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Genotoxic activity of halogenated phenylglycine derivatives

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Abstract—The discovery of genotoxic amino acids derived from phenylglycine, and posessing halogen substituents, is described. The utility of hypervalent iodine reagents in the synthesis of this class of compounds is highlighted. The mechanism of action of the (haloaryl)glycines was studied in *Saccharomyces cerevisiae*.

Tumour cells have very active transport systems to capture the amino acids required for protein and nitrogen base biosynthesis. Since these systems are overexpressed with respect to most non-tumour cells, the development of cytotoxic amino acid derivatives[1] is a promising way to achieve more selective anticancer treatments.[2] For instance, melphalan **1**[3] (Fig. 1) is a clinically used alkylating agent whose uptake is performed by active amino acid transport systems.[4] This drug was developed in an effort to reduce the side effects produced by other mustards.

Once into the cell, the amino acid analogues disrupt different physiological processes. For instance, L-alanosine **2** interferes with aspartic acid metabolism.[5] The potent antibiotic and antitumoural acivicin **3**[6] is a specific inhibitor of γ-glutamyl transpeptidase and transmembrane glutathione transport, inducing apoptosis in human lymphoblastoid cells.[7]

In spite of their potential selectivity, the use of amino acids as anticancer agents has yet to be fully explored.[1] In an effort to develop new amino acid-based antitumoural drugs, we turned our attention to aromatic amino acids, such as phenylalanine, phenylglycine and tyrosine analogues. Few examples of cytotoxicity have been reported for this class of compounds. For instance, the azatyrosine derivatives **4** were patented for the treatment of pancreas, colon and thyroid cancer.[8] Recently, phenylglycine and other amino acids were reported to block the ATB^{0+} amino acid transport, which is overexpressed in tumoural cells. Since they were deprived of vital nutrients, a strong growth inhibition was observed for human colon and breast cancer cell lines.[9]

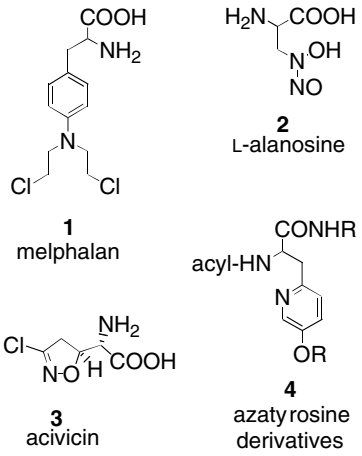

Figure 1. Some cytotoxic amino acid derivatives.

Now we report new cytotoxic agents derived from phenylglycine. The lead compound **5** (Fig. 2) was discovered

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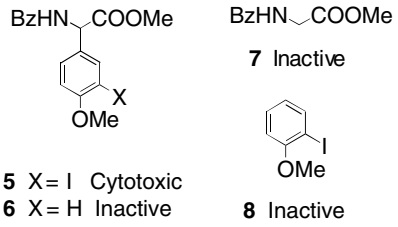


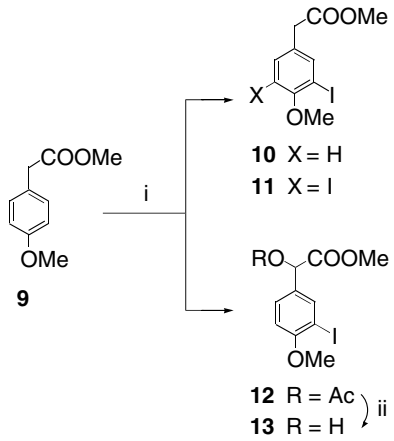
Figure 2. Discovery of the lead compound **5**.

during a screening of the cytotoxic activity of different amino acids in *Saccharomyces cerevisiae*. This yeast is used as a model system to study the mechanism of action of antitumour drugs, and to select, from a given batch, the most promising cytotoxic compounds. It was also observed that the deiodinated analogue **6** did not show activity, nor did methyl *N*-benzoylglycine **7** or 2-iodoanisole **8**.

In order to determine the structure–activity relationships (SAR), different analogues of compound **5** were prepared: (a) by replacement of the benzamide group by oxygen, sulfur and other nitrogen functions; (b) by replacing the ester group by amide, hydroxymethyl, acid, ketone and phosphonate groups; (c) changing the aromatic ring substitution pattern.

