cancer cells proliferate under conditions of OS, among them lung, kidney, prostate and skin cancer cells.[4,5] The disturbed redox balance in those cells may have several causes, including an increased metabolic activity, increased activities of pro-oxidant enzymes, down-regulation of antioxidant enzymes and inflammation around a tumour site with production of Reactive Oxygen Species (ROS).‡ Strategies exploiting OS in and against such cancer cells involve compounds able to generate ROS (*e.g.* As_2O_3), superoxide dismutase (SOD) inhibitors (*e.g.* 2-methoxyestradiol) and, perhaps most interestingly, the employment of catalysts able to convert OS in cancer cells into a lethal cocktail of cytotoxic chemical species.[7,8] At the same time, many unpleasant parasitic microorganisms, such as dermatophytes, plasmodia and bacteria, possess an intracellular redox network that differs significantly from the one found in the human cell.[9] In principle, such organisms may also be targeted with redox modulating agents.

OS is a 'multi-stressor event' which includes various ROS (*e.g.* $O_2^{\bullet -}$, H_2O_2), but also a range of nitrogen species and adventitious metal ions (*e.g.* iron and copper).[1,2] High efficiency and selectivity of compounds may therefore largely depend on their ability to 'recognize' the biochemical signature of OS in the cell, *i.e.* to respond to two or more of the ingredients of OS, and not just to one. Several attempts have been made to develop multifunctional agents able to recognize *various* ingredients of OS.[10,11] Unfortunately, the synthesis of such compounds encounters increasing difficulties when moving from just one or two to three or more redox sites – in particular if a combination of quinone, macrocycle, selenium and/or tellurium is involved. To resolve this obstacle, various synthetic avenues have been explored in order to synthesize compounds with three or even four OS-related functions. Depending on their structure and reactivity, several interesting lead compounds have now been identified

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‡ By contrast, cells found in hypoxic regions of tumours are often more *reducing* than normal cells, which is exploited in bioreductive drug therapy, *e.g.* by using Mitomycin C (MMC).[6] OS and bioreductive environments are not necessarily mutually exclusive.

which exhibit activity against cancer cells, dermatophytes and *Plasmodium falciparum*.

2. Results

2.1 Synthesis of agents combining two or more redox-active and metal binding sites

While there is a strong demand for multifunctional redox agents containing selenium or tellurium, synthetic avenues leading to such agents are often marred by difficulties and low yields. We have therefore explored five different avenues for the synthesis of agents combining two, three or even four redox/metal binding sites in one molecule.

2.1.1 Nucleophilic substitution by a redox-active chalcogen moiety at a quinone core structure. We have previously reported a method which relies on the direct attack of nucleophilic selenolate or tellurolate compounds at suitable bromoquinones.[10,11] This approach has enabled us to generate several compounds combining two redox centres relevant to OS, albeit in rather low yields (Fig. 1). In order to increase yields, the original method was improved by employing an heterogeneous solvent system (water and ethyl acetate (1:1)) and a phase transfer catalyst (PTC, tricaprylmethylammonium chloride). Under these conditions, the reaction proceeds in a more controlled manner, appears to avoid side-reactions and results in significantly higher yields. In the case of compound **1**, for instance, yields of 78–98% have been obtained with the two-phase-system, compared to a yield of just 9% in the original method. Related compounds, such as **2** and **3**, could also be obtained by this two-phase method and in similar yields. While this one-step synthetic method is attractive because of its comparable simplicity, its scope appears to be limited by the chemical diversity and number of compounds achievable.

2.1.2 Aminoalkylation. To overcome these limitations, aminoalkylation of the quinone by various chalcogen-containing amines has been explored (Fig. 2, **4–6**).[12,13] This reaction proceeds *via* nucleophilic attack of the amine-based nucleophile at the quinone. Mechanistically it resembles to the method described in section 2.1.1, with two significant differences: Firstly, aminoalkylation is a Michael addition reaction targeting quinones, and not bromoquinones. Secondly, the reactivity is controlled by the amine and not by the chalcogen (yet see below). This method was employed successfully to synthesize compounds combining a naphthoquinone with one or two sulfur or selenium moieties in rather good yields (20–35%). In the case of compounds **4** and **5**, two redox-centres – *i.e.* one quinone and a sulfur or selenium atom – are present, while compound **6** even combines three redox sites in one molecule. Although this method shows considerable promise for the synthesis of multifunctional sulfur and selenium agents, it could not (yet) be used for the synthesis of the corresponding tellurium analogues. One may speculate that the long reaction times of three days, combined with the oxidizing conditions required to re-oxidize the semi- or hydroquinone to quinone (*i.e.* presence of air), may adversely affect the tellurium moiety.

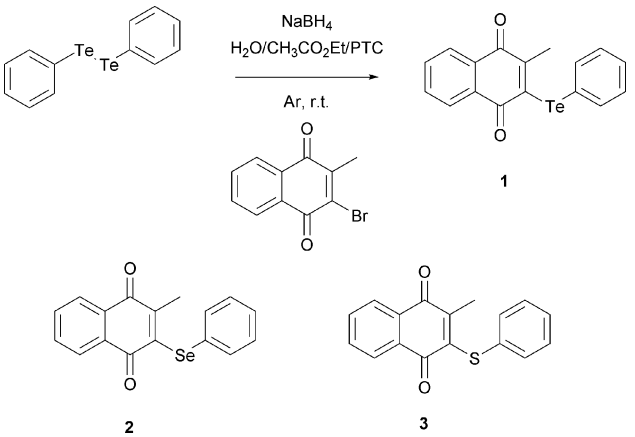

Fig. 1 Schematic overview of the synthetic method employing a bromoquinone and various chalcogen-based nucleophiles. A selection of compounds synthesized according to this method is shown. Experimental details are provided in the text and the ESI.†

2.1.3 Reductive amination. Alternative methods for 'coupling together' individual functional groups under mild conditions have therefore been explored. Reductive amination involves the reaction of amine and aldehyde to imine with subsequent reduction to amine (Fig. 3). This approach appears to be particularly

