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Identifying novel targets in renal cell carcinoma: Design and synthesis of affinity chromatography reagents

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ABSTRACT

Two novel scaffolds, 4-pyridylanilinothiazoles (PAT) and 3-pyridylphenylsulfonyl benzamides (PPB), previously identified as selective cytotoxins for von Hippel–Lindau-deficient Renal Carcinoma cells, were used as templates to prepare affinity chromatography reagents to aid the identification of the molecular targets of these two classes. Structure–activity data and computational models were used to predict possible points of attachment for linker chains. In the PAT class, Click coupling of long chain azides with 2- and 3-pyridylanilinothiazoleacetylenes gave triazole-linked pyridylanilinothiazoles which did not retain the VHL-dependent selectivity of parent analogues. For the PPB class, Sonagashira coupling of 4-iodo-(3-pyridylphenylsulfonyl)benzamide with a propargyl hexaethylene glycol carbamate gave an acetylene which was reduced to the corresponding alkyl 3-pyridylphenylsulfonylbenzamide. This reagent retained the VHL-dependent selectivity of the parent analogues and was successfully utilized as an affinity reagent.

1. Introduction

Identification of tumour-selective agents is a top priority in anticancer drug development. One approach is to leverage a genetic abnormality common to a particular tumour type and to identify agents that are selectively cytotoxic to tumour cells with this genetic abnormality. We recently used such a synthetic lethality approach[1–3] to discover two novel chemotypes that were selectively cytotoxic to Renal Cell Carcinoma (RCC) cells lacking the von Hippel Lindau factor (VHL) both in vitro and in vivo.[4,5]

RCCs are refractory to standard chemo- and radiotherapy and advanced RCC has an extremely poor prognosis.[6] Although new 'targeted' anti-angiogenic agents such as sunitinib and sorafinib have been approved for use against the highly vascularised advanced RCC, these agents provide limited efficacy and patients eventually relapse and succumb to their disease. Thus, there is still a cogent need for drugs with increased efficacy in the treatment of advanced RCC. Common to a majority of RCCs is the loss of function of the von Hippel–Lindau (VHL) tumour suppressor gene.[7] The VHL protein regulates a variety of proteins,[8] including the activity of the Hypoxia Inducible Factor (HIF) family of transcription factors, by targeting them for degradation. Loss of this control increases HIF activity and increases transcription of a wide range of genes.[9] This genetic response mimics the impact of tumour hypoxia and promotes reprogramming of tumor metabolism, progression, invasion, and metastasis, resulting in an aggressive phenotype, poor prognosis and resistance to therapeutic agents,[10,11] and so the VHL-deficient RCC cell line also provides a model of tumour cells under hypoxic stress.

The two chemotypes identified in our synthetic lethal screen, 4-pyridylanilinothiazoles (PAT) (**1**) and 3-pyridylphenylsulfonyl benzamides (PPB) (**2**) (Fig. 1) displayed selective cytotoxicity for VHL-deficient RCC4 cells compared to RCC/VHL VHL-proficient cells (Table 1) but displayed different phenotypic behaviour. In the first case, PAT cytotoxicity was independent of HIF-1 status. PAT compounds induced autophagy, as measured by LC3 immunostaining and Western blot analysis, and this led to cell death. Functional analysis of the activity of the PAT class using a yeast deletion pool implicated proteins involved in Golgi body processing as important in the induction of autophagy, but failed to unequivocally identify the target protein(s) of the PATs.[4]

Abbreviations: BOC, *tert*-butyloxycarbonyl; DCM, dichloromethane; DMF, dimethylformamide; HTS, high throughput screening; PAT, pyridylanilino thiazole; PPB, pyridylphenylsulfonyl benzamides; PEG, polyethylene glycol; RCC, renal cell carcinoma; SAR, structure–activity relationship; TBTA, tris-(benzyltriazolyl)amine; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TMS, trimethylsilyl.

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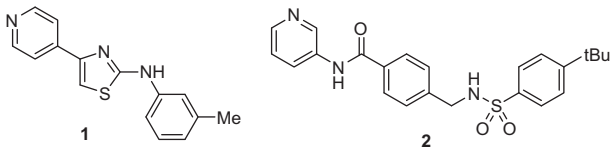

Figure 1. PAT and PPB chemotypes identified from RCC HTS.

Table 1
IC_{50} values and selectivity ratios in Renal Cell Carcinoma cells

No	RCC4 IC_{50} (μM)	RCC4/VHL IC_{50} (μM)	Ratio[a]
1[b]	2.1	40	19
2	0.16	>40[c]	>250
7	1.7	2.6	1.5
8	>40	>40	ND
11	>40	>40	ND
16	2.9	3.9	1.3
19	>40	>40	ND
30	5.8	>40	>7
32	7.9	>40	>5
38	>40	>40	ND

[a] Ratio = IC_{50} (RCC4/VHL)/IC_{50} (RCC4).
[b] Data from Ref. 15.
[c] Solubility prevented determination of IC_{50} values.

