



Diarylthiophenes as inhibitors of the pore-forming protein perforin



Christian K. Miller^{a,b}, Kristiina M. Huttunen^{a,c}, William A. Denny^{a,b}, Jagdish K. Jaiswal^a, Annette Ciccone^d, Kylie A. Browne^d, Joseph A. Trapani^{d,e}, Julie A. Spicer^{a,b,*}

^a Auckland Cancer Society Research Centre, Faculty of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand

^b Maurice Wilkins Centre for Molecular Biodiscovery, A New Zealand Centre for Research Excellence, Auckland, New Zealand

^c School of Pharmacy, Faculty of Health Sciences, University of Eastern Finland, PO Box 1627, FI-70211 Kuopio, Finland

^d Cancer Immunology Program, Peter MacCallum Cancer Centre, St. Andrew's Place, East Melbourne, Victoria 3002, Australia

^e Sir Peter MacCallum Department of Oncology, The University of Melbourne, Parkville, Victoria 3052, Australia

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ABSTRACT

Evolution from a furan-containing high-throughput screen (HTS) hit (**1**) resulted in isobenzofuran-1(3*H*)-one (**2**) as a potent inhibitor of the function of both isolated perforin protein and perforin delivered in situ by intact KHYG-1 NK cells. In the current study, structure–activity relationship (SAR) development towards a novel series of diarylthiophene analogues has continued through the use of substituted-benzene and -pyridyl moieties as bioisosteres for 2-thioxoimidazolidin-4-one (**A**) on a thiophene (**B**)-isobenzofuranone (**C**) scaffold. The resulting compounds were tested for their ability to inhibit perforin lytic activity in vitro. Carboxamide (**23**) shows a 4-fold increase over (**2**) in lytic activity against isolated perforin and provides good rationale for continued development within this class.

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Perforin (PRF) is a calcium-dependent pore-forming glycoprotein found within granules of cytotoxic T lymphocytes (CTL) and natural killer (NK) cells.^{1,2} These cytotoxic effector cells play an important role in immune-surveillance and homeostasis via a granule exocytosis pathway responsible for the elimination of neoplastic/virus-infected cells and intracellular pathogens.³ Numerous autoimmune diseases (e.g., insulin-dependent diabetes) and therapy-induced conditions (e.g., allograft transplantation rejection, graft-vs-host disease) adopt a synonymous biochemical pathway.^{4,5} An integral part of this biological cascade is perforin oligomerisation in which monomeric perforin binds calcium upon entry into the immune synaptic cleft and forms highly ordered oligomers with 19–24 adjacent perforin monomers. These in turn form cylindrical-type pores which subsequently penetrate target cell membranes, allowing rapid delivery of cytotoxic granzymes into the cytosolic compartment, a process which culminates in

apoptosis of the target cell. Highly specific inhibitors of perforin function are thus of interest as selective immunosuppressive drugs.

Isobenzofuran-1(3*H*)-one-based inhibitor **1** (Fig. 1) was a hit selected from a high-throughput screen of approximately 100,000 compounds sourced from commercial 'lead discovery' libraries.⁶ Subsequent SAR development led to the discovery of potent inhibitor **2** in which an ~8-fold improvement in inhibition of isolated perforin protein function was achieved through replacement of a central furan ring with a 2,5-thiophene moiety.⁷ In more recently published work⁸ this template was elaborated further through variation of the isobenzofuranone C-subunit where an extensive SAR programme was conducted and the mechanism of perforin inhibition investigated using real-time microscopy.^{8,9} The resulting isoindolinone-containing inhibitors were shown to have improved lytic activity against perforin delivered in situ by intact KHYG-1 NK cells and furthermore, were employed as probes to elucidate the role of perforin in the granule exocytosis pathway. This work resulted in the conclusion that this class of compounds target perforin after its release into the immune synapse.

