Structure–based design and synthesis of 2-benzylidene-benzofuran-3-ones as flavop...

Profession

Introduction

Flavopiridol, a well-established inhibitor of cyclin-dependent kinases (CDK’s), is currently undergoing clinical trials. The inhibition of CDK’s, which are involved in the cell division cycle, is a vital goal in anticancer agents and therefore having potent drugs which can selectively inhibit these is crucial. One of the aims is to synthesize 2-benzylidene-benzofuran-3-ones so that they are more potent at inhibiting some of the CDK’s but also selective in nature, something which flavopiridol lacks. To date falvopiridol has been found to inhibit CDK’s 1, 2 and 4 all with the same potency.

Flavopiridol acts on the CDK2 enzyme by mimicking the actions of the purine group of ATP. It is the keto and hydroxyl groups of the compound which form the same bidendate hydrogen bonds with the backbone of the CDK2 residues as the nitrogen atoms of the purine functional group of ATP. Having obtained this information it was clear to see that some variations of the benzafuranone structure would be able to mimic the interactions that flavopiridol has with CDK’s. The main objective of testing structural analogues was to obtain new CDK inhibitors that are more selective in discriminating between CDK2 and CDK4. Different substituents were attached to the phenyl ring, with modelling suggesting that a hydrogen bond acceptor group in para position would interact favourably whereas a bulky and positively charged para substituent would have a detrimental effect on CDK2 inhibition but not for CDK4.

Experimental Procedure

The derivatives of benzafuranone are synthesized in a set of continuous reactions starting with the acid-catalysed condensation of dimethoxy phenol and 1-methyl-4-piperidone in acetic acid. This produces an unsaturated derivative at 62% yield which is then followed by hydrogenation and treatment with chloroacetyl chloride and aluminium chloride to produce a derivative at 50% yield. The compound is then condensed with 4-bromobenzaldehyde to produce a derivative at 41% yield. Finally the aryl bromide derivative is reacted with 1-methylpiperazine and pyridinium hydrochloride to produce a benzofuranone with one R group being the methylpiperazine and the other being a hydrogen group at a 40% yield (8). The compound 8 and 4 others with different R groups were tested in kinase inhibition assays to see their effect on CDK's 1, 2 and 4.

Results

The derivative with both R groups being hydrogen's (4), proved to be as potent as flavopiridol at inhibiting CDK1 but not as effective against CDK2 and CDK4. The variation of potency of the derivative shows that there was a difference in the sensitivity between the enzymes. Moreover it proved that the synthesized benzofuranone derivatives whilst being potent maintained the high selectivity which was desired. When a chlorine atom was added at the ortho position on the phenyl ring the potency generally went down against all the CDK enzymes. The reason for this is that due to the presence of the chlorine there is some steric hinderence preventing the favourable conformation being adopted. Two other compounds which contain SO2NH2 and NO2 respectively as their R groups proved to be more potent than the

first derivative in inhibiting CDK1 and CDK2. Both compounds contained hydrogen bond acceptor groups in their para positions and so this acted favourably in terms of conserving lysine residue in the enzyme. The reason for a lower potency against CDK4 was due to the lack of conservation of lysine. The slight increase when a sulphonamide group was present as opposed to a nitro group is the result of its additional capacity to donate hydrogen bonds. Compound 8 was again more potent than the derivative with two hydrogen groups as the side chains. This was more prominent in the inhibition of CDK1 as there seemed to be a greater amount of repulsive interaction with the lysine at the 89th position. There wasn’t any noticeable difference in the potency between the compound 8 and 4 due to the ability of compound 8 being able to access the free solvent space due to the replacement of the lysine (at the 89th position) by a threonine.

Conclusion

The compounds obtained from the generalised structure of 2-benzylidene-4, 6-dihydroxy-7-benzofuran-3-ones showed inhibitory characteristics which are true of flavopiridol mimetics. Through careful manipulation of the structure it was possible to increase the inhibitory potency against the CDK1 and CDK2 enzymes and obtain selectivity against CDK4 due to the lack of conservation of lysine. However it was often difficult to increase the inhibition at CDK4 due to shape of the ATP binding site which prevented favourable interactions between the ligand occurring.