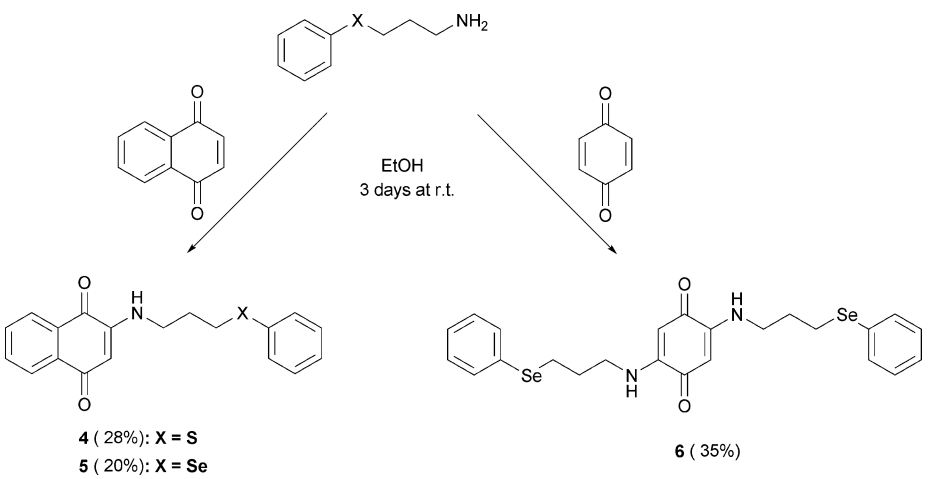

Fig. 2 The aminoalkylation method. A selection of compounds synthesized according to this method is shown. Experimental details are provided in the text and the ESI.†

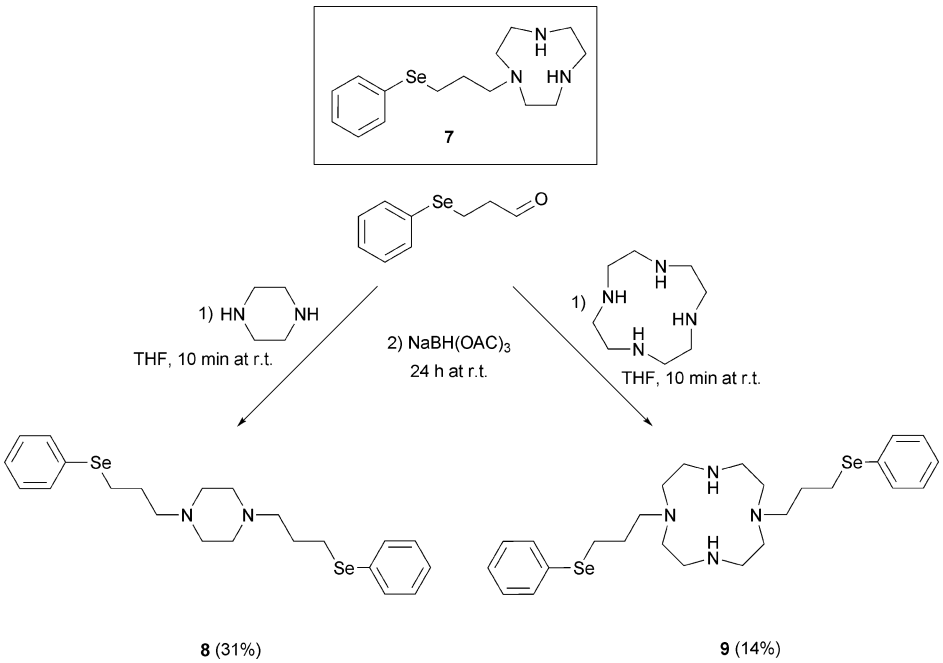

Fig. 3 Reductive amination as a synthetic route for agents incorporating nitrogen-based macrocycles.

suitable for molecules combining redox and metal binding sites, such as compound **7**.[14] Reductive amination takes advantage of the nucleophilic character of amines, which react efficiently with aldehydes under mild conditions and may also form a site for metal binding. Several new compounds containing one or two selenium atoms and a metal binding site (*e.g.* compounds **8** and **9**) were synthesized by this method, with yields around 30%.

2.1.4 Amide coupling of appropriate building blocks. Reductive amination demonstrates that it is possible to employ coupling of two or more pre-formed 'building blocks' for the synthesis of higher functionalized agents. The 'coupling' approach is generally rather promising: By incorporating redox and metal binding centres into different building blocks, it is possible to synthesize the individual blocks separately, even under harsh conditions. They may then be 'mixed and matched' to generate a range of target molecules (*e.g.* a small 'library'), which can be assembled under mild reaction conditions. Importantly, coupling does not have to rely on the chemistry of the redox or metal binding sites themselves, hence avoiding the problems discussed in section 2.1.1.

Alternative coupling methods have therefore been considered. The amide coupling method is based on amide bond formation between acid and amine, a reaction used extensively in peptide synthesis and renowned for its wide applicability and mild conditions (Fig. 4). This method was used to synthesize a range of compounds, some of which are shown in Figs. 4a–c. In the first step, chalcogen-containing amines[15] and quinone-containing carboxylic acid building blocks were obtained: It is possible, for instance, to attach a carbon chain to the quinone *via* oxidative decarboxylation of an acid, such as glutaric acid (Fig. 4a).[16] Similarly, primary amines and thiols may be attached to quinones to form secondary amines and sulfides, respectively (Figs. 4b and 4c). In the second step, amide coupling can be carried out under mild conditions to yield compounds combining two or three 'active' groups. Compounds **14** and **15**, for instance, combine one quinone redox centre with two chalcogen centres, while compound **21** contains two redox centres (quinone and sulfur) and one metal binding site.

Amide coupling works well for sulfur, selenium and tellurium and provides compounds in rather good yields of around 50 to 60% (Fig. 4). The preparation of the individual building blocks and the coupling reaction itself can be performed under rather mild conditions. It is also possible to 'switch' the acid and amine functions, *i.e.* to generate amine-bearing quinones and chalcogen-containing acids. This method is suitable for the creation of a small library of agents, but is limited by the number of building blocks which can be assembled at one time.

