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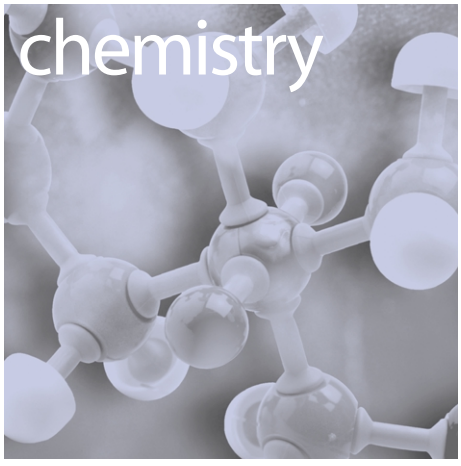
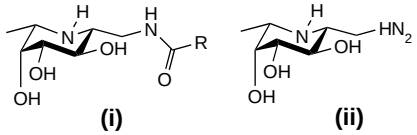


MONITOR CHEMISTRY

Tight-binding inhibitors of α-fucosidase

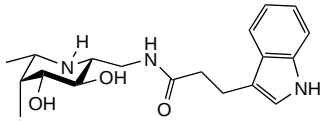
A number of significant biological activities associated with pathologies such as metabolic disorders, infectious diseases, tumor formation and metastasis are frequently under the control of glycosidases. These enzymes can catalyse the cleavage of the non-reducing terminal sugar of oligosaccharides or glycan conjugates. Several of these enzymes have been considered to be pivotal in pathogenesis and have therefore been adopted as potential drug targets.

One of the best examples of drug development through glycosidase inhibition is the neuraminidase inhibitors Tamiflu™ (oseltamivir phosphate) for the treatment of influenza. Thus, the development of potent glycosidase inhibitors is of interest. Amongst members of the glycosidase family, α-fucosidase is involved in removal of non-reducing terminal L-fucose residues that are α1,2-, α1,3-, α1,4-, or α1,6 linked to oligosaccharides. Due to the great biological and pathological diversity associated with fucose-containing glycoconjugates, α-fucosidases have attracted attention as potential drug targets. For example, anomalous distribution of α-fucosidases has been postulated to be significant in inflammation, colorectal cancer and cystic fibrosis. These enzymes have been studied as potential diagnostic and prognostic serum markers for the colorectal and hepatocellular cancers. Recently, slow and tight binding inhibitors of α-fucosidase have been discovered using an combinatorial chemistry approach [1]. This work centred on screening for an optimal aglycon attached to a fuconojirimycin (FNJ)-based structure **(i)**. These FNJ-based structures mimic the transition state of enzymatic glycosidase cleavage.



(i)

(ii)



(iii)

Using 1-aminomethyl FNJ **(ii)** as the scaffold for diversity-orientated synthesis, a library of amides was prepared by reaction with carboxylic acids. The library compounds were screened without purification. Several potent and competitive inhibitors against α-fucosidase from *Corynebacterium* sp. were discovered with IC_{50} values in the low nm range, for example compound **(iii)** has a K_i of 0.32 nM. It was noted that following prolonged periods of enzyme inhibition, there was a time-dependent decrease in the reaction rate as a function of the inhibitor concentration. Upon re-synthesis and purification of a number of library members exhibiting this time-dependent phenomenon, it was shown that the initial collision-complex (E.I) isomerizes to a tighter complex (E.I*). Here, E stands for free enzyme, I is free inhibitor, E.I is a rapidly forming pre-equilibrium complex and E.I* is the final enzyme-inhibitor complex.

Thus, the rapid diversity-based synthesis and subsequent *in situ* screening enables rapid identification of suitable hydrophobic groups for inhibition enhancement. Further work on the determination of the structure of the enzyme-inhibitor complex will allow a better understanding of the mechanism of inhibition. The slow-releasing feature of the inhibition process described here could also provide new directions in drug design.

DNA-dependent protein kinase inhibitor

The repair of DNA damage by cancer cells has a great influence on determining how sensitive they will be to either chemo- or radio-therapy or combination treatment. The DNA-dependent protein kinase (DNA-PK) acts to detect and repair DNA double-strand breaks via the non-homologous end-joining pathway. DNA-PK is a member of the phosphatidylinositol 3-kinase-related kinase family, and has a role in the signalling of cellular stress responses. The holoenzyme is composed of a catalytic unit and a heterodimeric regulatory moiety. The binding of these regulatory factors to DNA double stranded breaks allows recruitment of DNA-protein kinase to generate the active serine/threonine kinase.

Following binding to the DNA double stranded break, DNA protein kinase promotes the 3-dimensional interaction of the other components of the non-homologous end-joining molecular machinery. The activity of DNA protein kinase appears essential for DNA double-strand break repair, as DNA protein kinase-defective cell lines are hypersensitive to DNA-damaging agents. Chemo- and radio-sensitization of cancer cells can be achieved through inhibition of DNA protein kinase and this also results in the potentiation of cytotoxicity brought about by ionizing radiation

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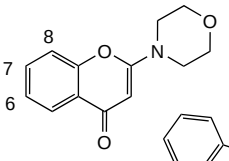
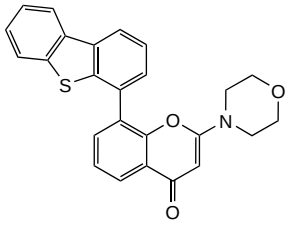
[2]. Recent work has seen the introduction of functionality at the 6-, 7-, and 8-positions on the chromenone template **(iv)**, a known inhibitor template for DNA protein kinase [3].



(iv)



(v)

A solution phase parallel synthesis of a small library of 152 compounds was undertaken using a Greenhouse™ reactor station (Radleys). Compounds were pre-screened for DNA-protein kinase inhibitory activity at an initial concentration of 0.5 μM. Of those compounds screened, the most potent activity was seen with substitution at the 8-position, as opposed to the 6- and 7-positions on template **(iv)**. The most potent compounds were re-screened to determine their IC_{50} values. One of the most potent compounds found was **(v)**, which possessed an IC_{50} of 14 nM against DNA protein kinase. Compound **(v)** is also selective for DNA protein kinase over other phosphatidylinositol 3-kinase-related kinase enzymes. This work is of interest, and warrants further investigation, because useful levels of biological potency and selectivity have been obtained for a DNA protein kinase inhibitor. These small molecule DNA protein kinase inhibitors represent useful tools for elucidating the role of DNA double-strand breaks.

1 Chang, C.-F. *et al.* (2004) Discovery of picomolar slow tight-binding inhibitors of α-fucosidase. *Chem. Biol.* 11, 1301–1306
2 Boulton, S. *et al.* (1996) Wortmannin is a potent inhibitor of DNA double strand break but not single strand break repair in Chinese hamster ovary. *Carcinogenesis* 17, 2285–2290
3 Leahy, J.J.J. *et al.* (2004) Identification of a highly potent and selective DNA-dependent protein kinase (DNA-PK) inhibitor (NU7441) by screening of chromenone libraries. *Bioorg. Med. Chem. Lett.* 14, 6083–6087