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1. Current literature highlights

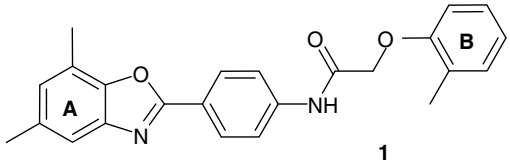
1.1. 2-Arylbenzoxazoles as new cholesteryl ester transfer protein inhibitors

The treatment of coronary heart disease utilising a lipid modifying modality has focused on lowering circulating levels of low-density lipoprotein cholesterol (LDL-C). In turn, drug discovery efforts have focused on investigating mechanisms that have the potential for raising high-density lipoprotein cholesterol (HDL-C) in plasma in the belief that there is an inverse relationship between HDL-C levels and the risk of coronary heart disease. Current therapies such as fibrates or niacin exert only a moderate effect on increasing HDL-C levels while displaying significant side effects.

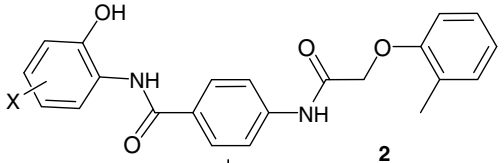
Thus there remains a need for a safer and more efficacious method of increasing HDL-C levels. In this regard, cholesteryl ester transfer protein (CETP), which is a 74-kDa plasma glycoprotein secreted by the liver, has been investigated. CETP facilitates the transfer of the cholesterol ester from HDL to LDL and VLDL in exchange for triglycerides and furthermore, CETP inhibition leads to increased HDL-C in humans. HDL particles can accept cholesterol from peripheral tissues such as macrophages, and thus CETP inhibitors could promote reverse cholesterol transport and therefore be anti-atherogenic.

Recent work has led to the identification and optimisation of a novel series of 2-arylbenzoxazole-based CETP inhibitors.[1] This publication describes a screening campaign, carried out using a fluorescence assay, and potent compounds discovered were evaluated for activity in a human whole plasma assay (WPA). A 2-arylbenzoxazole hit (**1**), with an IC_{50} value of 10 μM in the WPA assay, was obtained as a starting point for further investigation. The modular structure of this chemotype was well suited for rapid exploration of its SAR through the application of parallel synthesis.

The strategy involved developing chemistry that allowed exploration of substitutions on either the fused phenyl of the benzoxazole (A) or the phenyl ether (B), marked on **1**. The synthetic strategy to study various A ring substitutions involved the formation of two amide bonds to deliver compounds of generic structure **2** which then underwent a microwave assisted acid-promoted cyclisation of the anilide-alcohol (**2**) using *p*-toluenesulphonic acid to deliver compounds of general structure **3**. The average purity (by HPLC-UV) of the final products was 94%.



1



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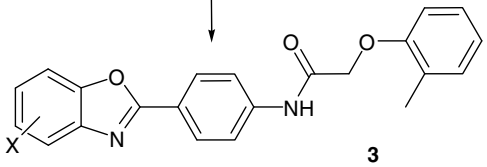

3

The synthetic approach used to vary the B-ring proceeded via formation of an aniline (**4**) and then exploration of the B-ring through the parallel synthesis of aryloxyacetyl chlorides, to provide the desired products of generic structure **5**.

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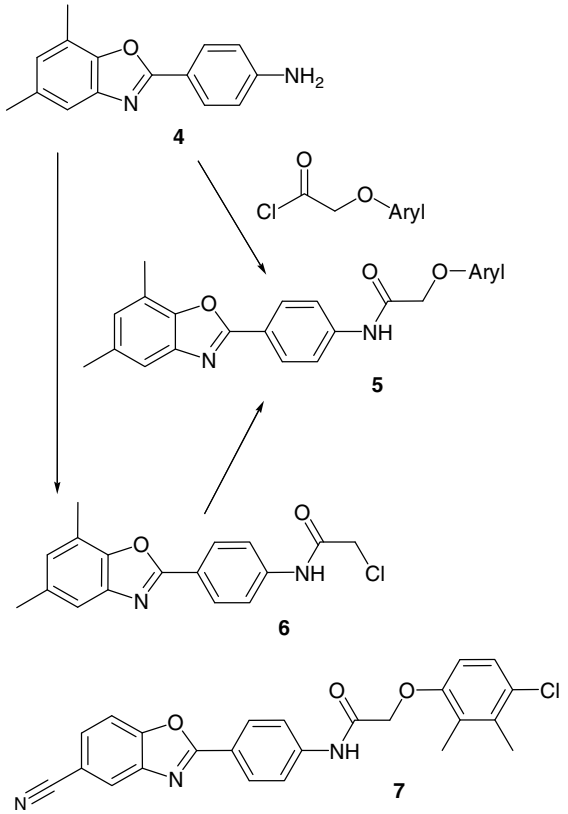
Alternatively, and in order to improve the range of diversity contained within final compounds, the aniline (**4**) was acylated with chloroacetyl chloride to give **6**. This compound then underwent a displacement of chlorine with phenols using parallel synthesis to give the desired products with generic structure **5** with an average purity of 98%, as determined by HPLC-UV. From these libraries, several compounds were obtained that displayed reasonable activity: one of the most potent tested was **7** which revealed an IC_{50} of 0.91 μM in the WPA assay.