While substantial advances have been made in the development and understanding of the thioxoimidazolidinone-based series, more detailed in vitro and in vivo assessment^{7,10} revealed several properties requiring further optimisation. Although well-tolerated in healthy mice, even those compounds deemed suitable

Abbreviations: HTS, high-throughput screen; SAR, structure–activity relationships; PRF, perforin; CTL, cytotoxic T lymphocytes; NK, natural killer cells; PAINS, pan-assay interference compounds; AgNO₃, silver(I) nitrate; KF, potassium fluoride; PFP, pentafluorophenyl; DCC, *N,N'*-dicyclohexylcarbodiimide; HOBt, hydroxybenzotriazole; EDCl, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; DMF, dimethylformamide; KOAc, potassium acetate; DMSO, dimethyl sulfoxide; THF, tetrahydrofuran.

* Corresponding author. Tel.: +64 9 3737599; fax: +64 9 3737502.

E-mail address: j.spicer@auckland.ac.nz (J.A. Spicer).

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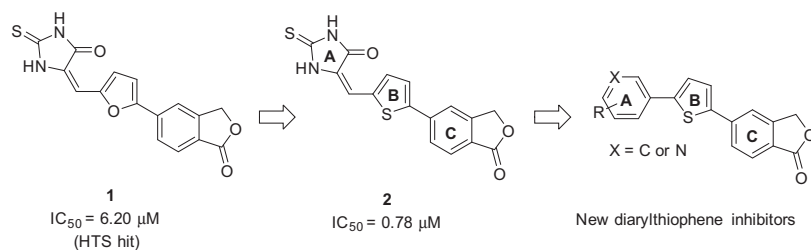


Figure 1. The development of new diarylthiophene inhibitors of perforin.

candidates for further *in vivo* studies showed some toxicity toward whole NK cells.⁸ It is therefore likely that toxicity would also be observed in the immunocompromised mice required for an efficacy study. Also desirable would be the identification of new compounds with significantly improved potency to deliver a lower efficacious dose and better control of potential dose-related toxicity. Finally, while the thioxoimidazolidinone series passed various pan-assay interference compound (PAINS) filters,¹¹ they still contain a potentially reactive Michael acceptor and exist as an inseparable (and interconverting) mixture of *E*- and *Z*-isomers.

To address these aforementioned issues the need for a 2-thioxoimidazolidin-4-one bioisosteric replacement was apparent and herein we report on the latest developments towards an exciting new class of nontoxic inhibitors of perforin function that show improved inhibition of the function of both isolated perforin protein and perforin delivered *in situ* by intact KHYG-1 NK cells.

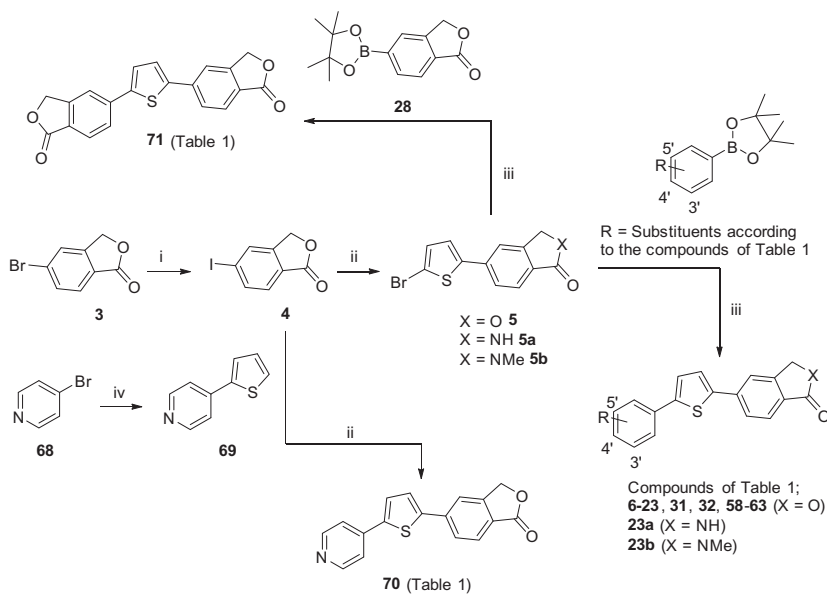
Synthesis: Target compounds **6–23** (Scheme 1) were obtained in a linear four-step sequence starting with the conversion of commercially available 2-methyl-4-bromobenzoic acid to 5-bromoisoindolin-1(3*H*)-one **3**.¹² A sequential Finkelstein halogen exchange reaction¹³ gave 5-iodoisoindolin-1(3*H*)-one **4** in 87% yield. This can also be prepared in high yield through diazotization of the corresponding 5-aminoisoindolin-1(3*H*)-one using a literature procedure.¹⁴ Lactone derivative **4** was then utilised in a palladium-catalysed arylation reaction with 2-bromothiophene in the presence of a silver(I) nitrate/potassium fluoride (AgNO₃/KF) activator system¹⁵ to yield key intermediate bromide **5**. Subsequent Suzuki couplings with a variety of commercially

