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Research paper

Substituted arylsulphonamides as inhibitors of perforin-mediated lysis

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ABSTRACT

The structure-activity relationships for a series of arylsulphonamide-based inhibitors of the pore-forming protein perforin have been explored. Perforin is a key component of the human immune response, however inappropriate activity has also been implicated in certain auto-immune and therapy-induced conditions such as allograft rejection and graft versus host disease. Since perforin is expressed exclusively by cells of the immune system, inhibition of this protein would be a highly selective strategy for the immunosuppressive treatment of these disorders. Compounds from this series were demonstrated to be potent inhibitors of the lytic action of both isolated recombinant perforin and perforin secreted by natural killer cells *in vitro*. Several potent and soluble examples were assessed for *in vivo* pharmacokinetic properties and found to be suitable for progression to an *in vivo* model of transplant rejection.

1. Introduction

Perforin is a 67 kDa, calcium-dependent glycoprotein expressed by only the natural killer (NK) cells and cytotoxic T lymphocytes (CTLs) of the mammalian immune system [1,2]. These "killer lymphocytes" utilise the pore-forming ability of perforin as a critical component of the granule exocytosis pathway; the principal mechanism used by NK and CTL cells for tumour immunosurveillance and as a defence against viral infection and intracellular pathogens [3]. Identification of a target cell by an effector cell results in the formation of an immune synapse whereupon CTL (or NK) secretory granules polarise to the site of contact. These granules contain both perforin and a group of pro-apoptotic serine proteases known as granzymes, and upon fusion with the CTL plasma membrane, release their luminal contents into the synapse [2,4]. Perforin performs a key role in this process because entry of the granzymes required for cell death into the target cell cytosol is solely dependent on its presence [1,5].

Although perforin is synthesized and secreted into the immune synapse as a monomer, it rapidly binds to the target cell membrane through its calcium-dependent C2 domain [6,7] and oligomerises into large transmembrane pores composed of approximately 24 perforin monomers. This process was elucidated using a combination of the perforin X-ray crystal structure and cryoelectron microscopy to reconstruct an entire perforin pore [8]. Electron microscopy, X-ray crystallography and functional studies have also shown that the process involves electrostatic interactions which include a salt bridge formed between R213 on the 'front' surface of one monomer interacting with E343 on the 'back' surface of the adjacent monomer [9]. Similarly, mutational studies reveal that D191, which is immediately adjacent to R213, makes interactions that are key to oligomerisation and that substitution with a bulky hydrophobic residue (D191V) abrogates this process [9].

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Until recently, the precise mechanism of granzyme entry into the target cell was debated, but it is beyond any doubt that the pore-forming activity of perforin is indispensable. In essence, secreted perforin forms large (18 nm diameter) transmembrane pores on the surface of the target cell, through which the granzyme monomers (4 nm diameter) diffuse into the cytosol [10,11]. Once internalised the granzymes cleave key substrates to initiate rapid apoptotic death [5,10–13]. Unlike the granzymes, which are encoded by many genes and are, therefore, subject to considerable redundancy of function, the gene encoding perforin (PRF1) is present as a single copy in all mammals. Gene-targeting studies in mice [1] and naturally occurring disease-causing mutations in humans [14,15] confirm that perforin deficiency cannot be compensated by any other protein. This makes perforin an ideal target for therapeutic intervention.

While perforin is a key component of the immune response, inappropriate activity has also been implicated in a number of human immunopathologies and therapy-induced conditions. These include cerebral malaria, insulin-dependent diabetes, juvenile idiopathic arthritis and postviral myocarditis [16–18], as well as therapy-induced conditions such as allograft rejection and graft versus host disease [19–21]. Our current goal is to seek small molecule inhibitors of perforin as potential immunosuppressive agents for the treatment of autoimmune diseases and other conditions characterised by dysfunction of this pathway. This should be a highly selective strategy since perforin is expressed exclusively by CTL and NK cells, in contrast to approaches using conventional immunosuppression treatments which indiscriminately depress immune function [22–24].

Based on an initial hit from a mass screen [25], we have previously designed and optimised inhibitors of perforin that can; (i) block recombinant purified perforin, (ii) block perforin delivered by intact NK cells and, (iii) withstand incubation in serum (e.g. **1**; Fig. 1) [26–30].

