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Pyrazino[1,2-*a*]indole-1,4-diones, Simple Analogues of Gliotoxin, as Selective Inhibitors of Geranylgeranyltransferase I

David M. Vigushin,[a,*] Greg Brooke,[a] David Willows,[b] R. Charles Coombes[b] and Christopher J. Moody[b,*]

[a]*Department of Cancer Medicine, 6th Floor MRC Cyclotron Building, Imperial College of Science, Technology and Medicine, Hammersmith Hospital Campus, Du Cane Road, London W12 0NN, UK*

[b]*Department of Chemistry, University of Exeter, Stocker Road, Exeter EX4 4QD, UK*

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Abstract—Some pyrazino[1,2-*a*]indole-1,4-diones, structurally simplified analogues of the natural mycotoxin gliotoxin, have been synthesised and investigated as inhibitors of prenyltransferases; one compound, 3-acetylthio-9-methoxy-2-methyl-2,3-dihydropyrazino[1,2-*a*]indole-1,4-dione **10** shows slightly greater selectivity (8-fold) for geranylgeranyltransferase type I (GGTase I) than gliotoxin tself.
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Activating point mutations in the human *ras* proto-oncogenes are associated with 20–30% of all human cancers, including 90% of pancreatic adenocarcinomas and 50% of colorectal carcinomas.[1,2] Ki-*ras* is the most commonly mutated oncogene in human cancers and is therefore an attractive potential therapeutic target.[3] All four mammalian Ras isoforms, Ha-Ras, N-Ras and the splice variants of Ki-Ras, Ki4A-Ras and Ki4B-Ras require prenylation (post-translational modification by an isoprenoid lipid) for subcellular plasma membrane attachment and biological activity.[4] Protein prenylation is catalysed by farnesyltransferase (FTase) or geranylgeranyltransferase I (GGTase I), members of the protein prenyltransferase family that covalently attach a farnesyl or geranylgeranyl unit, respectively to the C-terminal tetrapeptide of Ras and other specific proteins in eukaryotic cells. The related GGTase II specifically attaches two geranylgeranyl groups to newly synthesised Rab proteins. Both wild type Ras and oncogenic Ras variants require prenylation for their biological and/or transforming activities.[5–8] These observations have led to the development of prenyltransferase enzyme inhibitors for the treatment of cancer.

Of the Ras isoforms, only Ha-Ras is exclusively farnesylated. However, Ki-Ras is more commonly associated with human cancers and both Ki-Ras and N-Ras are also substrates for GGTase I.[9,10] Whereas Ki-Ras and N-Ras are normally farnesylated in cells, in cells treated with an FTase inhibitor (FTI) they are also geranylgeranylated by GGTase I.[11,12] Selective FTase inhibition is therefore insufficient to inhibit Ki-Ras dependent cell proliferation. Treatment with a GGTase I inhibitor (GGTI) can inhibit proliferation of oncogenic Ki-Ras or N-Ras transformed tumour cells in culture or in nude mouse xenograft models.[13] However, inhibition of Ki-ras prenylation in human cancer cells and in normal and tumour tissues in animal models requires a combination of an FTI and GGTI.[14,15]

Gliotoxin is a natural epidithiodiketopiperazine mycotoxin with immunosuppressive and antimicrobial activity. Gliotoxin and related fungal metabolites gliovirin and sporedesmin are low molecular weight non-polar compounds characterised by an intramolecular disulfide bridge that is the active moiety.[16–18] The observation that gliotoxin inhibits FTase at low micromolar concentrations,[19–21] and has antiproliferative activity in lymphosarcoma cells[22] stimulated our interest in this compound as a potential anticancer agent. Our recent work has shown that gliotoxin is a dual FTI-GGTI with potent antitumour activity against breast cancer and limited toxicity in vivo in a rodent mammary carcinoma

*Corresponding author. For biology: fax: +44-208-383-4650; email: d.vigushin@imperial.ac.uk (D. Vigushin); for chemistry: tel.: +44-1392-263429; fax: +44-1392-263434 email: c.j.moody@ex.ac.uk (C.J. Moody).