available phenyl-boronates/boronic acids in the presence of catalytic PdCl₂(dppf), afforded the respective diarylthiophene targets **6–23** in generally good yields. Isoindolinone-based versions of **23** (**23a** and **23b**) were also prepared in exactly the same manner, using the isoindolin-1-one and 2-methylisoindolin-1-one-based analogues¹⁶ of key bromide **5** (**5a** and **5b**; Scheme 1).

Analogous methodology was adopted to generate secondary and tertiary carboxamide targets **29** and **30**, by first reacting iodide **24** with 2-bromothiophene in the presence of AgNO₃/KF (Scheme 2). The afforded bromide **25** was then converted to carboxamide intermediates **26** and **27**¹⁷ through activation of the carboxylic acid to a pentafluorophenyl (PFP) ester followed by subsequent addition of the corresponding amine (methylamine or dimethylamine) in pyridine for 2–3 h at room temperature. Key boronate **28** was prepared in multi-gram quantities from bromide **3**¹⁸ (Scheme 1) and subsequently used in a Suzuki coupling with intermediates **26** and **27** to furnish carboxamide targets **29** (75%) and **30** (57%), respectively.

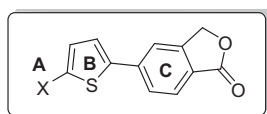
Compounds **31** and **32**, where the carboxamide side-chain was elaborated further by incorporating a series of basic side-chains, were accessed by applying the same chemistry as summarised in Scheme 1. A final step Suzuki coupling of key intermediate bromide **5** with the corresponding phenyl boronate (as according to the compounds of Table 1) gave **31** and **32** in good to modest yields, respectively, (61% and 42%).

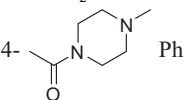
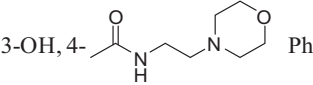
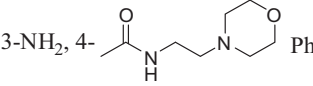
With the chemistry now established for the mono-substituted A-ring targets shown in Schemes 1 and 2, a series of di-substituted targets were investigated. Phenyl iodides **33–35** were prepared via



Scheme 1. Reagents and conditions: (i) sealed tube, 1,4-dioxane, CuI (5.0 mol %), NaI, racemic *trans*-*N,N'*-dimethyl-1,2-cyclohexanediamine (10 mol %), 110 °C, N₂, 18 h; (ii) 2-bromothiophene or **69**, DMSO, KF, PdCl₂(PPh₃)₂, AgNO₃, 100 °C, N₂, 2 h; (iii) toluene/EtOH, 2 M Na₂CO₃, PdCl₂(dppf)·CH₂Cl₂, 85–90 °C, N₂, 2–6 h; (iv) thiophene-2-boronic acid, toluene/EtOH, 2 M Na₂CO₃, PdCl₂(dppf)·CH₂Cl₂, 85 °C, N₂, 2 h.