The cytotoxic activity of these analogues was then studied with three tumour cell lines: MCF7 (breast), NCI-H460 (lung) and SF-268 (glioma). Their mechanism of action was characterised using mutant strains of *S. cerevisiae*, as will be commented below.

The first analogues were prepared to determine the influence of the nitrogen function on the cytotoxic activity. Thus, the aromatic ring of compound **9** (Scheme 1) was iodinated with hypervalent iodine reagents and iodine, generating products **10**–**12**. The acetate group in product **12** was hydrolysed, yielding the alcohol **13**.

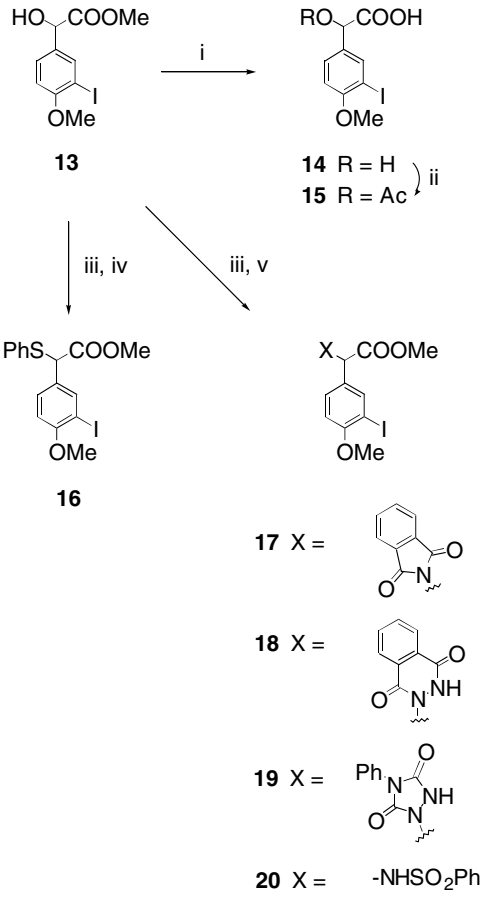


Scheme 1. Replacement of the nitrogen function by hydrogen or oxygen functions. Reagents and conditions: (i) DIB, I_2, CH_2Cl_2, dark, 20 h **10** (55%), **11** (14%), **12** (30%); (ii) MeONa, MeOH, 92%.

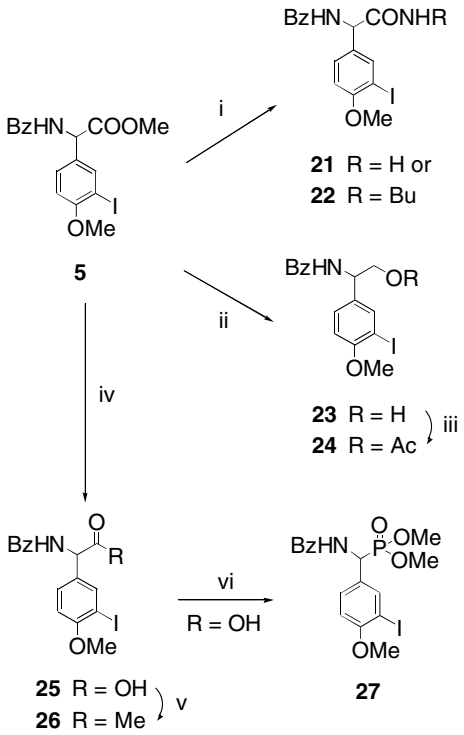
Compound **13** was then transformed into derivatives **14**–**20** (Scheme 2), where the benzamide group was replaced by oxygen functions (compounds **14** and **15**), a thioaryl group (product **16**) and other nitrogen functions (compounds **17**–**20**). Some of these functions have a volume similar to the NHBz group, but lack the ability to form hydrogen bonds and present different polarity (such as the PhS or the phthalimide groups). Others are able to form hydrogen bonds (OH, hydrazide, sulfonamide, etc.) but differ in volume and polarity.

In other group of analogues, the ester function in product **5** was replaced by amide, hydroxymethyl, acid, or ketone groups (Scheme 3), using conventional methodologies. The resulting products **21**–**26** present differences in hydrosolubility, volume and metabolization, with respect to the lead compound.

