Bioorganic & Medicinal Chemistry Letters 28 (2018) 2636–2640

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Merging of ruxolitinib and vorinostat leads to highly potent inhibitors of JAK2 and histone deacetylase 6 (HDAC6)

Lianbin Yao[a], Pondy Murugappan Ramanujulu[a,b], Anders Poulsen[c], Sten Ohlson[d], Brian W. Dymock[a,*]

[a] Department of Pharmacy, National University of Singapore, 18 Science Drive 4, Singapore
[b] Life Sciences Institute, Centre for Life Sciences Level 5, 28 Medical Drive, National University of Singapore, Singapore
[c] Experimental Therapeutics Centre, 31 Biopolis Way, 03-01 Nanos, Singapore
[d] School of Biological Sciences, Nanyang Technological University (NTU), Singapore

ARTICLE INFO

Keywords:
Janus kinase
Histone Deacetylase
Designed Multiple Ligand

ABSTRACT

Inhibition of more than one pathway in a cancer cell with a single molecule could result in better therapies with less complex dosing regimens. In this work multi-component ligands have been prepared by joining together key pharmacophores of two different enzyme inhibitors in a way which increases potency against the individual pathways. Selective JAK1/2 inhibitor, ruxolitinib (**3**), and pan-HDAC inhibitor vorinostat (**4**) were linked together by a single nitrogen atom to create a new series of compounds with very potent JAK2 and HDAC6 inhibition with selectivity against HDAC1. A preferred compound, **13b**, had unprecedented sub-nanomolar JAK2 potency with an IC_{50} of 41 pM and a sub-nanomolar IC_{50} against HDAC6 of 200 pM. Binding models show a good fit into both JAK2 and HDAC6.

Modern cancer therapy typically requires combinations of more than one drug in carefully designed dosing regimens.[1] Despite good progress with this approach in many disease indications a strong need for better treatments still exists, for example in drug resistant disease.[2] Where a drug is administered together with a second agent careful scheduling is usually required to optimise efficacy with acceptable toxicity. However it is resource intensive and time consuming to identify the best doses and schedules and minimizing problems such as drug-drug interactions.[3] Another approach to achieving multi-target inhibition, which may alleviate some of the problems associated with combination therapy, is to design molecules capable of selectively inhibiting multiple targets.[4] The so-called 'multi-component ligand' requires judicious choice of biological targets known to be important when inhibition is combined in disease pathways while having complimentary pharmacophores to enable design of a single molecule able to bind both targets.[5] We have been active in designing such molecules targeting janus kinases (the JAK-STAT signaling pathway) and the epigenetic enzyme histone deacetylase (HDAC).[6,7] JAK kinases are direct activators of STATs[8] and inhibition of HDACs is reported to reduce STAT levels.[9] Combination inhibition of JAKs and HDACs could provide multiple blockage of the JAK-STAT pathway.

We recently reported dual inhibitors of JAK kinases and HDACs, EY3238 (**1**) and YLB343B (**2**), with low nanomolar potency and selectivity against members of both target families (Fig. 1).[6,7] Our investigations leading to **1**[6] were based on the kinase inhibiting macrocycle pacritinib[10] and the pan-HDAC inhibitor vorinostat (**4**).[11] In further work we focused our studies on new combination molecules based on the smaller JAK1/2 inhibitor ruxolitinib (**3**).[7] In this work we expand on our studies merging **3** with **4** to produce exceptionally potent dual inhibitors with sub-nanomolar inhibition of JAK2 and HDAC6.

Analysis of published crystal structures of **4**[12] suggests the most solvent exposed position is the terminating phenyl ring. There are no published crystal structures of **3** in complex with JAK kinases but structures of similar molecules such as tofacitnib are available.[13] These structures strongly support the central carbon of the pyrimidine having a direct path out to solvent hence linking the pharmacophores of **3** and **4** via these positions could result in a single compound series with activity against both JAK and HDAC enzymes (Fig. 2).

In our previous work we developed a suitable template for JAK-HDAC dual inhibitors based on **3**[7] but we had not explored alternative attachment points for the hydroxamate bearing chain. In this work we report dual inhibitors with an in-depth exploration of the pyrazole substituent.