2.1.5 Multicomponent Passerini and Ugi reactions. Although amide coupling may be limited by the fact that just two blocks can be combined at a time (more if equivalent sites are present), similar building blocks can be used in multicomponent reactions, such as the three-component Passerini and four-component Ugi reactions (Fig. 5). The Passerini reaction combines an aldehyde, an acid and an isonitrile to an α-acyloxy amide, and the Ugi reaction combines an aldehyde, an acid, an isonitrile and an amine to amide-bonded α-aminoacyl amide structures.[17–20] Although the synthesis of appropriate quinone- and chalcogen-bearing building blocks is not always straightforward, a range of such compounds have now been reported in the literature. Of particular importance is the tellurium-containing isonitrile **23**.[20] By assigning the tellurium moiety to the isonitrile building block, the remaining building blocks, which are chemically more accessible, can be designed at will, for instance to incorporate further exciting functional groups. The building blocks required were synthesized in sufficient yield and purity, with analytical data according to literature.[20] The various building blocks were used to synthesize a series of representative compounds **26–29**, whose chemical structures are shown in Fig. 5. All of these compounds were produced under mild conditions (H_2O as solvent, room temperature) and, from

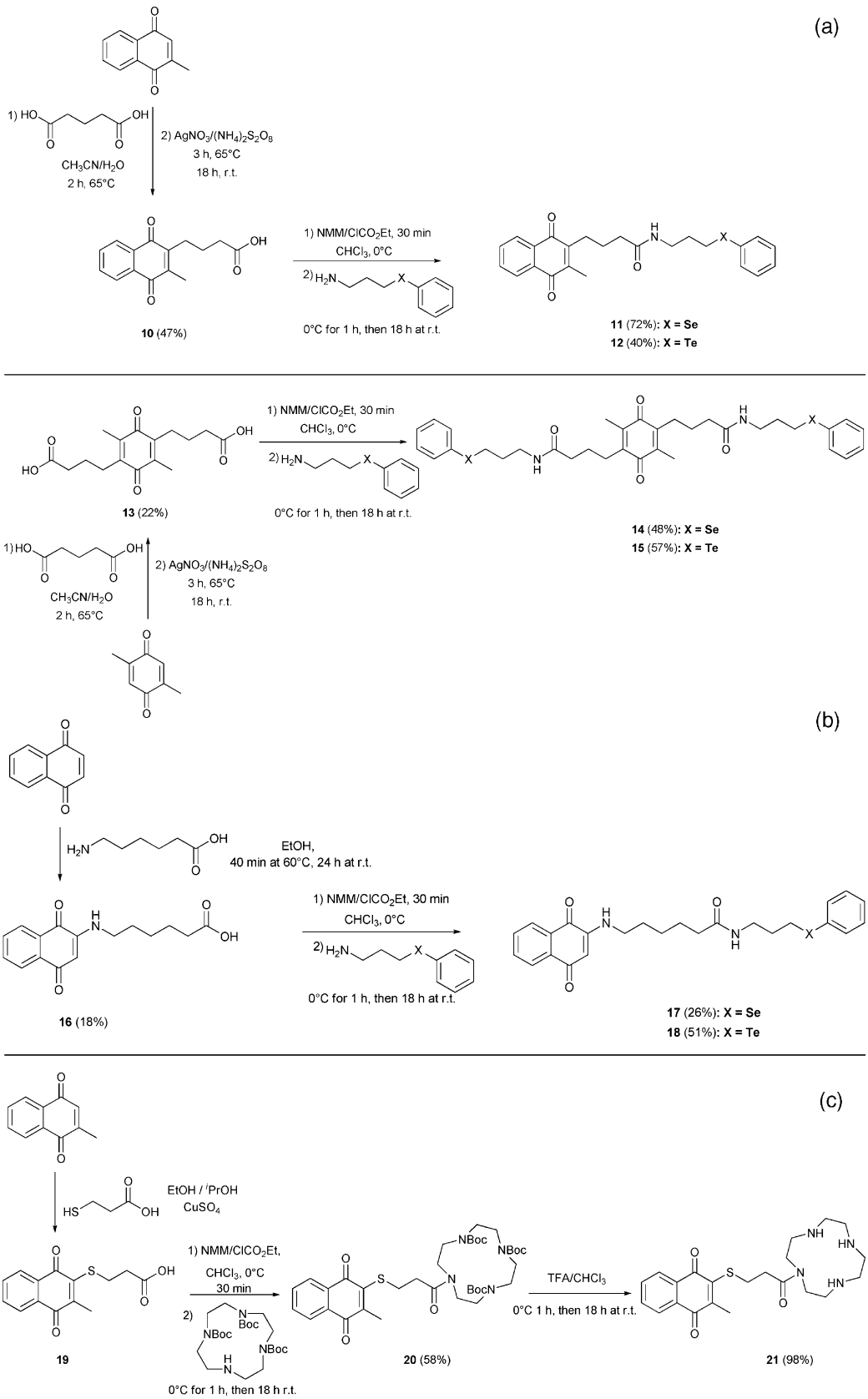

Fig. 4 The two-step amide coupling method, which follows the 'building block' approach, whereby individual components carrying different functional groups are synthesized first and then assembled in a final, straightforward step. This method employs 'linkers', which attach to the quinone *via* a carbon, nitrogen or sulfur atom (Fig. 4a, 4b and 4c, respectively). A selection of compounds synthesized according to this method is shown. Experimental details are provided in the text and the ESI.†