In contrast, PPB cytotoxicity was dependent on HIF-1 status and resulted in necrotic cell death. The PPBs decreased glycolysis in a VHL-dependent manner and inhibited the uptake of glucose.[5]

Further development of these novel chemotypes into viable anticancer agents is critically dependent on the identification of the molecular target of action. In this study we report our synthetic efforts to use structure activity relationships (SAR), in combination with molecular design, to develop chemical biology tools suitable for use in an affinity chromatography approach[12] for target identification for both PAT and PPB chemotypes.

2. Molecular design

We expanded on the initial hit compound **1** and explored the SAR for the PAT chemotype to identify more potent and selective analogues, but were hampered in this by the lack of an identified molecular target.[13] This handicap led us to use a comparative molecular field analysis (CoMFA) to determine possible bioactive conformations to aid our studies. We identified a positive steric contour (Fig. 2, green volume) adjacent to the pyridine ring as a potential feature for further analogue development and this feature was further explored, along with the orientation of the thiazole B-ring and the roles of the heteroatom substituents, in a subsequent study.[14] We used Suzuki–Miyaura and Sonogashira Pd-mediated cross coupling reactions and nucleophilic displacement reactions to prepare series of aryl-, alkynyl-, alkoxy- and alkylamino-substituted pyridines, respectively, to explore the steric and electronic contours adjacent to the pyridyl ring. Although we failed to improve the predictivity of the CoMFA model, we did identify several analogues with increased potency and/or selectivity to **1**. The CoMFA model predicts the 2- and 3-positions of the pyridine ring were the most favorable for functionalization with a variety of groups at these positions providing potent and selective compounds. Although, two acetylenic compounds displayed selectivity for VHL-negative cells, a lack of selectivity for higher homologues dissuaded us from using an acetylene linker. Either pyrazole or triazole groups in the 2-position provided similar or improved potency and selectivity compared to the parent **1** [IC_{50} (RCC4) ca. 2 μM, selectivity (RCC4VHL/RCC4), 6 to 30-fold)[14] and suggested a 'Click'[15] strategy to incorporate a long chain linker suitable for affinity chromatography.

Our SAR studies on the PPB chemotype identified considerable steric tolerance at the 4-position of the phenyl sulfonamide[16] These data suggested molecules with a long chain linker attached to this position may retain activity and may provide a plausible strategy for attachment of linkers required for affinity reagents. The overexpression of the facultative glucose transporter GLUT-1 in VHL-deficient RCC cells, combined with evidence for inhibition of glucose uptake, led us to consider the possibility that the cytotoxicity of the PPBs was mediated through an interaction with GLUT-1.[5] We were able to use a homology model of GLUT-1[17] to identify a binding mode consistent with the SAR (Fig. 3) and our approach to position a long chain linker at the 4-position.

3. Results

3.1. Chemistry

3.1.1. PAT synthesis

We elaborated the 2-acetylene PATs using 'Click' chemistry to generate a substituted triazole. We had previously demonstrated

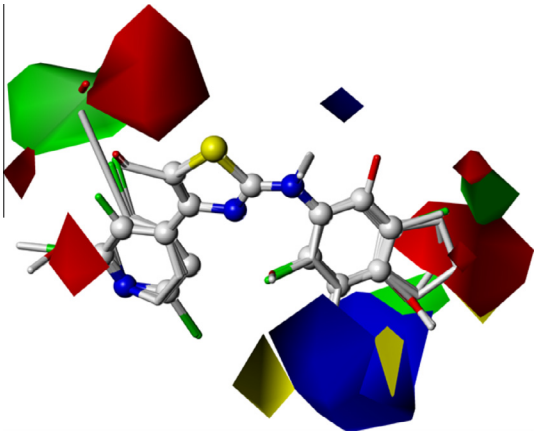

Figure 2. CoMFA model 3D-QSAR conformer model relating PAT conformation to cytotoxicity. Electrostatic fields are contoured at 80% favored (blue) and 20% disfavored (red). Steric fields are contoured at 80% favored (green) and 20% disfavored (yellow).

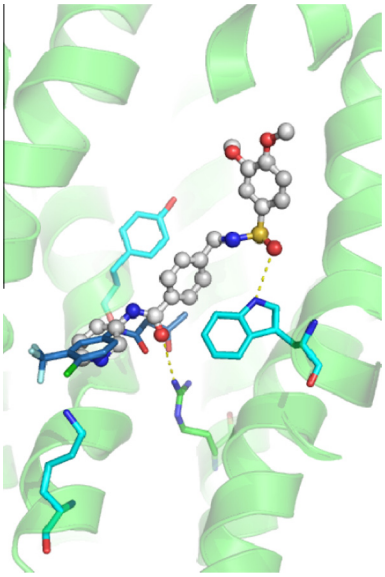

Figure 3. A representation of PPB **S3** (white ball and stick) and fasentin (blue stick) modelled in the central transport channel of GLUT-1. Possible interactions with Trp412 and Arg126 are shown.