Table 1
Inhibitory activities of isobenzofuran-1(3*H*)-ones with various A-subunits



Number	Scheme	X ^a	Inhibition of Jurkat cell lysis IC ₅₀ ^b (μM)
6	1	Ph	>20
7	1	2-Me Ph	>20
8	1	3-Me Ph	>20
9	1	4-Me Ph	>20
10	1	2-Cl Ph	>20
11	1	3-Cl Ph	>20
12	1	4-Cl Ph	>20
13	1	2-OMe Ph	>20
14	1	3-OMe Ph	>20
15	1	4-OMe Ph	9.36
16	1	3-NH ₂ Ph	13.97
17	1	4-NH ₂ Ph	11.82
18	1	3-OH Ph	5.89
19	1	4-OH Ph	>20
20	1	3-CN Ph	20
21	1	4-CN Ph	6.87
22	1	3-CONH ₂ Ph	2.97
23	1	4-CONH ₂ Ph	0.18
23a ^c	1	4-CONH ₂ Ph	6.04
23b ^d	1	4-CONH ₂ Ph	>20
29	2	4-CONHMe Ph	10.7
30	2	4-CONMe ₂ Ph	>20
31 ^e	1		>20
32	1	4-CONH(CH ₂) ₃ NMe ₂ Ph	>20
39	3	4-COOEt Ph	10.97
40	3	3-OH, 4-COOEt Ph	>20
46	3	3-NH ₂ , 4-COOH Ph	5.84
48	3	3-OH, 4-CONH ₂ Ph	0.67
49 ^e	3	3-OH, 4- 	12.75
50 ^e	3	3-OH, 4-CONH(CH ₂) ₂ NMe ₂ Ph	>20
51	3	3-OH, 4-CONHCH ₂ CH ₂ OH Ph	3.31
52	3	3-OH, 4-CONHCH ₂ CH(OH)CH ₃ Ph	>20
53	3	3-OH, 4-CONHCH ₂ CONH ₂ Ph	2.65
54	3	3-OH, 4-CONHCH ₂ CH ₂ CONH ₂ Ph	7.12
55	3	3-NH ₂ , 4-CONH ₂ Ph	1.20
56 ^e	3	3-NH ₂ , 4- 	14.71
57 ^e	3	3-NH ₂ , 4-CONH(CH ₂) ₂ NMe ₂ Ph	>20
58	1	4-SMe Ph	>20
59	1	4-CH ₂ OH Ph	0.90
60	1	4-SO ₂ Me Ph	>20
61	1	3-SO ₂ NH ₂ Ph	4.85
62	1	4-SO ₂ NH <i>tert</i> -Bu Ph	6.09
63	1	4-NHSO ₂ Me Ph	1.09
67	4	4-CONH ₂ , 3-pyridyl	0.92
70	1	4-Pyridyl	4.53
71	1	5-Isobenzofuran-1(3 <i>H</i>)-one	6.24

^a Structure of the A-subunit.

^b Testing was carried out over a range of doses, with the IC₅₀ being equal to the concentration at which 50% inhibition of the lysis of Jurkat cells by perforin was observed, as measured by ⁵¹Cr release. Values are the average of at least two independent IC₅₀ determinations.

^c Isobenzofuran-1(3*H*)-one C-subunit replaced with an isoindolin-1-one.

^d Isobenzofuran-1(3*H*)-one C-subunit replaced with an 2-methylisoindolin-1-one.