In order to obtain more structural diversity, the acid **26** was transformed into the phosphonate **27**, using a one-pot fragmentation–phosphorylation reaction developed by our group.[10]



Scheme 2. Replacement of the benzamide group in the lead compound **5** by other functionalities. Reagents and conditions: (i) 2 N NaOH, MeOH, 92%; (ii) Ac_2O, Py, 84%; (iii) MsCl, CH_2Cl_2, Et_3N; (iv) Cs_2CO_3, DMF, reflux, PhSH, 58% for both steps; (v) Cs_2CO_3, DMF, reflux, nucleophile (phthalimide or phthalohydrazide or phenylurazole or phenylsulfonamide). Compounds **17** (74%); **18** (74%); **19** (64%); **20** (46%).



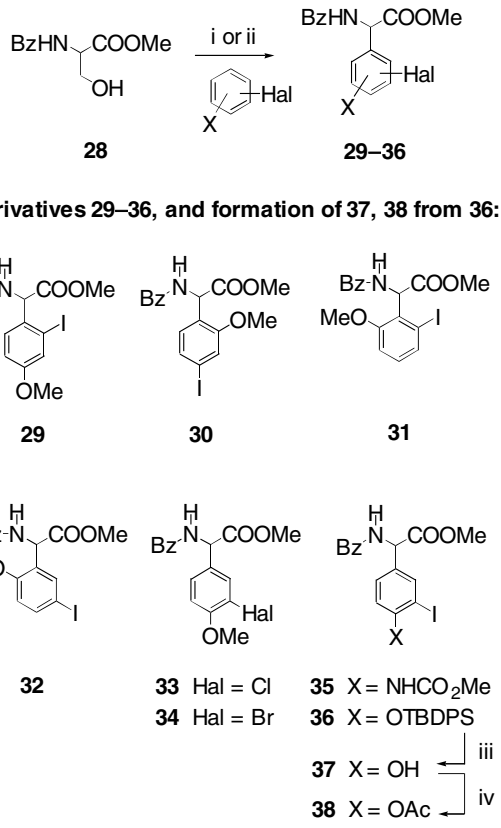
Scheme 3. Replacement of the ester group in the lead compound **5** by other functionalities. Reagents and conditions: (i) RNH_2, THF, reflux; **21** (31%), **22** (81%); (ii) DIBAL-H, CH_2Cl_2, 37%; (iii) Ac_2O, Py, 57%; (iv) NaOH, MeOH, 92%; (v) BuLi, THF, −78 °C; then MeMgI, THF, −78 °C → rt; **26** (20%), **25** (61%); (vi) DIB, I_2, hν, CH_2Cl_2, then $BF_3{\cdot}OEt_2$, $P(OMe)_3$, 84%.

The influence of the aromatic substituents was studied afterwards. Different halogen and X groups were introduced, and their positions changed, as shown in Scheme 4. Thus, starting from serine derivative **28**, a one-pot fragmentation–arylation reaction[11] was carried out, yielding the arylglycines **29**–**36**. The silyl ether **36** was then cleavaged to give the phenol **37**, and this was transformed into the acetate **38**.

The cytotoxic activity of all derivatives was studied (Fig. 3), and those compounds in which the nitrogen function was replaced by hydrogen, oxygen or sulfur functions (products **10**–**16**) proved to be inactive. The phthalimide **17** also was inactive, suggesting that hydrogen bonding was needed for activity. However, the hydrazide **18** and urazole **19** showed little activity, probably due to changes in the position of the NH group or by polarity reasons, since the heteroatom attached to the NH group exerts a strong electron-withdrawing effect. The sulfonamide **20**, which was able to form hydrogen bonds, was cytotoxic. The derivatives **21**–**27**, where the ester group has been replaced by amide, hydroxymethyl, acid, ketone, or phosphonate groups, showed little activity.

The derivatives **29**–**38**, where modifications of the aromatic ring were made, showed that changes in the iodine position were deleterious for activity, and thus products **29**–**31** showed little cytotoxicity.

The changes in the halogen were also important, and the chloro derivative **33** was less active than the lead compound **5**. However, the bromo analogue **34** presented similar activity.



Scheme 4. Changes in the aromatic ring substituents. (i) (a) DIB, I_2, hν, CH_2Cl_2, then MeOH; (b) $BF_3{\cdot}OEt_2$, 3-iodoanisole, CH_2Cl_2, **29** (31%), **30** (35%) and **31** (7%); (ii) DIB, I_2, hν, CH_2Cl_2, then $BF_3{\cdot}OEt_2$, Ar. With Ar = 4-iodoanisole: **32** (43%); with Ar = 2-chloroanisole: **33** (71%); with Ar = 2-bromoanisole: **34** (76%); with Ar = 2-iodoaniline methyl carbamate: **35** (30%); with Ar = 2-iodo-*O*-tertbutyldiphenylsilylphenol: **36** (50%); (iii) TBAF, THF, 61%; (iv) Ac_2O, Py, 65%.

