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MOLECULES

The use of combinatorial chemistry methodologies to discover novel chemotherapeutic agents

N-(4-hydroxyphenyl) retinamide derivatives as potential chemotherapeutic agents for rhabdoid tumours

Rhabdoid tumours (RTs) are rare, mostly incurable pediatric malignancies that arise in brain, kidneys and soft tissues [1]. These tumours arise mainly in children younger than five years of age, with a peak incidence of up to three years of age. RTs are characterised by the presence of sheets of rhabdoid cells. The tumours also exhibit biallelic deletions and/or mutations in the INI1/hSNF5 gene [2]. Current therapeutic regimens to treat RTs involve empirically selected combinations of chemotherapeutic agents that are toxic and rarely curative, leading to low survival rates. Therefore, there is a great need for novel therapeutics to treat RTs, particularly if based on the understanding of molecular factors responsible for the genesis, growth and survival of these tumours. It has been demonstrated previously that Cyclin D1 is essential for the genesis and survival of RTs and that chemotherapeutic agents such as *N*-(4-hydroxyphenyl) retinamide (i), which targets Cyclin D1, are effective in inhibiting the growth of RTs [3]. *N*-(4-hydroxyphenyl) retinamide (i) is currently under phase III clinical trials for many types of cancer. For example, in clinical studies with pediatric neuroblastoma patients, *N*-(4-hydroxyphenyl) retinamide (i) has demonstrated prolonged stabilisation of the disease [4]. Thus, the clinical studies preformed to date indicate that *N*-(4-hydroxyphenyl) retinamide (i) is a promising anticancer drug. To improve activity as a chemotherapeutic agent against RTs, analogues of (i) were generated to assess the series SAR using (i) as the lead. Recent work has been undertaken to generate small libraries of synthetic derivatives of *N*-(4-hydroxyphenyl) retinamide and screen for their ability to induce cytotoxicity in rhabdoid tumour cell lines *in vitro* [5]. Additionally, studies were initiated to determine the compounds potential for conjugation, such as linking to nanoparticles, to facilitate the compounds crossing of the blood–brain barrier freely. Thus, series of anilide analogues of (i) were synthesised in small libraries starting from the carboxylic acid (ii), with reaction proceeding via the acid chloride (iii), then anilide bond formation through reaction with a set of anilines, to give library compounds of general structure (iv). These compounds were then assayed against the rhabdoid tumour cell line MON. A cell survival assay was carried out testing the effect of compounds and comparing their effect on MON cells to that of the parent compounds *N*-(4-hydroxyphenyl) retinamide (i) and retinoic acid. From these assays, several active compounds were obtained. One of the most potent compounds was (v) which possessed an IC_{50} of 3 μM. The SAR provided by this works suggests that the 4-hydroxy position of *N*-(4-hydroxyphenyl) retinamide (as in compound (v)) is important for activity. This work is of interest because there exists the possibility of using the synthetic derivatives retaining activity in cell culture models to derive clinically important conjugates for radiotherapy, and also to identify target proteins responsible for mediating *N*-(4-hydroxyphenyl) retinamide activity in rhabdoid tumour cells.

Discovery of an Aurora kinase inhibitor by dynamic combinatorial chemistry

In recent times, the use of fragments for the discovery of leads has become common practice amongst the medicinal chemistry community [6]. A potentially useful approach to lead discovery couples fragment-based lead discovery with dynamic combinatorial chemistry. Dynamic combinatorial chemistry allows multimeric compounds to assemble reversibly from constituent reactants [7]. The assembly should be guided by experimental conditions that enrich a

