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Design and synthesis of triple inhibitors of janus kinase (JAK), histone deacetylase (HDAC) and Heat Shock Protein 90 (HSP90)

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ABSTRACT

Inhibition of multiple signaling pathways in a cancer cell with a single molecule could result in better therapies that are simpler to administer. Efficacy may be achieved with reduced potency against individual targets if there is synergy through multiple pathway inhibition. To achieve this, it is necessary to be able to build multi-component ligands by joining together key pharmacophores in a way which maintains sufficient activity against the individual pathways. In this work, designed triple inhibiting ligands are explored aiming to block three completely different target types: a kinase (JAK2), an epigenetic target (HDAC) and a chaperone (HSP90). Although these enzymes have totally different functions they are related through inter-dependent pathways in the developing cancer cell. Synthesis of several complex multi-inhibiting ligands are presented along with initial enzyme inhibition data against 3 biological target classes of interest. A lead compound, **47**, was discovered which had low micromolar activity for all 3 targets. Further development of these complex trispecific designed multiple ligands could result in a 'transient drug', an alternative combination therapy for treating cancer mediated via a single molecule.

Combination drug treatment is the mainstay of modern cancer therapy.[1] However even with the welcome progress made with immune-oncology therapeutics,[2] there remains a high unmet need in treatment of many cancers and especially drug resistant disease.[3] Combination therapies are typically given as two or more separate drugs which need to be scheduled to ensure maximal efficacy with the lowest possible toxicity. However this is not always successful and drug-drug interactions can be a particular problem.[4] New ways of delivering combination therapy are therefore urgently needed. The new concept of the transient drug, where less potency at the target is balanced with multiple target inhibition creating a synergistic effect which is efficacious but without overt toxicity, could be one such new approach.[5] To approach such a challenge molecules able to inhibit multiple targets will need to be designed and synthesized. The choice of target is crucial and must include targets important in disease pathways, or known to be important when combined with inhibition of certain other pathways.[6] In this regard, we have been interested in inhibition of kinase signaling pathways, epigenetic targets and chaperone proteins.

Our previous work has led to dual inhibitors of JAK (Janus) kinases and HDACs (histone deacetylases), EY3238 (**1**) and YLB343B (**2**), with low nanomolar potency against members of both target families (Fig. 1).[7,8] In this present work, we aimed to stretch the possibilities of small molecule chemistry to assess the feasibility of preparing single molecules which could have potential to be inhibitors of 3 different pathways by inhibition of 3 different types of enzyme target.

We aimed to build upon our previous work in JAK-HDAC dual inhibitors by adding on a third activity against the chaperone Heat Shock Protein 90 (HSP90). HSP90 is massively upregulated in developing cancer cells due to its importance in ensuring the correct folding of a wide array of client proteins many of which are oncoproteins.[9] HSP90 promotes activation of the oncogenic inflammatory mediator STAT-3.[10] JAK kinases are direct activators of STATs[11] hence inhibition of HSP90 and JAK could be synergistic. Inhibition of HSP90 has been shown to be effective in cells with an activated JAK-STAT pathway.[12] HDACs, in particular HDAC6, are known to be important in modulating HSP90 complexes.[13,14] Hence exploring a triple inhibitor of all three enzymes could have value in treating cancer with JAK-STAT and/or HSP90 dependency. We selected the marketed drugs ruxolitinib (**3**),[15] vorinostat (**4**)[16] and the published HSP90 inhibitor BEP800 (**5**)[17] as the base

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molecules for initial exploratory work towards a triple inhibitor of JAK-HDAC-HSP90.

Analysis of published crystal structures of **4**[18] and **5**[19] indicates clearly which regions of the structures are solvent exposed. There are no published crystal structures of **3** in complex with JAK kinases but structures of similar molecules such as tofacitnib are available.[20] These structures indicate that there are two regions of the structure of **3** which are solvent exposed, or with a direct path out to solvent: the nitrile group and the central carbon of the pyrimidine. These were therefore considered as viable attachment points for either an HDAC or HSP90 binding moiety. Compound **4** has a solvent exposed terminal phenyl group which we have established as an attachment point for a JAK inhibiting moiety. HSP90 binds small molecules in its ATP site which is a deep pocket but the structure of molecules related to **5** indicates two solvent exposed regions: the amide alkyl group and the *meta* position of the phenyl.[19] Using this information our initial designs focused on using a simplified core based on **3** with attachment of an HSP90 binding moiety, based on **5**, on the pyrimidine ring and an HDAC binding moiety, based on **4**, on the pyrazole (compare with **2**[8]) (Fig. 2a). Alternatively we planned to explore the HDAC binding group attached to the pyrimidine and the HSP90 binding group attached to the pyrazole (Fig. 2b).