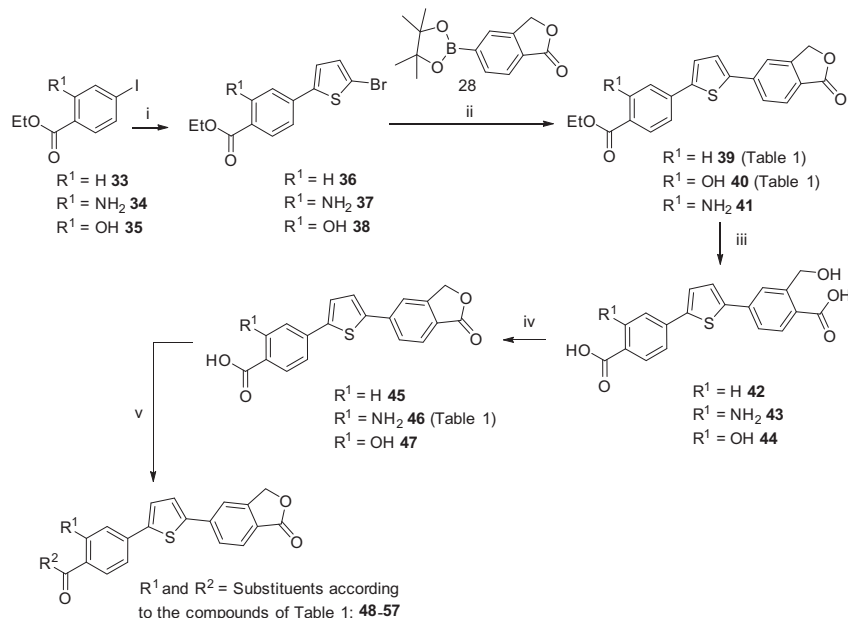
^e Detailed structures for compounds, **31**, **49**, **50**, **56** and **57** can be found in the [Supplementary information \(Experimental Section\)](#).

and 2-methylisoindolin-1-one (**23b**) derivatives were also prepared. As expected this modification resulted in a loss of activity. Conversion of the primary amide of **23** to a secondary derivative (**29**) results in a loss of efficacy, while the presence of a tertiary amide (**30**, **31**) abolishes activity completely. Ester **39** showed limited activity (10.97 μM), while compounds **40**, **46**, **48** and **55** were designed in an effort to combine the outstanding potency of **23** with the preferred 3-OH of **18** or 3-NH₂ of **16**, thereby introducing an H-bond donor at the 3-position and adding an ionisable centre to assist solubility. While this approach did generate potent (and slightly more soluble—see [Supplementary data](#)) compounds, none were an improvement on **23**. In an extension of this strategy, solubilising sidechains were introduced in examples **32**, **48–54** and **56**, **57**. Disappointingly, compounds with strongly or weakly basic sidechains (**32**, **49**, **50**, **56**, **57**) displayed poor activity or were inactive, while neutral compounds **51** and **53** showed only moderate activity (IC₅₀s 3.31 and 2.65 μM), respectively.

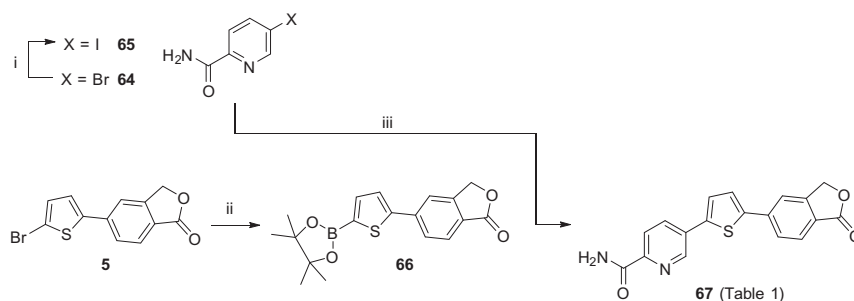
In order to further increase diversity and enrich the SAR of the diarylthiophene series, a range of commercially available boronates were deployed in Suzuki reactions with key intermediate **5** ([Scheme 1](#)), generating analogues **58–63**. Results were mixed, with the methyl alcohol **59** and methyl sulfonamide **63** (IC₅₀s 0.92 and 1.09 μM, respectively) the best of this set. Finally, given similarities in SAR between the A- and C-subunits⁷ the symmetrical analogue **71** was synthesised but proved only moderately potent as well as extremely insoluble.