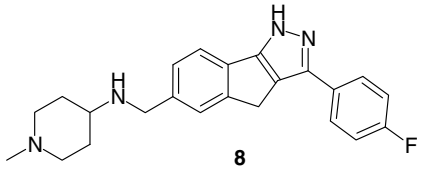
1.2. 1,4-Dihydroindeno[1,2-c]pyrazoles as potent and selective checkpoint kinase 1 inhibitors

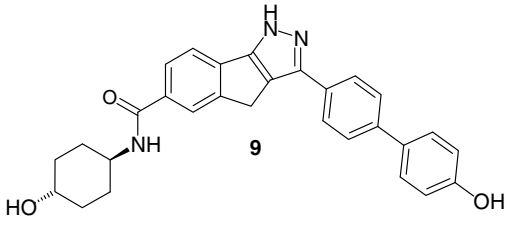
A stratagem used in the battle against cancer is the application of treatments that cause selective DNA damage. The effectiveness of these cytotoxic treatments, such as ionising UV radiation and chemotherapy, is compromised by severe side effects and drug resistance. A solution to these problems may lie in sensitisation of DNA-damaging treatments to make them more potent and/or more selective toward tumour cells.

Recently, there have been studies on the role of checkpoint kinase 1 (CHK-1) in the cell cycle as a response to genotoxic stresses. These studies demonstrated that inhibition of CHK-1 offers a mechanism for sensitising various DNA-damaging therapies. Human CHK-1 is a serine/threonine kinase that is phosphorylated by the upstream kinases ATR and/or ATM following DNA damage. CHK-1 subsequently phosphorylates Cdc25A and induces its degradation. This results in inhibition of the downstream cyclin E/Cdk2 or cyclin B/Cdc2 kinases, leading to cell cycle arrest at the S phase or G2/M phase, respectively. As tumour cells can arrest at different cell cycle checkpoints mediated by CHK-1, they have a chance to repair themselves. Thus, inhibition of CHK-1 to remove S and G2/M checkpoints will cause tumour cells to undergo premature mitotic entry, leading to cell death.

Tumour cells and normal cells differ in that tumour cells are often deficient in p53, preventing arrest at the G1 checkpoint, whereas, in response to DNA damage, normal cells are capable of arresting at G1 checkpoint through the p53-mediated pathway, ensuring genomic integrity. This difference provides the potential for a therapeutic window for sensitising DNA-damaging treatments of cancer using CHK-1 inhibitors. A recent publication reports the discovery of 1,4-dihydroindeno[1,2-c]pyrazoles as a new class of CHK-1 kinase inhibitors.[2]

The program used the screening hit **8** as a starting point for further design. This compound possessed an IC_{50} of 510 nM against CHK-1. An x-ray co-crystal structure of **8** bound to the CHK-1 active site indicated to the project team that they should focus their efforts on elucidating SAR around the fluoro group because it occupied a region where extra hydrogen bonding between the inhibitor and several polar residues of the backbone protein could be envisioned. A hit-to-lead process was then undertaken supported by x-ray crystallography. From these rounds of medicinal design, a number of potent compounds were obtained that possessed inhibitory properties against CHK-1.





One of the most potent compounds was **9** which possessed an IC_{50} value of 6.2 nM. Compounds active in the enzymatic inhibition assay, such as **9**, were then tested in two cellular assays. A cell proliferation assay (MTS assay) in HeLa cells (a human cervical cancer cell line) was used to measure the ability of CHK-1 inhibitors to sensitise cells to the DNA-damaging agent, doxorubicin. An EC_{50} was determined for the inhibitor as a single agent and the inhibitor in combination with doxorubicin; with the ratio being a relative scale of an inhibitor's ability to potentiate the DNA-damaging agent. Additionally, a cell cycle analysis (FACS assay) in H1299 cells (a human lung cancer cell line) was performed to determine if the mechanism of doxorubicin sensitisation is through abrogation of G2/M check-

point. Here, the EC_{50} was either the concentration of a CHK-1 inhibitor that reduces doxorubicin-induced G2/M cell population by half, or it was measured in the absence of doxorubicin. Compound **9** possessed an EC_{50} of 1.8 μM in the MTS assay, and an EC_{50} of 770 nM in the FACS assay (with doxorubicin present).