Synthesis of a JAK-HDAC dual inhibitor based on the design of Fig. 2, started with advanced intermediate **5** (Scheme 1).[14] Conversion of the methyl ester of **5** to THP-protected hydroxamate **6** was achieved

* Corresponding author at: QEDDI, Uniquest Pty Ltd, General Purpose South Bldg, Staff House Road, The University of Queensland, Brisbane, QLD 4072, Australia.
E-mail address: bwdnus@gmail.com (B.W. Dymock).

https://doi.org/10.1016/j.bmcl.2018.06.037
Received 25 April 2018; Accepted 17 June 2018
Available online 19 June 2018
0960-894X/

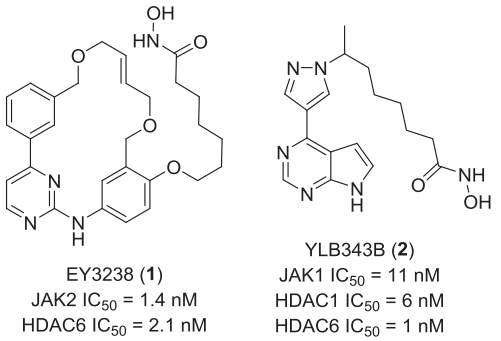


Fig. 1. Structures of the JAK2-HDAC dual inhibitors EY3238 (**1**) and (**2**).

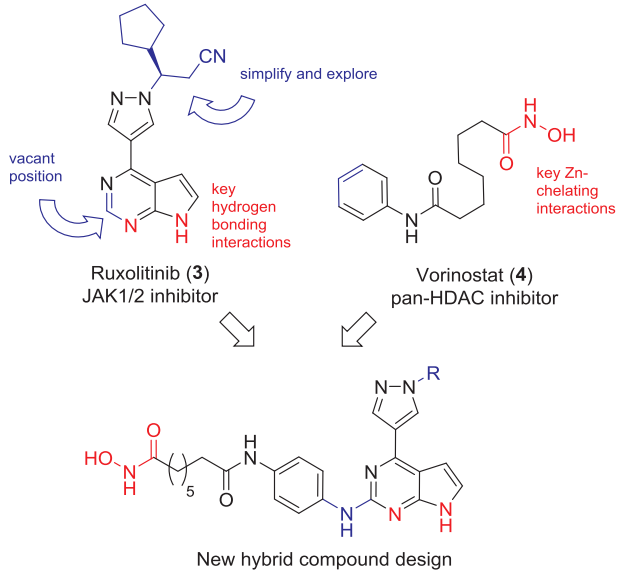
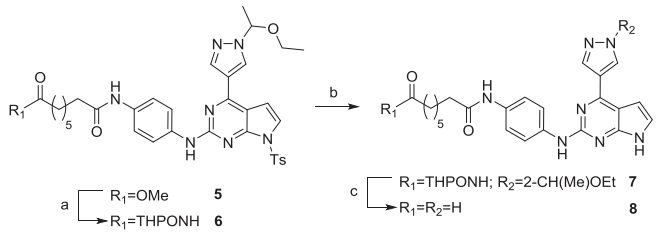


Fig. 2. Known SAR of reference compounds ruxolitinib (**3**) and vorinostat (**4**) indicates solvent exposed areas (blue colour) of each inhibitor which can be used to as connection points for combination compounds. Preservation of key hydrogen bonding groups (red colour) ensures important binding interactions are maintained at each target. A new hybrid compound design with the HDAC binding component of **4** attached through an amine to the pyrimidine of the JAK inhibitor core of **3**.



Scheme 1. Preparation of compound **8**. Reagents and Conditions: (a) (i) LiOH, THF/H_2O, rt, overnight; (ii) *O*-(tetrahydro-2H-pyran-2-yl)hydroxylamine, HATU, Et_3N, DMSO, rt, overnight, 88%; (b) MeONa, MeOH, 70 °C, 3 h, 96%; (c) HCl/1,4-dioxane, rt, overnight, 26%.

via saponification with LiOH followed by amide coupling using HATU in good overall yield. Deprotection of the tosyl group with sodium methoxide furnished **7** which was globally deprotected to the target **8** with HCl in dioxane. Purification of polar **8** required preparative reverse phase HPLC.