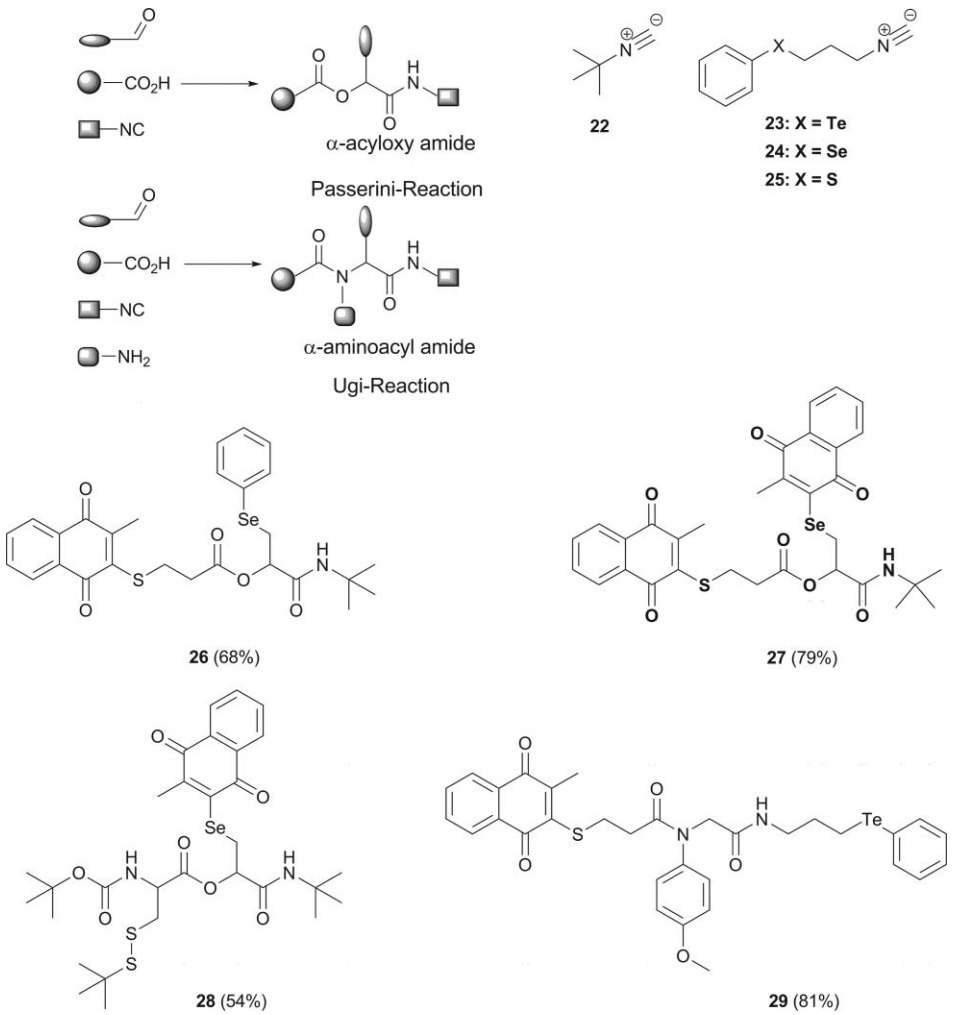

Fig. 5 Schematic overview of the Passerini and Ugi multicomponent reactions. These reactions expand the concept of 'building blocks' and allow a wide range 'mix and match' approach of readily available, functionalized aldehyde, acid, isonitrile and – in the case of the Ugi reaction – amine building blocks. Only a few selected examples of compounds synthesized by this method are shown, with experimental details provided in the text and the ESI.†

the perspective of synthetic selenium and tellurium chemistry, in good yields (54–81%).

2.2 Activity screens in cancer cell lines

Ultimately, it is important to show that the chemistry developed leads to 'useful' molecules. A selection of the structurally most interesting compounds was therefore evaluated for biological activity. Since previous studies have linked combined quinone/chalcogen compounds with (selective) cytotoxicity, the main focus of these studies was on cancer cells and infectious microbes. Due to the fact that selenium compounds are on occasion considered as antioxidants, some of the compounds were also tested for antioxidant activity. It should be emphasized from the outset that the biological data presented here is of an early-stage, preliminary nature, and requires more in-depth pharmacological studies in the future.

2.2.1 Activity in cancer cell line screen. In order to gain an initial insight into the 'usefulness' of multifunctional agents, a broad-spectrum, one-dose (10 μM) cell culture screen was performed at the National Cancer Institute (NCI) of the National Institute of Health (NIH) in Bethesda, MD, USA. This screen includes 58 cell lines grouped into leukaemia, non-small-cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer and breast cancer cell lines.[21,22] In this screen, compounds **18** (see ESI for screening data†), **27** and **28** exhibited a significant cytotoxicity against cancer cell lines and were subsequently selected for 5-dose testing and LC_{50} determination. The LC_{50} values obtained at the NCI were in the range 5–10 μM. It should be noted that compounds **27** and **28** feature a direct attachment of the selenium to the quinone, in contrast to the structurally similar, yet less active **26**, where the selenium atom is located further away from the quinone moiety.

2.2.2 Activity against human SK-Mel-5 melanoma cells. The initial activity screen indicated that compounds combining quinone and chalcogen redox centres may be active against certain types of cancer cells. To investigate this activity further, human SK-Mel-5 melanoma cells were used as model. This cell line was chosen for several reasons. Skin cancer is rather 'easy' to target with drugs, either systemically or by topical applications. Furthermore, the initial screens (section 2.2.1) have already shown

some activity of quinone and/or chalcogen compounds against melanoma cell lines. And finally, multifunctional selenium agents appear to respond well to ROS present in skin cells, as studied previously in a different, antioxidant context in skin fibroblast cells.[14,23]

The SK-Mel-5 cell culture studies have been designed to measure toxicity as well as to evaluate a possible pro- or antioxidative response in the presence of ROS. As a result, compounds can be classified overall as either (a) inactive, (b) generally toxic or (c) toxic in response to elevated levels of ROS. Compounds combining sulfur and quinones, and compounds containing either just quinones or just sulfur were mostly inactive at concentrations up to 200 μM, which was the highest concentration tested. In contrast, compounds containing tellurium, including compounds **12**, **15** and **18** (see section 2.2.1) were generally toxic at concentrations of 5 μM, which was the lowest concentration tested.