Some changes in the aromatic X group were also performed. When X = $NHCO_2Me$ or OH (products **35** and **37**) the compounds were not active. On the contrary, compound **38** (X = OAc) retained some cytotoxicity, and the analogue **36** (X = OTBDPS) was more active than the lead compound **5**. Clearly, both substituent volume and polarity are important for activity.

With these results in hand, chiral derivatives[12] of the most active arylglycine, compound **36**, were prepared (Scheme 5). The synthesis was performed from commercial L-(4-hydroxyphenyl)glycine (*S*)-**39**, which was N-benzoylated to compound (*S*)-**40** and then esterified and O-silylated. The resulting product (***S***)-**41** was iodinated, affording product (*S*)-**36** in satisfactory yield. The synthesis of the D-enantiomer (*R*)-**36** was performed in a similar way.

The L-enantiomer (*S*)-**36** showed similar activity to the D-epimer (*R*)-**36**, although this result could be due to in vivo epimerization.

The mechanism of action for the arylglycines and their simplified analogues was then studied in *S. cerevisiae*.

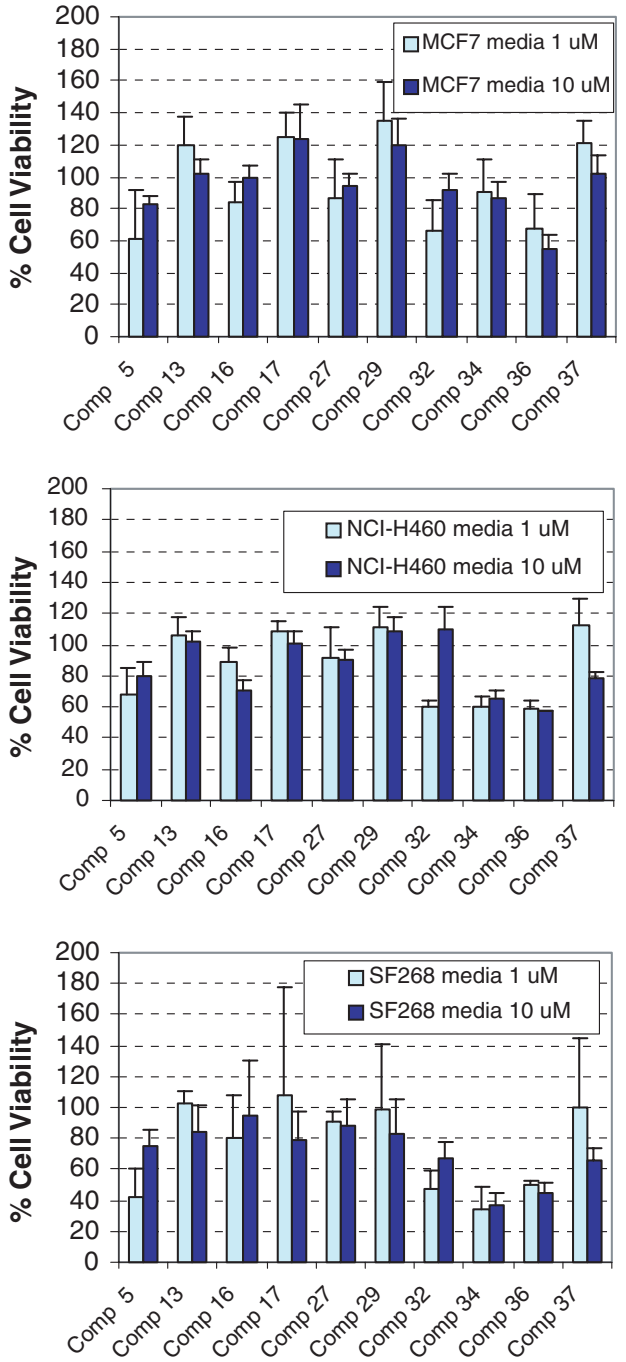
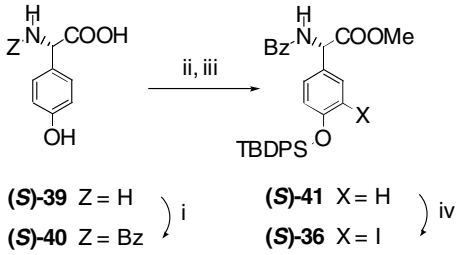


Figure 3. Table of cell viability with respect to control (%) of tumour cell lines SF680, MCF7 and NCI-H460 in the presence of representative compounds **5**, **13**, **16**, **17**, **27**, **29**, **32**, **34**, **36** and **37**.

