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A 2,6,9-hetero-trisubstituted purine inhibitor exhibits potent biological effects against multiple myeloma cells

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ABSTRACT

A focused library of hetero-trisubstituted purines was developed for improving the cell penetrating and biological efficacy of a series of anti-Stat3 protein inhibitors. From this SAR study, lead agent **22e** was identified as being a promising inhibitor of MM tumour cells (IC_{50}'s <5 μM). Surprisingly, biophysical and biochemical characterization proved that **22e** was not a Stat3 inhibitor. Initial screening against the kinome, prompted by the purine scaffold's history for targeting ATP binding pockets, suggests possible targeting of the JAK family kinases, as well for ABL1 (nonphosphorylated F317L) and AAK1.

1. Introduction

Purines feature in biological molecules that are critically involved in many essential cellular processes. Considered a privileged structure in biological systems, the purine heterocycle represents an intriguing molecular starting point for new pharmaceutical agents.[1] Purines have been incorporated into therapeutic structures for applications in nucleotide anti-metabolites and play a key structural role in inhibitors of cancer-promoting dysregulated proteins in cancer.[2–6] For example, hetero-tri-substituted purines have been used successfully to inhibit cell cycle initiators, cyclin-dependant kinases which have been implicated in cancer.[7] In addition, purine inhibitors have successfully suppressed the activity of Heat Shock Protein 90, a protein critical for tumour survival.[8]

We have demonstrated that purine scaffolds could be functionalized as part of a pharmacophore model to bind and inhibit the Src Homology 2 (SH2) domain of the signal transducer and activator of transcription 3 (Stat3) protein. Stat3 has been shown to play a key role in regulating cancer cell growth and differentiation. Hyperactivation of Stat3 protein levels has been shown to be transformative, leading to uncontrolled cell proliferation and apoptotic resistance in a multitude of human cancer cells.[9,10] Many types of cancer cells exhibit Stat3 'addiction' and are widely acknowledged to be hypersensitive to Stat3 inhibition that leads to programmed cancer cell death. Thus, potent Stat3 inhibitors have tremendous potential as novel therapeutics.[11,12]

In previous work, a quantitative structure activity relationship (QSAR) of a 2,6,9-hetero-trisubstituted purine core was conducted for targeting the Src Homology 2 domain 'hot spot' of Stat3 (Fig. 1).[13] Computational docking studies suggested that trisubstituted purine inhibitors may best access the sub-pockets of Stat3's SH2 domain and thus prevent Stat3 cellular function. Specifically, inhibitors were prepared to access two predominantly hydrophobic pockets (various appendages at R_1 and the cyclohexylbenzyl substituent at the N2 position), as well as the polar phosphotyrosine (pTyr) binding site with an N9-carboxylate substituent. The purines prepared showed potent Stat3 protein binding in vitro, as assessed by surface plasmon resonance (SPR: Biacore 3000), with K_d values ranging from 1 to 10 μM. However, lead Stat3 binders **8a–g** (Fig. 1) displayed only modest anti-proliferative effects in cancer cell lines. To determine the origins of the lower than expected IC_{50} values, experiments were conducted to assess the cell penetrating properties of lead purine inhibitors. In particular, Caco2 cell penetrating studies using a Waters Xevo quadrupole time-of-flight (QTof) mass spectrometer and an ACQUITY UPLC system revealed that inhibitors poorly traversed the cell membrane.

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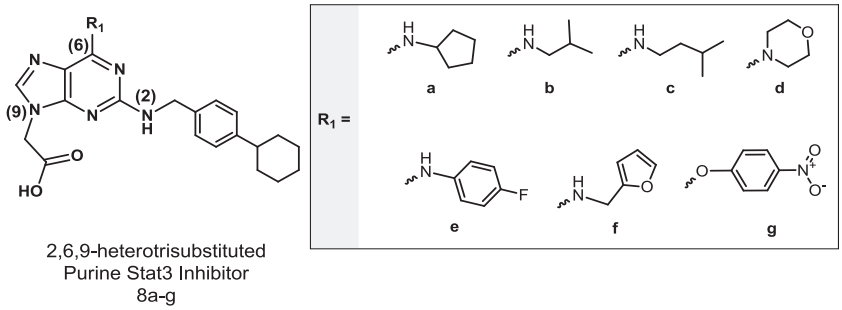

Figure 1. 1st generation 2,6,9-heterotrisubstituted purine inhibitors of Stat3.

Herein, synthetic efforts to prepare second-generation anti-Stat3, purine-based inhibitors, with improved cell permeability and enhanced cytotoxicity toward cancer cells are presented. Specifically, synthetic efforts were undertaken to mask the anionic N9-carboxylic acid substituent, present on lead Stat3 inhibitors prepared in the previous study. Prodrug and bioisostere strategies were employed to furnish a family of purine-based small molecules that retained the top ranked R1 and R2 substituents from the previous studies. However, while non-carboxylate containing analogs did not exhibit Stat3 binding affinity, compound **22e**, substituted with a sulfamate group, exhibited potent cytoxicity in multiple myeloma (MM) whole cell tumour studies. Efforts to delineate the intracellular targets are described.