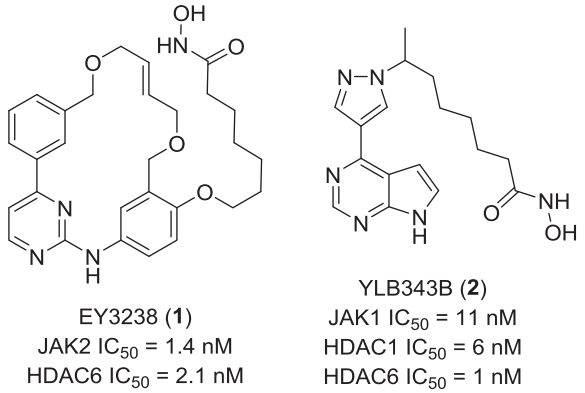

Fig. 1. Structures of the JAK2-HDAC dual inhibitors EY3238 (**1**) and YLB343B (**2**).

In our previous work we had developed a suitable template for JAK-HDAC dual inhibitors based on **3**[8] but we had not developed methods for dual HSP90 inhibitors therefore before embarking on the challenging synthesis of a triple inhibitor we decided to firstly explore JAK-HSP90 dual inhibitor chemistry.

Synthesis of a prototype JAK-HSP90 dual inhibitor (based on the designs of Fig. 2a), started by building the HSP90 portion from aldehyde **6** through a SNAr reaction using 2-mercaptoethylacetate and cyclisation of the intermediate to form thienyl pyrimidine **7** in good yield (Scheme 1). Suzuki coupling with hydroxyphenyl boronic acid gave **8** followed by ethyl amide formation gave phenol **9** in

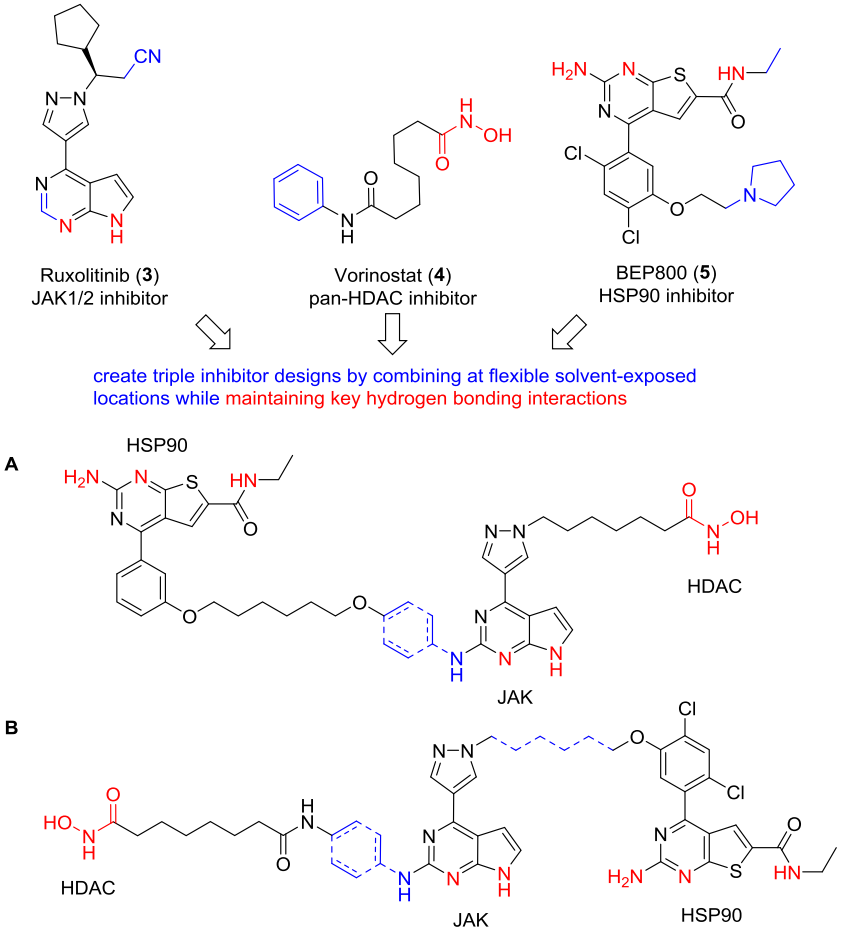

Fig. 2. Known SAR of reference compounds ruxolitinib (**3**), vorinostat (**4**) and BEP800 (**5**) enables design of multiple ligands. Solvent exposed areas (blue colour) of each inhibitor can be used to identify connection points for combination compounds. Key hydrogen bonding groups (red colour) are preserved to ensure maximum chance of binding at each target. A: A design based on the compact structure of **2**[8] with attachment of the HSP90 binder to the pyrimidine ring of the JAK binding motif, used for synthesis of compound **39**; B: HDAC binder is attached through the pyrimidine of the JAK inhibitor while the HSP90 binding component can be attached to the pyrazole via a linker, used for synthesis of compounds **17**–**19**, **26**, **27**, **46**, **47**.