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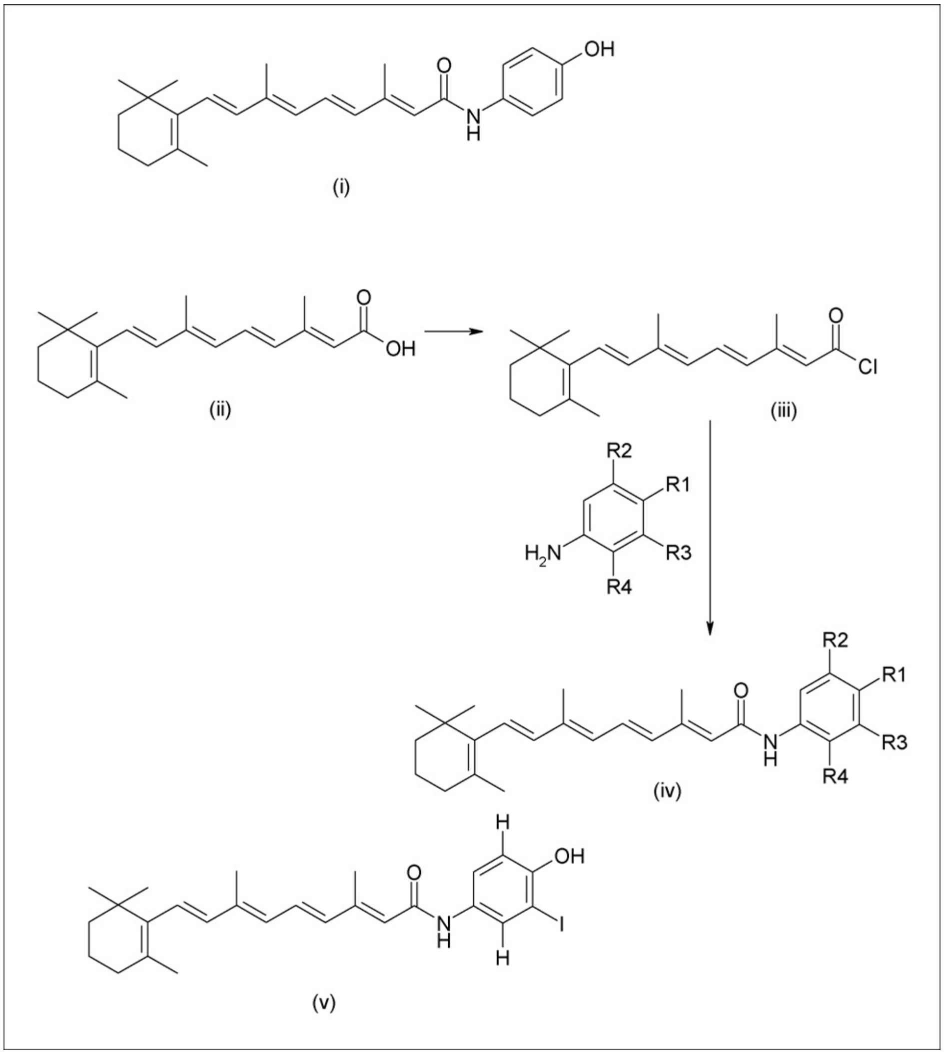

reaction mixture for molecules with desired characteristics, such as binding to a receptor.

A previously reported fragment-based discovery technology, called tethering with extenders, identifies companion fragments in the presence of a known binding moiety (extender) [8]. A disadvantage with this approach has been that the extender must both irreversibly modify a target cysteine residue and reversibly capture companion fragments that bind to an adjacent site. This requires two sequential rounds of protein modification which could disrupt a protein's structure. Recently disclosed work [9] describes tethering using dynamic extenders where the irreversible electrophile of the extender is replaced with a disulfide, thus enabling both targeted and reversible cysteine modification. This methodology combines dynamic combinatorial chemistry with fragment-based lead discovery in a generally applicable way. These authors [9] proceeded to demonstrate this methodology on a protein kinase, an actively pursued target class. Kinase inhibitors can bind at non-conserved adaptive regions away from the active site. When compounds bind in locations such as these, they typically demonstrate high specificity for individual kinases. Identifying such compounds has, however, been difficult. Specifically, these authors have applied dynamic combinatorial chemistry to identify a fragment that bound in the adaptive pocket of Aurora A, a kinase that has been shown to have a crucial role in regulating mitosis [10]. Their approach commenced with the introduction of a cysteine residue near the ATP-binding site (T217C) through site-directed mutagenesis of the Aurora A construct. Next, the protein was screened against a library of around 4500 disulfide-containing fragments under partially reducing conditions in the presence of dynamic extender (vi). Added to the extender were two disulfide-containing appendages, one of which anchored the extender to the protein in the purine-binding site through the introduced cysteine residue, whilst the other was placed to capture disulfide-containing fragments. The screening occurred in pools of approximately ten fragments where each fragment had a unique mass. Several hits were obtained from this screening effort. The disulfide between the extender and the fragment was replaced with a flexible alkyl linker and the other disulfide was removed from the diaminopyrimidine to generate soluble compounds. One of the most potent compounds formed in this way was (vii) which inhibited Aurora A with an IC_{50} of 17 μM. Further work sought to replace the diaminopyrimidine moiety with a purine and shortening of the linker by one methylene, which led to a compound (viii) with an IC_{50} = 2.9 μM. Thus, compound (viii) represents a new chemotype that is amenable to further optimisation and so represents a proof of concept for this technology in its ability to rapidly identify ligands that could serve as starting points for a medicinal chemistry program. Further work is warranted because this methodology could be applied to any kinase and so used to discover new leads.

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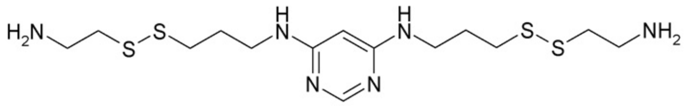

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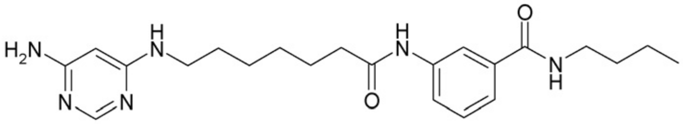

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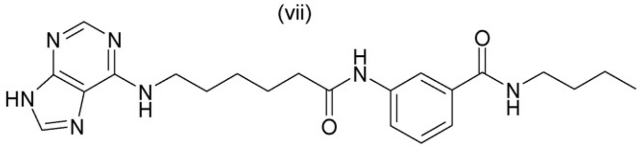

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