Biological activity and stability: The five most potent inhibitors of isolated recombinant perforin (**23**, **48**, **59**, **63**, and **67** from [Table 1](#)) were then subjected to more advanced assessment. Preliminary stability studies were carried out by incubation in human or mouse plasma at 37 °C with the percentage parent remaining measured at 24 h ([Table 2](#)). All five inhibitors were significantly more stable in mouse plasma compared to human. This result is not unexpected, as the anticancer agent camptothecin which also contains a lactone moiety has been shown to co-exist in both the closed and ring-opened form and that this equilibrium is distinctly different for human and mouse plasma. In mouse plasma the ratio of open to closed is 50:50%, while in human plasma this shifts to 90:10 due to the strong affinity of human serum albumin for the ring-opened form.²⁰ It is likely that the same phenomenon is operating in the present case and while not necessarily an issue for mouse studies, will need to be addressed in future work.

The compounds were then evaluated for their ability to block the effect of perforin delivered by whole KHYG-1 NK cells ([Table 2](#)). Employing whole NK cells is a more rigorous model of conditions in vivo than the use of isolated recombinant protein since effector cell identification of the labelled target cell and formation of an immune synapse are required for perforin release. Any putative inhibitor must also possess the ability to access perforin released into the synaptic cleft. After co-incubation of inhibitor with KHYG-1 NK cells in medium for 30 min at room temperature, ⁵¹Cr-labelled K562 leukaemia target cells were added and cell lysis evaluated after 4 h incubation at 37 °C by measuring ⁵¹Cr release. The viability of the NK cells in the presence of inhibitor was also assessed 24 h. later to confirm that any inhibitory activity exhibited by the compounds was due to blocking the action of perforin and not nonspecific killing of the effector cells. Viable and dead cells were counted and percent viability calculated based on total cell count (for further details see [Supplementary data](#)). Of the five compounds selected for further testing, three showed poor or no activity; the 3-OH, 4-CONH₂ compound **48**, 4-methyl alcohol **59** and methanesulfonamide **63**. Gratifyingly, the most potent compound against isolated perforin (**23**) showed comparable activity (60% inhibition of lytic activity at 20 μM concentration) to many of the active compounds contained in our previous report on the thioxoimidazolidinone-based series.⁸ In addition, pyridinecarbox-



Scheme 3. Reagents and conditions: (i) 2-bromothiophene, DMSO, KF, $\text{PdCl}_2(\text{PPh}_3)_2$, AgNO_3 , 100 °C, N_2 , 2–4 h; (ii) toluene/EtOH, 2 M Na_2CO_3 , $\text{PdCl}_2(\text{dppf})\cdot\text{CH}_2\text{Cl}_2$, 85–90 °C, N_2 , 1–6 h; (iii) MeOH, 2 M NaOH, 100 °C, 2 h, HCl-workup; (iv) TFA-DCM (1:1), rt, 3–4 h; (v) THF, pyridine, pentafluorophenyltrifluoroacetate, rt, 2 h, $\text{R}^2\text{-NH}_2$, rt, 2 h, **48**; or pyridine, DCC, HOBT, 75 °C, 5–6 h, **49–54**; or anhydrous-DMF, EDCI-HCl, HOBT, rt, 2 h, $\text{R}^2\text{-NH}_2$, 0 °C → rt, 1–2 h, **55–57**.



Scheme 4. Reagents and conditions: (i) sealed tube, 1,4-dioxane, CuI (5.0 mol %), NaI, racemic *trans*- N,N' -dimethyl-1,2-cyclohexanediamine (10 mol %), 110 °C, N_2 , 24 h; (ii) DMSO, bis(pinacolato)diboron, KOAc, $\text{PdCl}_2(\text{dppf})\cdot\text{CH}_2\text{Cl}_2$, 90 °C, N_2 , 4 h; (iii) toluene/EtOH, 2 M Na_2CO_3 , $\text{PdCl}_2(\text{dppf})\cdot\text{CH}_2\text{Cl}_2$, 85–90 °C, N_2 , 1.5 h.