The more interesting compounds – exhibiting a differentiated toxicity in response to H_2O_2 – included a range of multifunctional selenium compounds incorporating quinone and macrocycle moieties. Noteworthy in particular are compounds **8** and **21** followed by compounds **7** and **11**. Compounds **7** and **8**, as well as **21**, combine redox catalysis with metal binding properties. These compounds appear to respond well to the presence of OS in form of H_2O_2. This effect is best exemplified by compound **21**, which combines a quinone, sulfur and macrocycle group in one molecule. The results obtained for this compound are shown in Fig. 6: In the absence of H_2O_2, **21** exhibits little toxicity against SK-Mel-5 cells when used in concentrations up to 25 μM (97% cell survival). In the presence of H_2O_2, however, cell survival is reduced dramatically to 38%. This effect is not additive, but due to a synergy of **21** and H_2O_2. The latter enhances the cytotoxicity of **21** by about 2–3-fold.

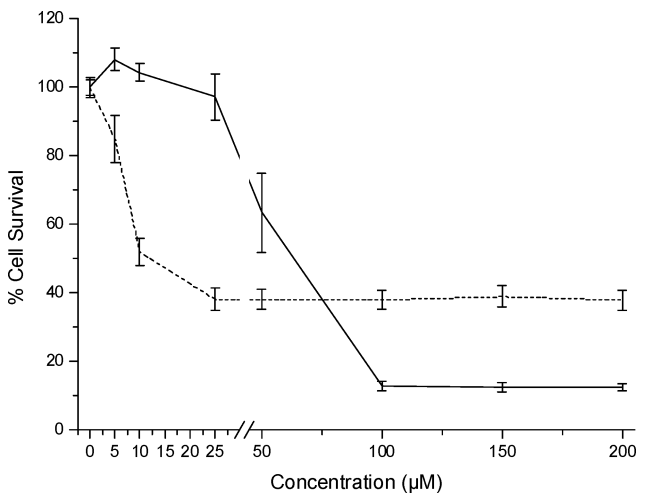

Fig. 6 Cytotoxic effects of compound **21** against SK-Mel-5 cells in the absence (solid line) and presence (dashed line) of H_2O_2. The presence of H_2O_2 enhances the toxicity of **21** about 2–3-fold. Please note that the results are normalized to 100% survival for cells both in the absence and presence of H_2O_2. n = 3.

Similar results were obtained for compound **8**. At concentrations up to 50 μM, **8** is virtually non-toxic against SK-Mel-5 cells in the *absence* of H_2O_2 (91% survival at 25 μM), yet shows a 2–3-fold increased toxicity in the *presence* of H_2O_2 (65% survival at 25 μM). Interestingly, the combination of redox centre(s) with metal binding site(s) appears to be particularly effective (see also section 2.2.4): Compared to **21** and **8**, compounds **7** and **11** were less active – although they still exhibited a significant synergistic effect between compound and H_2O_2.

It should be mentioned that the activity of compounds depends on the cell type used. In HL-60 human leukaemia cells,[24] for instance, some of the compounds tested showed a *lower* toxicity rather than enhanced toxicity in the presence of H_2O_2, which was also lower than the toxicity of H_2O_2 on its own. These compounds include **17**, but also compound **7**, which in SK-Mel-5 cells is *more toxic* in the presence of H_2O_2. Such results underline the differences seen in the 58-cell screen. They also confirm a certain antioxidant effect of **7**, which has been observed previously in skin fibroblasts.[14] Issues surrounding such pro- and antioxidant effects of catalysis are explained in section 3.

Considered together, the results obtained in the cancer cell culture screen and SK-Mel-5 cell line studies point towards a range of different activities. Worth considering are responses towards the presence of OS, either in form of an enhanced cytotoxicity (**8** and **21**) or as possible antioxidants (**7**). It is therefore sensible to expand the scope of biological assays for redox modulators beyond cancer cell lines and to consider other diseases linked to unusual redox states or events, such as infectious diseases.

2.2.3 Activity against *Plasmodium falciparum*. The apicomplexan parasite *Plasmodium falciparum* is a unicellular organism multiplying – after an initial liver stage – in human erythrocytes. *Plasmodium falciparum* is the causative agent of tropical malaria and responsible for approximately 350 million cases of malaria and around one million human deaths each year. Novel drugs, vaccines, and insecticides, as well as deeper insights into parasite biology are essential to counteract increasing drug resistance.[25] As demonstrated by glucose-6-phosphate dehydrogenase deficiency, a genetic disorder protecting from malaria, the parasite host-cell unit is very sensitive to disturbances in its redox balance. This feature is actually shared by many rapidly growing and dividing cells. In *Plasmodium*, the lack of catalase and a typical glutathione peroxidase certainly add to this sensitivity.[26] This also implies that the *redox metabolism* of this organism is an excellent target for anti-malarial drug development.[26,27]

Since both quinones and chalcogens are known to act as redox modulators able to generate ROS, the toxicity of our multifunctional agents against *Plasmodium falciparum* was studied *in vitro*. Most of the compounds exhibited activity in the lower micromolar range, which was not studied in more detail (Table 1). Two compounds combining a naphthoquinone with a selenium or tellurium moiety, however, showed excellent anti-parasitic activity in the nanomolar range (compounds **11** and **12**). The difference in IC_{50} values between the selenium (225 nM) and the tellurium compound (216 nM) was found to be minor, which is equally surprising and important, since tellurium compounds are usually more active when compared to their selenium analogues. In contrast, structurally similar compounds **6**, **17** and **18** were not particularly active in this assay ($IC_{50} > 2$ μM).