2. Results and discussion

2.1. Inhibitor design

To overcome the poor cell penetrating properties of first generation purines, two design strategies were adopted. First, the N9 carboxylic acid substituent was masked using prodrug strategies.[14] Since lead purine compounds were considered relatively non-polar ($c\log P = -0.2$–4.1), and that masking the charged appendage might significantly reduce water solubility, we prepared a range of prodrugs with varying polarity to circumvent possible inhibitor aggregation. We reasoned that a successful prodrug approach will facilitate inhibitor cell membrane penetration, improve inhibitor half-life, and increase cellular potency.

Second, bioisosteres of the carboxylic acid were introduced for purposes of increasing inhibitor lipophilicity, reducing anionic character and improving binding potency through potentially more favorable and increased intermolecular interactions with the protein surface. In this study, the N9-carboxylic acid appendage was replaced with either a tetrazole or sulfamate appendage. Tetrazole, while possessing a similarly acidic proton to the carboxylate, possesses significantly greater lipophilicity, and potentially improved cell penetrative properties.[15] In addition, a neutral, hydrogen bonding sulfamate group was selected for making contacts with the pTyr binding pocket of Stat3's SH2 domain.

2.2. Preparation of 2,6,9-tri-heterosubstituted purines

Access to final molecules was achieved through four synthetic routes each starting from 2-amino-6-chloropurine (Fig. 2). Near quantitative BOC protection of the N9 position was achieved using BOC anhydride and catalytic dimethylamino-pyridine to give **2** (Scheme 1).[16] As previously reported by our group,[17] treatment with NaH efficiently mediated BOC group transfer from N9 to N2 (**3**). Mitsunobu reaction conditions using ethyl glycolate afforded the N9 alkylated product **4**, over the less reactive N7 position. Next, **4** was subjected to a successive round of Mitsunobu conditions, this time with cyclohexyl benzyl alcohol, to furnish the N2 alkylated product **5**. Using microwave mediated nucleophilic aromatic substitution, **5** was treated with a select series of previously identified alkylamines,[13] to give **6a**–**g** in good yields. Quantitative BOC deprotection of **6a**–**g** was achieved using standard trifluoroacetic acid/CH_2Cl_2 conditions to give the ethyl ester prodrug final molecules, **7a**–**g**. To access acyloxymethyl ester classes of carboxylic acid prodrug, the ethyl ester of compounds, **7a**–**g** was hydrolysed using LiOH mediated saponification to give carboxylic acids **8a**–**g**, then intermediates subjected to treatment with either acetoxymethyl bromide to yield the acyloxymethyl ester prodrugs (**9a**–**g**), or pivaloyloxymethyl iodide to yield the pivaloyloxymethyl ester prodrugs (**10a**–**g**). Due to stability issues, all prodrug analogs were used immediately after HPLC and lyophilisation. Purity levels were confirmed by analytical HPLC prior to in vitro or biological testing.

Preparation of N9-sulfamate-containing purine inhibitors were prepared by Mitsunobu-mediated installation of the sulfamate substituent (Scheme 2). Briefly, compound **3** was selectively alkylated on N9 with freshly prepared monosilyated ethylene glycol using Mitsunobu conditions as previously reported to acquire **17** in good yields.[18] Next, **17** was subjected to a subsequent Mitsunobu reaction with cyclohexyl benzyl alcohol to yield the N2 alkylated product **18**. Analogous to the prodrug approach, nucleophilic aromatic substitution was employed to incorporate alkylamines at C6 to yield compounds **19a**–**g**. TBDMS deprotection using TBAF in THF afforded the free alcohol **20a**–**g** in excellent yields. Finally, primary alcohols **20a**–**g** were subjected to NaH and treated with sulfonamide chloride (as prepared from chlorosulfonyl isocyante **23**)[19] to yield the sulfamate products **21a**–**g** in good yields (35–66%).[20] Finally, BOC deprotection using trifluoroacetic acid provided final molecules **22a**–**g** in excellent yields (73–89%).

Derivatives featuring the lipophilic tetrazole bioisostere utilized a modified alkylation procedure. Precursor **3**, was alkyated with trityl protected tetrazole **30**, proceeding through an S_N2 reaction after Mitsunobu conditions proved poor yielding (Scheme 3). The resulting mixture of N7 and N9 alkylated products were separated by chromatography, with the N9 product isolated in good yields providing **31**. Formation of **32** follows conventional N2 alkylation using Mitsunobu chemistry, and **33a**–**g** were formed using an aromatic substitution diversification step. Final molecules **34a**–**g** was obtained in good yields following a global de-protection of BOC and trityl groups using trifluoroacetic acid.

Several lead derivatives of the past library possessed a cyclohexyl carbonyl moiety in the N2 position To successfully acylate these molecules, the N2 nitrogen was liberated of protecting groups using trifluoroacetic acid or a Lewis acid ($AlCl_3$). The remaining steps follow logically from the methods just described and are detailed in Supplementary data.