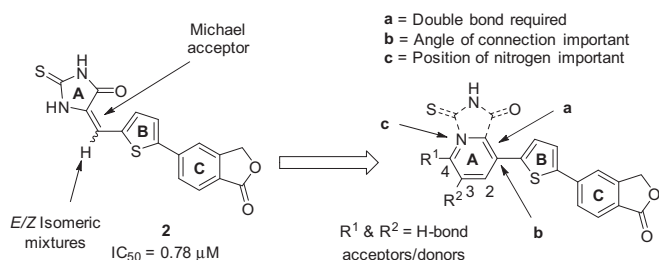


Figure 2. Rationale for the development of new diarylthiophene inhibitors.

amide **67** was essentially equivalent to our previously most potent in vivo candidates (80% inhibition of lysis). Most importantly, this activity was observed in the absence of toxicity toward the effector cells (90% and 82% viability, respectively). For the purposes of this work, compounds are classified as toxic if NK cell viability falls below 70%. This is due to the importance of CTLs and NK cells in the overall immunological response which means that it is essential that any pharmacological intervention allows rapid recovery of these cytotoxic effector cells for normal immunological function.

In summary a new series of benzene- and pyridine-carboxamides have been designed as isosteric replacements for our previous

Table 2
Capacity of selected compounds to inhibit perforin delivered by KHYG-1 NK cells

Number	Jurkat IC_{50}^a (μM)	KHYG-1 inhibition ^b (% at 20 μM)	KHYG-1 viability ^c (%)	Plasma stability ^d (% at 24 h)	
				Mouse	Human
23	0.18	63	90	75	46
48	0.67	20	92	75	49
59	0.90	13	79	87	73
63	1.09	No activity	—	80	70
67	0.92	80	82	66	39

^a Data given for five most potent compounds as determined by the Jurkat assay.

^b Inhibition by compound (20 μM) of the perforin-induced lysis of K562 target cells when co-incubated with KHYG-1 human NK cells (see [Supplementary data](#)). Percent inhibition calculated compared to untreated control.

^c Viability of KHYG-1 NK cells after 24 h by Trypan blue exclusion assay (see [Supplementary data](#)).

^d Data given as % parent measured at 24 h in mouse and human plasma (see [Supplementary data](#) for further details).

thioxoimidazolidinone-based series. Several of these compounds showed improved inhibition of perforin-mediated lysis, with benzene-carboxamide **23** demonstrating a 4-fold increase (0.18 μM) over the corresponding thioxoimidazolidinone inhibitor **2** (0.78 μM). Compound **23** and its pyridine analogue **67** also showed

excellent activity against the lytic action of whole human NK cells, at a level comparable to our previously identified *in vivo* candidates.⁸ Use of an isostere in place of the thioxoimidazolidinone A-subunit has also enabled removal of a potentially reactive Michael acceptor moiety and elimination of *E*- and *Z*-isomeric mixtures. Finally, these compounds do not appear to possess the marginal toxicity associated with many of the thioxoimidazolidinones.

We can conclude that we have identified viable replacements for the thioxoimidazolidinone subunit while improving potency. We have also overcome several key concerns in relation to this historic series and now seek to expand on these findings to identify a suitable *in vivo* candidate. The mechanism of action of these inhibitors is still under investigation, however given our previous conclusion that this class of compounds target perforin after its release into the immune synapse it is most likely that inhibition occurs by either (1) blocking the monomers from association with the target cell membrane, (2) counteracting the oligomerisation process that forms the pre-pore, or (3) by blocking of the conformational changes required for pore formation. This work is currently ongoing and further developments will be reported in the future.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bmcl.2015.12.003>.