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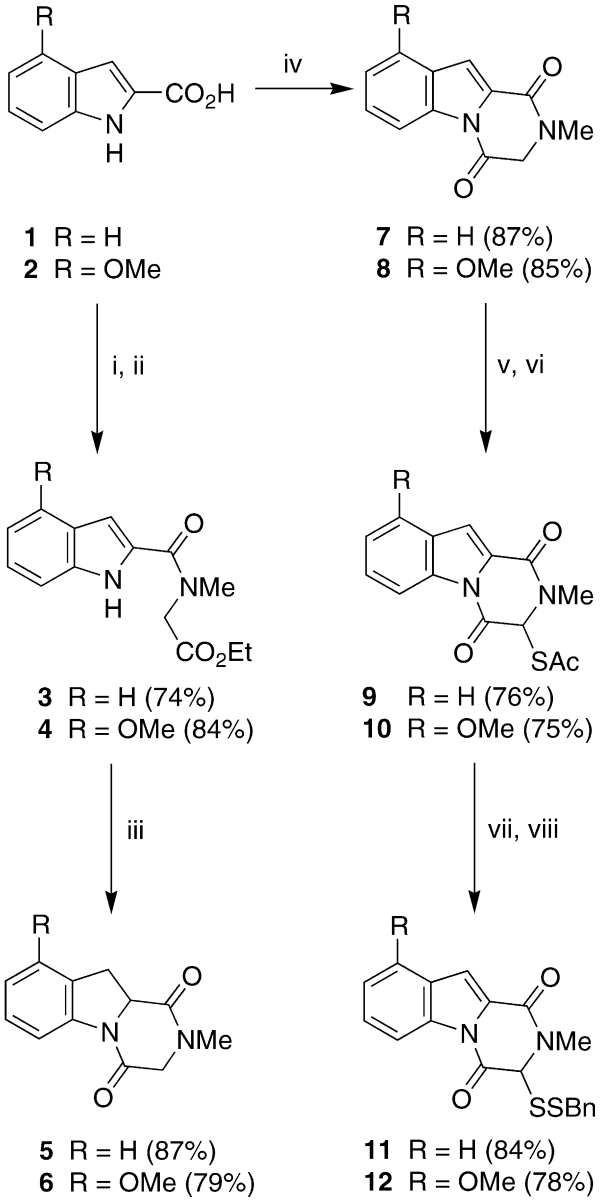
model.[23] In contrast, other investigators have found that concurrent in vivo administration of an FTI and GGTI is very poorly tolerated and often lethal in nude mouse tumour xenograft models.[24] The antitumour efficacy and remarkable lack of toxicity of gliotoxin therefore led us to design, synthesise and evaluate a series of simple structural analogues.

Chemistry

The analogues chosen for study share the tricyclic pyrazino[1,2-*a*]indole-1,4-dione ring structure of gliotoxin, and are either unsubstituted in the diketopiperazine ring or contain a sulfur substituent. The starting materials were commercially available indole-2-carboxylic acid **1** and the 4-methoxy derivative **2**.[25] Formation of the acid chloride by treatment with thionyl chloride in toluene followed by reaction with sarcosine ethyl ester gave the amides **3**[26] and **4**[27] in 74 and 84% yield, respectively (Scheme 1). Treatment with magnesium in methanol resulted in reduction of the indole ring,[28] and cyclisation of the resulting indoline to give the tetrahydro pyrazino[1,2-*a*]indoles **5** and **6**.[27]

Alternatively, the indole-2-carboxylic acids could be converted directly into the dihydropyrazino[1,2-*a*]-indoles **7**[26] and **8**[27] by carbodiimide mediated coupling to sarcosine ethyl ester followed by cyclisation. Sulfur substituents were introduced by initial radical bromination at C-3,[29] followed by reaction with excess potassium thioacetate to give the 3-acetylthio derivatives **9** and **10**. Hydrolysis of the thioacetate gave the corresponding thiols which were converted into the *S*-benzyl disulfides **11** and **12** by reaction with *S*-benzylthiophthalimide[30] (Scheme 1).