2.2.4 Activity against *Trichophyton rubrum*. Finally, a selection of compounds was evaluated for possible activity against the dermatophyte *Trichophyton rubrum*, a parasitic fungus affecting humans.[28] Fungi are, on occasion, sensitive to ROS and, at the same time, require considerable amounts of trace metal ions

Table 1 Activity of selected compounds against *Plasmodium falciparum in vitro*

Compound	IC_{50} on *P. falciparum*
4	>2 μM
5	>2 μM
6	>2 μM
7	>2 μM
8	>2 μM
11	225 nM
12	216 nM
14	>2 μM
15	>2 μM
17	>2 μM
18	>2 μM
21	>2 μM

for normal growth. The use of compounds with a combined ROS generating and metal sequestering activity was therefore envisaged as an alternative to current antifungal treatment. The latter includes the drug ketoconazole, which is taken systemically or topically over a time course of several weeks.

In order to test the compounds in a 'real-life situation', *Trichophyton rubrum* samples were obtained from patients treated at the Dermatology Unit at Saarland University Hospital. As a consequence, each dermatophyte sample differed as to the exact nature of the fungus. The results obtained as part of the antifungal tests were therefore to a certain degree dependent on each patient and their individual dermatophyte. Nonetheless, the results overall point towards a reasonable antifungal activity of compounds **7** and **9** when used at concentrations of 100 μM (or above). Fig. 7 shows a representative concentration-dependent activity of **7** against the fungus. It is worth noting that the benchmark compound ketoconazole is active in these assays at similar concentrations (188.2 μM). The other compounds tested against *Trichophyton rubrum* were inactive at concentrations up to 10 mM. Interestingly,

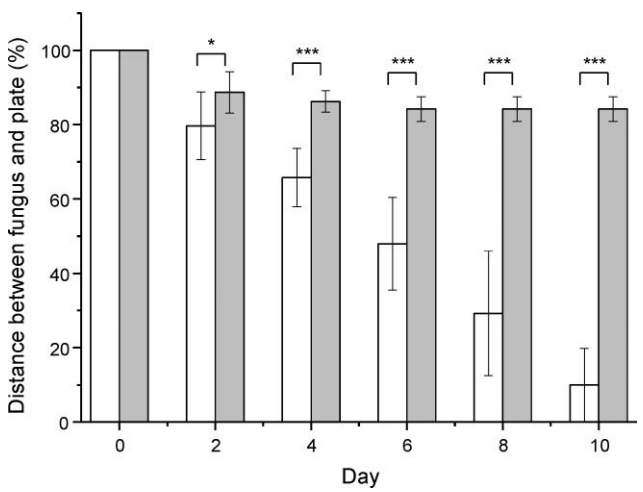

Fig. 7 Activity of compound **7** against *Trichophyton rubrum*. Fungal growth towards the plate containing **7** is inhibited (grey bars). In contrast, the fungus grows readily towards the plate containing the DMSO control, as measured by the (rapidly decreasing) distance between fungus and plate (white bars). Please note that this compound was able to inhibit growth (*i.e.* maintain the distance between the fungus and the point of application) for at least ten days. Students t-test, n = 8, * $P < 0.05$, *** $P < 0.001$. Experimental details are provided in the text and the ESI.†

compound **7** also shows good antioxidant activity, especially on human skin cells, which may provide an interesting lead for a combined antifungal/protective activity on skin (see section 3).

3. Discussion

3.1 Comparison of the synthetic avenues

A range of synthetic methods have been explored which allow the synthesis of highly functionalized redox and metal binding agents. While all of them are, in principle, able to generate molecules combining redox and/or metal binding sites, there are obvious differences between them.

The most basic approach employs direct 'coupling' of the chalcogen (sulfur, selenium, tellurium) to the quinone as part of a simple, one-step procedure. The chalcogen serves as nucleophile and the resulting product features a quinone–chalcogen bond, a structural feature that appears to be particularly active in some of the biological assays. This coupling method can be employed for the synthesis of a range of mono- and di-substituted quinones, including benzo- and naphthoquinones. Good yields (75% and more) may be obtained when the procedure is carried out in a water–ethyl acetate mixture in the presence of a phase transfer catalyst. Nonetheless, this method is ultimately limited in scope by the availability of suitable and reactive quinones and chalcogen-derivatives. It is also questionable if higher substitutions, such as three- or four-substituted quinones, may be accessible by this method or stable in aqueous media, especially in the case of tellurium. Although the method appears to work well for simple compounds, the attraction of one-step synthesis is lost once rather complex starting materials have to be synthesized first.

Nonetheless, nucleophilic attack at the quinone provides an excellent entry point for the synthesis of bifunctional agents, especially when more reactive and stable amines are used. Amines avoid some of the problems encountered with selenium and tellurium nucleophiles, and the aminoalkylation method (using quinones instead of bromoquinones) works rather well for sulfur and selenium compounds **4–6**. It appears to fail, however, for the corresponding tellurium analogues, possibly due to the instability of 3-(phenyltelluryl)propane-1-amine and/or the product under the reaction conditions employed.

The aminoalkylation method has therefore been developed further. By initially 'separating' the quinone from the chalcogen and macrocycle chemistry, it has been possible to synthesize appropriate building blocks which in a final step are coupled together under mild conditions. Employing such coupling methods, it is possible to take full advantage of the specific quinone, chalcogen and macrocycle chemistries – which must be kept separately at the beginning to avoid the kind of complications arising once these groups encounter each other (see above). Reductive amination, a rather straightforward assembly method which couples a (functionalized) aldehyde to a (functionalized) amine, has been used successfully to synthesize compounds containing macrocycles, such as **8** and **9**. Nonetheless, the scope of the method is rather limited.

By using the amide coupling approach, it has been possible to successfully synthesize various selenium and tellurium compounds, including compounds **11**, **12** and **21**, which show interesting biological activities. This method works rather well

with a linker containing an amine (for attachment to the quinone) and acid (for amide coupling), and it has also been possible to employ a linker containing a carbon or thiol instead of an amine group. The use of a thiol allows a two-fold 'functionalization' of the quinone: Compound **21**, for instance, combines three important groups in one molecule, namely a quinone redox-centre, a sulfur redox-centre and a cyclen metal binding site. At the same time, **21** also highlights the limitations of this method: Although further groups of interest may be introduced – such as a selenium atom in the linker – more diverse or higher functionalization may become difficult. This is due to the fact that generally just two building blocks are assembled *via* amide coupling, and additional groups would have to be introduced *via* more complex building blocks. Ultimately, the issue of generating highly functionalized molecules would simply shift from the synthesis of the final product to the synthesis of the individual building blocks.