Substitution of the vacant pyrazole nitrogen was achieved by alkylation of tosyl protected ester **9** with alkyl halides **9a-g** or addition to either vinyl sulfone (**9h**) or acrylonitrile (**9i**) using DBU as base (Scheme 2). Subsequent THP hydroxamate formation followed by deprotection with HCl gave access to desired analogues **13a-i**.

Prepared target compounds **13a-i** were tested in isolated enzyme assays against HDAC1, HDAC6 and JAK2 and compared with reference compounds **3**, **4** and the HDAC6-selective Tubastatin A (Table 1). Surprisingly, **8** was very potent against all tested enzymes with an IC_{50} of 2 nM for HDAC1, 0.2 nM for HDAC6 and 0.96 nM against JAK2. When the pyrazole was substituted with propyl (**13a**) both HDAC1 and HDAC6 activity dropped but JAK2 potency increased to significantly sub-nanomolar (0.15 nM). The slightly longer homoallyl **13b** enjoyed a further significant increase of JAK2 potency into the low picomolar range (41 pM) with additional very good HDAC6 potency (0.25 nM) and selectivity over HDAC1 of > 300-fold. This remarkable JAK2 potency surpasses even that of **3** (56 pM tested in the same assay) and could be due to the extra hydrogen bond donor from the pendant pyrimidine NH. Hence we carried out docking studies to improve our understanding of the possible binding modes of **13b** to JAK2 and HDAC6 (Fig. 3).

When docked into JAK2 **13b** forms 3 hydrogen bonds with the hinge residues Glu930 and Leu932 (Fig. 3A).[15] From this model it appears clear that the additional pendant NH does indeed form an additional hydrogen bond with Leu932. Furthermore, the homoallyl side chain is completely buried in a pocket formed from the glycine rich loop (Fig. 3B). In HDAC6, the hydroxamate of **13b** chelates the Zn at the base of the substrate pocket in the mode of **4** as expected (Fig. 3C/D). Intriguingly, the homoallyl pyrazole substituent engages in a shallow hydrophobic pocket around Thr678 and Met682. This interaction appears to be very complementary possibly explaining the very strong observed inhibition of HDAC6.

Similar results are seen with the similar propionitrile **13i** (JAK2 IC_{50} = 40 pM), however methoxyethyl **13c** loses the exquisite potency of **13b**/**13i** but in itself is a very useful analogue since JAK2 and HDAC6 activities are very similar while still retaining 30-fold selectivity over HDAC1. Steric tolerances were challenged with cyclopentylmethyl **13d** where JAK2 potency was fully retained compared with **13c**. HDAC6 only suffered a 3-fold drop in activity but HDAC1 selectivity returned to nearly 300 fold, similar to **13b**. Clearly the pyrazole substituent is a very influential tuning point for potency and selectivity. Continuing the investigation, we explored even larger substituents. Phenoxypropyl **13e** experienced a drop in JAK2 potency and when the carbon chain was extended (**13f**/**13g**) no significant change in the data was seen for either target enzyme. This could reflect the ability of long flexible pyrazole side chains to adopt a conformation toward solvent.[7] Finally, methylsulfone **13h** also had single digit nanomolar potency for JAK2 and HDAC6 but with less selectivity over HDAC1.

We next evaluated selected compounds for their ability to inhibit proliferation in a panel of 4 solid tumor cells lines including breast (MDA-MB-231, MCF7), colorectal (HCT-116) and prostate (PC3) cancer (Table 2). Reference compounds in these cell lines included the pan-HDAC inhibitor **4**, the HDAC6 selective inhibitor Tubastatin A and the JAK1/2 selective **3**. The panel of cells was not sensitive to compound **3** (IC_{50}s > 10 μM) indicating that these cells were not suitable to assess JAK kinase potency. However, these cells are sensitive to HDAC inhibition with the lowest IC_{50} value being 2.0 μM for Tubastatin against HCT-116 and 0.65 μM for **4** against MCF7. Of the new compounds, the most potent enzyme inhibitor, **13b**, was also the most potent in each of the cell lines. This activity is most likely reflecting the potent HDAC activity of **13b**. Further studies in other cell lines will be required to elucidate the best cellular applications of these dual inhibitors.

In conclusion, this preliminary work describes small molecules with highly potent sub-nanomolar inhibition of two different enzyme classes, exemplified by JAK2 and HDAC6, both strongly implicated in serious diseases such as cancer and immuno-inflammatory diseases.