While these compounds appeared highly promising, replacement of the 2-thioxoimidazolidinone moiety that contained a potential Michael acceptor and showed variable toxicity toward perforin-producing NK cells proved problematic. This issue was only overcome when we amalgamated our own finding that an aryl sulphonamide could act as a bioisosteric replacement [30] with a strategy implemented by GSK workers, where a thiazolidinedione was replaced with a pyridyl-linked benzenesulphonamide to give **2** [31]. This approach resulted in a new series of benzenesulphonamide-based perforin inhibitors, exemplified by **3**, which were potent, soluble and essentially non-toxic toward NK cells [32]. In the following report we extend our study to explore whether it is possible to further modulate activity and physicochemical properties through variation of the sulphonamide linker, linker position, and substitution on the central pyridine ring and terminal benzene (Fig. 2).

2. Results and discussion

2.1. Chemistry

The majority of the target compounds were constructed from right to left starting with our previously published key iodide **75** [32] (Scheme 1). Suzuki reaction of **75** with a variety of commercially available aminopyridine boronates under standard conditions gave the required amine intermediates **76**–**79** which were subsequently reacted with a range of substituted aryl sulphonyl chlorides. The 5-amino-3-pyridine derivative **78** [32] in particular was employed in the preparation of all compounds in Table 3. One exception was where the central pyridine ring was replaced with a benzene; in this case the Suzuki step was carried out with 2-methyl-5-nitrobenzeneboronic acid, the nitro compound (**80**) hydrogenated to give the amine (**81**), which was then reacted with either 2,4-difluorobenzenesulphonyl chloride or 2-pyridinesulphonyl chloride to afford **13** and **15** respectively. Finally, amido-linked compound **8** was prepared by reaction of **78** with 2,4-difluorobenzoic acid chloride.

In a smaller number of cases, mostly those examples with substitution on the central pyridine ring, the target compounds were effectively synthesized from two halves; the fully elaborated left-hand side benzenesulphonamide subunit (e.g **82**–**85**) and key iodide **75** as the right-hand side (Scheme 2). The intermediate bromides **82**–**85** and **91**–**93** were prepared from a variety of commercially available aryl sulphonyl chlorides and substituted 3-aminopyridines (or anilines) under standard conditions. In the case of **85**, the sulphonamide NH was methylated with NaH and MeI in DMF to give **86**, and for **91**–**93** where protection of this NH was required for the subsequent coupling to be successful, the alkylation was carried out with (chloromethoxy)ethane to give **94**–**96**. All bromides were converted to the corresponding boronates **87**–**90** and **97**–**99** under palladium-catalysed conditions using bis(pinacolato)diboron and KOAc in DMSO, then finally reacted in a Suzuki step with iodide **75** to introduce the thiophene-*N*-methyl-isoindolinone subunit (**6**, **10**–**12**, **17**, **18**, **20**). Where required (for **17**, **18**, **20**), deprotection was carried out under acidic conditions.

A limited number of “reverse” sulphonamides were also prepared (Scheme 3). In the case of target compound **7**, intermediate bromide **100** was prepared from 2,4-difluoroaniline and 5-bromopyridine-3-sulphonyl chloride. Protection of the sulphonamide was not required and conversion to the boronate **101** and

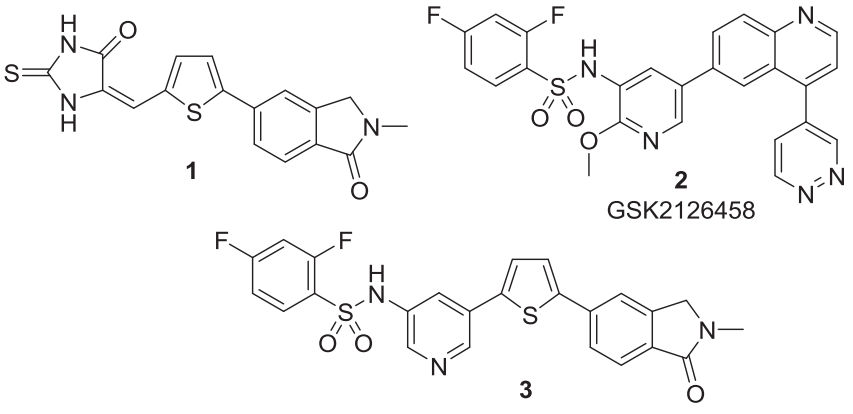

Fig. 1. Perforin inhibitors and PI3Kα clinical candidate GSK2126458.