A bolder approach has therefore been investigated. The Passerini or Ugi reactions allow the one-step assembly of three or four building blocks, respectively. If one building block were to carry just one function, three- to four-functional molecules may become accessible. The individual building blocks required can now be synthesized with comparable ease (including functionalized isonitriles) and one may 'mix and match' them according to individual design criteria. The subsequent Passerini and Ugi chemistry itself is fairly straightforward: It can be carried out under mild conditions in solvents such as water or chloroform, and yields are high when compared to the other methods evaluated in this study. Multicomponent chemistry ultimately could be used to generate molecules bearing four or more redox and/or metal binding sites in one molecule, as demonstrated by the synthesis of **27**. Then again, it may not always be the method of choice. The synthesis of the individual building blocks is fairly time-consuming. In addition, the Passerini reaction leads to an α-acyloxy amide, *i.e.* a compound containing an ester bond, which is not particularly stable and may be cleaved chemically or enzymatically. It is possible that such compounds are readily modified *in vivo*, either for the benefit of activation or, less desirably, in conjunction with a loss of activity.

In any case, the availability of a range of redox-active building blocks, such as numerous quinone- and selenium-containing acids, aldehydes and amines, allows the synthesis of a vast range of chemically diverse molecules *via* different synthetic avenues. Such molecules may be 'tailor-made' to respond to OS. Indeed, the biological data obtained so far highlights the potential and potential use(s) of such multifunctional redox/metal binding compounds.

3.2 Biological activity

Overall, the various compounds synthesized as part of this study have provided a number of interesting leads as far as possible drug design and development are concerned. First of all, compounds such as **8** and **21** have confirmed the feasibility of so-called 'sensor/effector' agents. The latter are catalytic agents able to recognize a particular intracellular state of cells – such as OS – and, by using this state, are able to kill these cells selectively. This property manifests itself in the cytotoxicity of **8** and **21** against SK-Mel-5 cells, which is enhanced significantly in the presence of H_2O_2. The most likely explanation for this effect is a combination of (a) oxygen radical and H_2O_2 formation at the quinone and (b) oxidation of cellular thiols catalyzed by selenium in the presence of H_2O_2. Together, this 'chemistry' is likely to significantly increase OS in cells already suffering from (mild) H_2O_2 stress – and ultimately to push those cells over a critical 'life and death' (redox) threshold. This explanation may also account for the differences in toxicity observed in the 58 cell screen: Cells particularly sensitive to compounds such as **18**, **27** and **28** may be cells with elevated intracellular levels of ROS and/or cells particularly sensitive to OS. For most compounds, the highest activity in mammalian and human cell culture is achieved when employed in concentrations between 1 and 50 μM. LC_{50} values obtained in 5-dose testing of the 58 cell screen are around 10 μM, and the best results in SK-Mel-5 cells are also obtained in this concentration range. These values are reasonable, yet still leave room for further improvement. It must be pointed out that the compounds used were selected initially to showcase the underlying synthetic chemistry. They are not optimized regarding their pharmacokinetic profile (*e.g.* Lipinski's rules) or metabolic stability.

Some compounds, such as **7** and **17**, also appear to counteract the cytotoxic effects exerted by H_2O_2 on HL-60 cells. Such compounds may be considered as 'antioxidants'. Compound **7**, in particular, has already shown this kind of activity in cultured mouse fibroblast cells exposed to UVA radiation stress.[14,23] In principle, the ability of compounds to catalyze the reaction between H_2O_2 and thiols may result in antioxidant protection as well as enhanced cytotoxicity. This apparent contradiction is easily resolved: The impact of this type of peroxidase-like activity is dependent on the concentrations of intracellular thiols and the nature of the thiols consumed. Peroxidase-like catalysis is antioxidative as long as *sacrificial* thiols (*e.g.* reduced glutathione, GSH) are affected, and lethal once *essential* thiols (*e.g.* zinc finger proteins) are consumed. Interestingly, the tellurium analogue of compound **17**, *i.e.* compound **18**, is toxic to cells.

The notion of redox modulation by our compounds as a possible approach towards antimicrobial therapy has been evaluated further by studying their effects on parasitic organisms. The IC_{50} values for growth inhibition obtained for compounds **11** and **12** against the malarial parasite *Plasmodium falciparum* were around 200 nM. These values are considerably lower than those obtained for cultured human cells, which represents an excellent starting point for further developing this class of compounds as potential anti-malarials which might act alone or in combination therapies. Indeed, a number of currently used drugs, including the quinoline and the endoperoxide anti-malarials, appear to act at least partially by increasing OS. Current international drug development strategies also include the synthesis of novel compounds with pro-oxidant activity such as peroxidic compounds, anthraquinones, and inhibitors of antioxidant enzymes.[26,27] Redox-active enzymes like glutathione reductase, thioredoxin reductase or peroxiredoxins rank among the currently most attractive drug targets against parasitic infections. As a next step, we envisage the synthesis of further compounds structurally related to **11** and **12** and a detailed evaluation for anti-malarial activity in order to understand the underlying structure–activity relationships.