References and notes

1. Lopez, J. A.; Brennan, A. J.; Whisstock, J. C.; Voskoboinik, I.; Trapani, J. A. *Trends Immunol.* **2012**, *33*, 406.
2. Law, R. H. P.; Lukoyanova, N.; Voskoboinik, I.; Caradoc-Davies, T. T.; Baran, K.; Dunstone, M. A.; D'Angelo, M. E.; Orlova, E. V.; Coulibaly, F.; Verschoor, S.; Browne, K. A.; Ciccone, A.; Kuiper, M. J.; Bird, P. I.; Trapani, J. A.; Saibil, H. R.; Whisstock, J. C. *Nature* **2010**, *468*, 447.
3. Voskoboinik, I.; Smyth, M. J.; Trapani, J. A. *Nat. Rev. Immunol.* **2006**, *6*, 940.
4. Barry, M.; Bleackley, R. C. *Nat. Rev. Immunol.* **2002**, *2*, 401.
5. Veale, J. L.; Liang, L. W.; Zhang, Q.; Gjertson, D. W.; Du, Z.; Bloomquist, E. W.; Jia, J.; Qian, L.; Wilkinson, A. H.; Danovitch, G. M.; Pham, P.-T. T.; Rosenthal, J. T.; Lassman, C. R.; Braun, J.; Reed, E. F.; Gritsch, H. A. *Hum. Immunol.* **2006**, *67*, 777.
6. Trapani, J. A.; Smyth, M. J. WO 2005083098 A1, 1 March, **2005**.
7. Spicer, J. A.; Huttunen, K. M.; Miller, C. K.; Denny, W. A.; Ciccone, A.; Browne, K. A.; Trapani, J. A. *Bioorg. Med. Chem.* **2012**, *20*, 1319.
8. Bull, M. R.; Lena, G.; Lyons, D. M.; Huttunen, K. M.; Miller, C. K.; O'Connor, P. D.; Bull, M.; Helsby, N. A.; Jamieson, S. M. F.; Denny, W. A.; Ciccone, A.; Browne, K. A.; Lopez, J. A.; Rudd-Schmidt, J.; Voskoboinik, I.; Trapani, J. *J. Med. Chem.* **2013**, *56*, 9542.
9. Lopez, J. A.; Jenkins, M. R.; Rudd-Schmidt, J. A.; Brennan, A. J.; Danne, J. C.; Mannering, S. I.; Trapani, J. A.; Voskoboinik, I. *J. Immunol.* **2013**, *191*, 2328.
10. Bull, M. R.; Spicer, J. A.; Huttunen, K. M.; Denny, W. A.; Ciccone, A.; Browne, K. A.; Trapani, J. A.; Helsby, N. A. *Eur. J. Drug Metab. Pharmacokinet.* **2015**, *40*, 417.
11. Baell, J. B.; Holloway, G. A. *J. Med. Chem.* **2010**, *53*, 2719.
12. Hayat, S.; Atta-ur-Rahman; Choudary, M. I.; Khan, K. M.; Bayer, E. *Tetrahedron Lett.* **2001**, *42*, 1647.
13. Klapars, A.; Buchwald, S. L. *J. Am. Chem. Soc.* **2002**, *124*, 14844.
14. Kumar, N.; Nayyer, S.; Mitu, G. Patent WO 2006103550 A1, 5 October, **2006**.
15. Kobayashi, K.; Sugie, A.; Takahashi, M.; Masui, K.; Mori, A. *Org. Lett.* **2005**, *7*, 5083.
16. Spicer, J. A.; Denny, W. A.; Miller, C. K.; O'Connor, P. D.; Huttunen, K.; Trapani, J. A.; Hill, G.; Alexander, K. Patent WO 2014028968 A1, 27 February, **2014**.
17. Ernst, G.; Fietze, W.; Jacobs, R.; Phillips, E. Patent WO 2005061510 A1, 7 July, **2005**.
18. Dally, R. D.; Dodge, J. A.; Hummel, C. W.; Jones, S. A.; Shepherd, T. A.; Wallace, O. B.; Weber, W. W. Patent WO 2005073205 A1, 11 August, **2005**.
19. Effenberger, F.; Endtner, J. M.; Miehlich, B.; Münter, J. S. R.; Vollmer, M. S. *Synthesis* **2000**, *9*, 1229.
20. Giovannella, B. C.; Harris, N.; Mendoza, J.; Cao, Z.; Liehr, J.; Stehlin, J. S. *Ann. N.Y. Acad. Sci.* **2000**, *922*, 27.