Scheme 1. Reagents and conditions: (i) $SOCl_2$, toluene, 50 °C; (ii) sarcosine ethyl ester (2 equiv), ether; (iii) Mg, MeOH; (iv) sarcosine ethyl ester hydrochloride, Et_3N, CH_2Cl_2, EDCI, C_6F_5OH; (v) NBS, $(PhCOO)_2$, CCl_4; (vi) KSAc (4 equiv), CH_2Cl_2; (vii) HCl, MeOH; (viii) PhthNSBn, toluene, 80 °C.

Table 1. In vitro inhibition of recombinant protein prenyl transferases

Compd	FTase IC_{50} (μM)	GGTase I IC_{50} (μM)
5	>100	>100
6	>100	>100
7	>100	>100
8	>100	>100
10	748±263	90.3±8.0
11	>100	>100
12	>100	>100

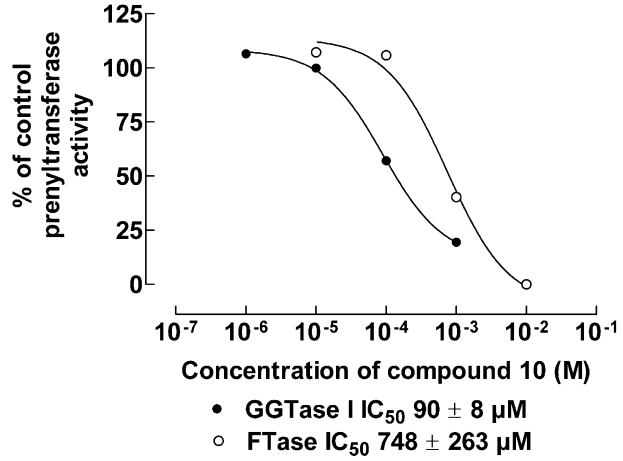


Figure 1. Inhibition of FTase (○) and GGTase I (●) enzyme activities by the pyrazino[1,2-*a*]indole-1,4-dione **10**. Recombinant human FTase and GGTase I were incubated in the presence of various concentrations of **10**. Transfer of farnesyl from [^{3}H]FPP and geranylgeranyl from [^{3}H]GGPP into recombinant human Ha-Ras-CVLS and Ha-Ras-CVLL, respectively, was determined. Each value represents a single incubation, except for control values taken as 100%, which are the mean of triplicate determinations. A blank value, determined in parallel reactions without protein substrate, was subtracted from each value. IC_{50} values were determined graphically using non-linear regression analysis to fit the inhibition data to the appropriate dose–response curves.

Biological Results and Discussion

FTase and GGTase I enzyme inhibition assays measuring the amount of [^{3}H]FPP and [^{3}H]GGPP incorporated into recombinant human Ha-Ras-CVLS and Ha-Ras-CVLL, respectively were performed as previously described,[31] and the results are shown in Table 1.

The preliminary data show that the compounds are not particularly potent inhibitors of FTase or GGTase I, with all except one compound having IC_{50} values >100 μM.[32] Compound **10**, however, is an inhibitor of GGTase I with an IC_{50} of 90.3 μM and interestingly exhibits ca. 8-fold selectivity over FTase (IC_{50} 748 μM) (Fig. 1).

Although the simple analogues are not as potent as gliotoxin itself (IC_{50} 80 and 17 μM vs FTase as GGTase I, respectively[23]), compound **10** shares the same selectivity for GGTase I (ca. 8-fold, cf. 5-fold for gliotoxin), and therefore forms the basis for the design of further analogues.

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32. Many of the compounds were insoluble in 5% DMSO at concentrations exceeding 500 μM. For the assay, a test compound must be soluble in 5% DMSO at 5 times the desired final concentration. We therefore had to report the IC_{50} results as >100 μM.