At the same time, the results obtained with *Trichophyton rubrum* point towards a possible strategy for fighting off this rather unpleasant and resilient parasitic fungus. Although the concentrations of the most active compounds **7** and **9** required for activity

ranged between 100 μM and 10 mM, *i.e.* were rather high, so were benchmark ketoconazol concentrations (188.2 μM), pointing towards a particularly resistant organism. Importantly, long-term activity against *Trichophyton rubrum* is of particular interest and **7** and **9** were able to stop individual fungi for several days from growing. Based on the assumption that compounds such as **7** and **9** may act by increasing OS and, at the same time, by depriving the fungus from essential trace metal ions (such as zinc, copper and iron), a topical rather than systemic drug treatment may become possible, whereby growth of the fungus would be stopped by deprivation of essential metal ions, and the remaining parts may be killed by ROS. The impact of ROS would be particularly severe, since the lack of metal ions such as copper and iron may weaken the fungi's antioxidant defence. At the same time, **7** is known to act as an antioxidant in skin cells, implying a protective role of **7** against ROS in human cells. A compound such as **7** may therefore attack *Trichophyton rubrum* by causing OS in this organism, yet protect the skin cells affected by counteracting OS in these cells. As mentioned above, pro- and antioxidant effects are not mutually exclusive, but depend on the availability and nature of intracellular thiols. In this respect, the fungus may lose out against the skin cells, which have an almost unlimited supply of GSH and trace metal ions available *via* the blood. Similar effects may be associated with other compounds combining redox-centres with metal binding sites. Although those considerations are necessarily speculative at this time, they provide a lead worth pursuing. It must be emphasized, however, that not all samples of *Trichophyton rubrum* were affected equally. Nonetheless, the use of patient samples, rather than cultivated, pedigreed fungi, provides a more realistic model of real-life infections, which, of course, is also more robust.

4. Conclusions

In summary, the results obtained as part of this study provide an overview of various synthetic strategies which can be employed to synthesize compounds combining several redox-centres and/or metal binding sites. These molecules are not only intriguing from a purely synthetic point of view. They also provide several interesting leads for possible drug development, which are not limited to cancer research, but also appear to include lesser exposed, yet equally important areas of pharmaceutical research, such as anti-malarial and anti-fungal therapies.

The studies presented here are, of course, only an entry point for considerably wider and more in-depth investigations, which ultimately may also consider mechanistic aspects and biochemical mode(s) of action. The evaluation of various synthetic strategies, for instance, provides fertile ground for future synthesis. While the multicomponent approach towards multifunctional agents may appear to be the most promising avenue – and certainly needs to be explored further – other methods, which may provide less sophisticated end-products yet are ultimately faster to conduct (*e.g.* amide coupling), also possess a certain attraction. The choice of synthetic method ultimately depends on the target at hand. In any case, the availability of several established avenues is a great asset for future studies to build upon.

As far as biological activity is concerned, several of the compounds tested appear to respond well to the presence of H_2O_2, and this may be exploited further for targeting tumour cells under OS. At the same time, results obtained for **7** support the notion that compounds featuring redox and metal binding sites may be able to attack fungi, such as *Trichophyton rubrum*, while simultaneously acting as antioxidant. This matter requires further investigation, especially in the context of skin disease. Here, future compounds may feature metal exchange instead of metal binding sites, for instance sites able to 'exchange' iron and copper for zinc. This exchange would not only scavenge iron and copper ions but also release (fungicidal) zinc ions, and hence hit the fungus particularly hard.[14] Since topical applications are possible, evaluation of antifungal properties may soon reach the stage of simple tests on volunteers. A more complex, yet equally important activity is found against *Plasmodium falciparum*. This species was chosen originally as an example of an organism highly sensitive towards OS. The rather low IC_{50} values obtained for **11** and **12** are promising and support the notion that organisms with a weak antioxidant defence may indeed be targeted by certain quinone-selenium agents. These and related compounds targeting the redox system of *Plasmodium falciparum* take advantage of an inherent biochemical 'weakness' of this malaria pathogen and may become useful in the future.

Ultimately, the studies presented here provide a fertile ground for various multidisciplinary follow-on projects in the area of synthetic chemistry, electrochemistry, biological chemistry, *in vitro* biochemical studies, cell culture and drug development.

5. Experimental

5.1 Materials and general methods

Chemical reagents for the synthesis of compounds were purchased from Sigma-Aldrich-Fluka (Darmstadt, Germany) and used without further purification unless stated otherwise. THF and $CHCl_3$ was refluxed with calcium hydride and freshly distilled before use. Reactions under inert atmosphere were carried out under argon (99.996%) using standard Schlenk techniques. Silica gel 60 (Macherey-Nagel, 50-200 μm) was used for column chromatography. Unless noted otherwise, the dimensions of columns used were 2.5 cm (diameter) and 25–30 cm (height of silica gel). TLC plates (silica gel 60 F_{254}, 0.20 mm) were purchased from Merck.

^{1}H NMR spectra were recorded at 500 MHz, and ^{13}C NMR spectra at 125 MHz on a Bruker (Rheinstetten) DRX 500 or Avance 500 spectrometer. Chemical shifts are reported in δ (ppm), expressed relative to the solvent signal at 7.26 ppm ($CDCl_3$, ^{1}H NMR) and at 77.16 ppm ($CDCl_3$, ^{13}C NMR), as well as 3.31 ppm (CD_3OD, ^{1}H NMR) and 49.00 ppm (CD_3OD, ^{13}C NMR). Coupling constants (*J*) are given in Hz.

LC-MS/MS analysis was performed using a TSQ Quantum mass spectrometer equipped with an ESI source and a triple quadrupole mass detector (Thermo Finnigan, San Jose, CA).

High-resolution mass spectrometry was performed on an Accela UPLC-system (Thermo-Fisher) coupled to a linear trap-FT-Orbitrap combination (LTQ-Orbitrap), operating in positive ionization mode.

Melting points were recorded using a Digital Melting Point Apparatus (IA9000 series, ThermoFischer Scientific, Rochford, UK) and are given without correction.

A detailed description of the synthesis of individual compounds, including extensive analytical data used for their characterization, is provided as part of the ESI†.

5.2 Biological assays

The cell survival (cytotoxicity) studies using SK-Mel-5 and HL-60 cell culture were performed according to the literature.[29] Activity against *Plasmodium falciparum* was determined with a method which has been developed recently and is now used routinely by the authors to screen for activity.[30,31] Details of these methods, together with a detailed description of the activity assay against *Trichophyton rubrum*, can be found in the ESI†. The 58 cell line screen at the National Cancer Institute (NCI) was carried out independently of the authors following a standard protocol, details of which can be obtained from the NCI website http://dtp.nci.nih.gov.

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