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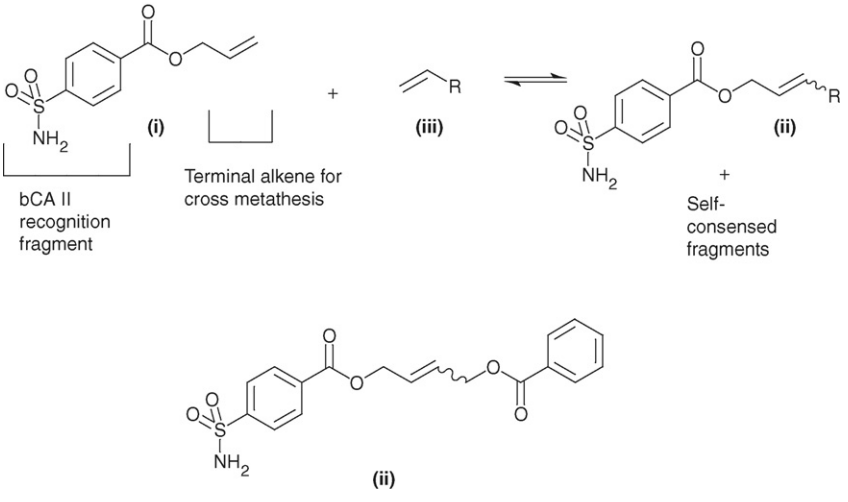
Fragment-based drug discovery of carbonic anhydrase II inhibitors by dynamic combinatorial chemistry

Dynamic combinatorial chemistry (DCC) is a relatively modern expression of combinatorial chemistry that exploits reversible covalent reactions of molecules to synthesize libraries. Unlike conventional synthesis, which seeks to deliver pure compounds from high-yielding reactions, DCC gives access to a diverse array of molecular interactions and enables amplification of the 'best binder' by the interaction of dynamic combinatorial library (DCL) constituents with a target in a self-screening protocol. Drug discovery applications of DCC require reaction conditions that do not disrupt the function or structure of the target. Particularly, this means that there must be compatibility with aqueous media and with the target functional groups; the reaction must proceed at a biologically relevant temperature; and the reaction must occur at, or close to, physiological pH. Together, all of these criteria are necessary to enable ligand amplification from DCLs when generated in the presence of a protein target. If these criteria cannot be met, then the concept of a pre-equilibrated DCL (pDCL) can be used. This technique involves the preparation of the DCL in the absence of the protein target, and the library screened post-synthesis for identification of components with affinity for the protein target. Although the pDCL approach does not enable the amplification and identification of the best binder(s), it does still benefit from the potentially greater diversity generated in DCC synthesis owing to reversible covalent reactions.

Recent work has extended these principles towards fragment-based drug discovery of carbonic anhydrase II (CA II) inhibitors utilising DCC with alkene cross metathesis as the reversible reaction. The carbonic anhydrase (CA) family of Zn(II) metalloenzymes catalyzes the interconversion of CO_2 and HCO_3^-, a reaction that underpins many physiological processes associated with pH control, ion transport and fluid secretion [1,2]. Successful development of small molecule inhibitors of CA has been achieved using a fragment-based drug discovery methodology that utilises various classical bCA II recognition motifs, such as aromatic or heteroaromatic sulfonamides ($ArSO_2NH_2$) [1,2]. The alkene metathesis reaction, using the carbene ruthenium catalysts developed by Grubbs, has been well studied for conventional organic chemistry applications [3].

Recent work [4] has incorporated the alkene metathesis reaction into drug discovery as follows: the classical bCA II recognition fragment (the aromatic sulfonamide $ArSO_2NH_2$) was incorporated into a scaffold building block [general structure (i)], which was subsequently derivatized by dynamic combinatorial chemistry to give compounds of general structure (ii), utilizing alkene cross metathesis with (iii) as the reversible reaction. These products were then screened against bCA II and the results enabled determination of the relative bCA II binding affinities of the cross metathesis products (iii), which contained the $ArSO_2NH_2$ fragment. A bCA II competitive binding assay validated these results with a representative number of pure compounds (freed from homo-coupled side products). The results for screening, without prior isolation of the active constituent, were in full agreement with those obtained for equilibrium dissociation constants (K_is) of pure compounds. Some of these compounds exhibited K_is in the low nanomolar range as exemplified by (iv), which had a K_i of 6.6 nM in the bCA II enzyme binding assay.

This work is of interest because it demonstrates that the application of fragment-based drug discovery to inhibitors of bCA II is effective utilizing a pre-equilibrated DCC strategy together with cross metathesis as the reversible reaction. Specifically, the results demonstrate that, with appropriate control experiments, the observed trend of bCA II affinity obtained when assaying the library without purification is a reflection of actual bCA II affinity.



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