Cancers accumulate a large number of genetic changes during progression towards malignancy due to an intrinsic genetic instability. These genetic alterations frequently affect DNA repair and cell cycle checkpoint pathways thereby increasing tumour cell sensitivity towards DNA damaging agents. As these pathways are conserved throughout evolution one can explore the therapeutic potential of molecules by using a panel of isogenic yeast strains with defined genetic alterations in DNA repair or checkpoint functions. Indeed, this approach has proven to be extremely useful in the analysis of well-known



Scheme 5. Synthesis of chiral arylglycines. Reagents and conditions: (i) BzCl, $NaHCO_3$ (aq satd), THF; (ii) MeOH, AcCl (80% for the two steps); (iii) TBDPSCl, imidazole, CH_2Cl_2, 96%; (iv) DIB, I_2, CH_2Cl_2, dark, 45% (plus 23% recovered starting material).

cytotoxic compounds which are currently used in cancer therapy.[13]

To further characterise the toxicity mechanism of these compounds, we explored the effect of compounds **5** and **34** on cell growth and viability, using a set of isogenic yeast strains defective in DNA repair (rad52, rad52-ku80 and rad14 strains) and DNA damage checkpoint (mec1-1 and rad53-11) pathways (Fig. 4).

As shown in Figure 4, treatment with both compounds affected yeast cell growth (Fig. 4A) and viability (Fig. 4B) in all strains, indicating that compounds **5** and **34** were cytotoxic in yeast. Interestingly, the growth defect produced by exposure to arylglycines **5** and **34** was exacerbated in the rad52 and rad52,ku80 mutants, respectively. These strains are hypersensitive to alterations in a number or processes involved in genome stability such as DNA replication, DNA damage signaling, double strand break repair, chromatin structure and

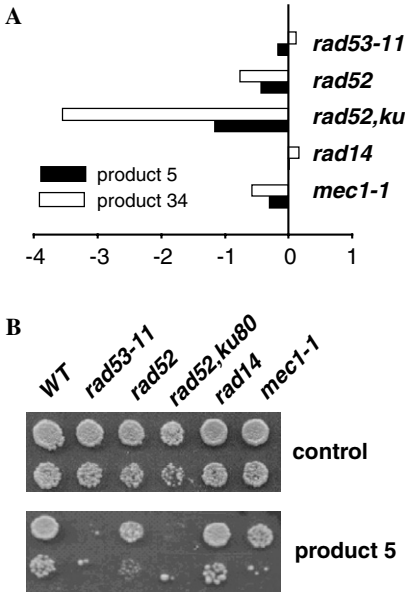


Figure 4. (A) Inhibitory effects of compounds **5** and **34** on the growth of selected DNA damage repair/checkpoint yeast mutants in liquid media (log[% yeast mutant growth/% WT growth]). (B) Growth of selected DNA damage repair/checkpoint yeast mutants in solid media (control vs compound **5**).

assembly, chromosome segregation, telomere maintenance and metabolism of reactive oxygen species.[14] Therefore, these data are consistent with compounds **5** and **34** behaving as genotoxins. Consequently, an attractive hypothesis would be that compounds **5** and **34** induce genomic instability through alteration of one/some of the above-mentioned processes. This hypothesis is currently being addressed.

In summary, new cytotoxic amino acids derived from 3-iodo- or 3-bromo-phenylglycine are described herein. Many of these compounds were synthesized using one-pot fragmentation–arylation or halogenation reactions with hypervalent iodine reagents. The mechanism of action of the (haloaryl)glycines was studied in *S. cerevisiae*, showing that these compounds were genotoxic.

Acknowledgments

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Supplementary data

Spectroscopic data for selected compounds, other cytotoxic activities, materials and general procedures for the determination of the cytotoxic activity and the mechanism of action studies. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.08.111.

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