2. A summary of the papers in this month's issue

2.1. Solid-phase synthesis

Chemical stability and reactivity of a bifunctional polymer conjugate containing an *ortho*-amino arylamide linkage have been successfully exploited to achieve a parallel synthesis of methoxycarbonylated head–tail bis-benzimidazoles. Regioselective alkylation of the two nitrogens in the benzimidazolone moiety has been carried out by *ipso*-fluoro displacement, and N-alkylation to generate two diversities, and cleavage of the polymer support has resulted in two libraries of di-and tri-substituted benzimidazolyl benzimidazolones in high purity and high yield.[3]

The solid-phase synthesis of several 5-aminoimidazole-4-(*N*-alkyl)carboxamide-1-ribosides (4-*N*-alkyl AICARs) and the corresponding 2′,3′-secoriboside derivatives has been reported. The method uses *N*-1-dinitrophenyl-inosine 5′-bound to a solid support, and has the C-2 of the purine base strongly activated towards attack of *N*-nucleophiles. This allows preparation of several N-1 alkylated inosine supports from which a small library of 4-*N*-alkyl AICAR derivatives has been synthesised.[4]

A collection of 131 small molecules, reminiscent of families of long chain *N*-acyl tyrosines, enamides and enol esters that have been isolated from heterologous expression of environmental DNA (eDNA) in *Escherichia coli*, has been prepared. The synthetic libraries of *N*-acyl tyrosines and their 3-keto counterparts were prepared via solid-phase routes, whereas the enamides and enol esters were synthesised in solution-phase.[5]

2.2. Solution-phase synthesis

No papers this month.

2.3. Scaffolds for combinatorial libraries

A positional scanning synthetic combinatorial library has been employed to determine the conformation of bicyclic guanidines with kappa-opioid receptor activity. A common bioactive conformation and putative pharmacophoric features have been suggested by means of 3D similarity methods. Comparison of this model with known opiates suggests a similar binding mode showing that the bicyclic guanidines presented in this work are suitable scaffolds for further development of new opioid receptors ligands.[6]

2.4. Solid-phase supported reagents

A wide range of commercial diazodicarboxylates and phosphines have been screened in an attempt to find purification-free conditions for application in parallel synthesis. The combination of immobilised triphenylphosphine and TMAD proved to be suitable for the synthesis of aryl ethers via the Mitsunobu reaction, and nine ethers were synthesised in good yield and excellent purity.[7]

Three polymer-supported quaternary ammonium mesylates have been synthesised for use as recyclable polymeric phase transfer catalysts (PTCs). Through a comparative study using nucleophilic fluorination, one tertiary alcohol-containing polymer proved to be the best catalyst with high activity and chemoselectivity.[8]

A facile method for the enantioseparation of functionalised Tröger's base analogues possessing various substitution patterns has been developed. The systematic separation of a library comprising 36 representatives on a commercially available chiral stationary phase provided valuable information on structure–enantioselectivity relationships.[9]

A one-pot, two-step protocol for the microwave-assisted solid-phase synthesis of substituted benzoxazoles has been developed. This approach starts from different polymer-bound esters previously designed as solid-supported reagents for the acylation of amines, alcohols and phenols. The combination of a parallel synthesiser and a microwave reactor allowed rapid preparation of a collection of substituted benzoxazoles in high purity and satisfactory yields.[10]

2.5. Novel resins, linkers and techniques

l,6-Dioxaspiro[4,4]nonane-2,7-dione has been found to react readily with alcohols in the presence of 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU). The 4-oxoheptanedioic acid tether obtained bears a free carboxy group, which enables anchoring to aminoalkylated resins. There is also a 4-oxobutanoate structural motif, which allows release of the target alcohol by a mild hydrazinium acetate treatment. Nucleosides have been derivatised with this simple difunctional linker arm, anchored to a solid phase and subjected to synthesis and subsequent release of oligonucleotides bearing base-sensitive biodegradable phosphate protection.[11]

Peptides are limited in their use as drugs due to low cell permeability and vulnerability to proteases. In contrast, peptoids are immune to enzymatic degradation and some peptoids have been shown to be relatively cell permeable. In order to facilitate future design of peptoid libraries for screening experiments, the strengths and limitations of a high-throughput cell-based permeability assay that registers the relative ability of steroid-conjugated peptides and peptoids to enter a cell have been assessed.[12]

2.6. Library applications

A heteroaromatic 6,7-diaryl-2,3,8,8a-tetrahydroindolizin-5(1*H*)-one library has been prepared and tested for cytotoxic properties against the HCT-116 colon cancer cell line, thus providing information pertaining to structure–activity relationships for this class of compounds.[13]

A combination of a literature survey, structure-based virtual screening and synthesis of a small library has been performed to identify hits for the potential antimycobacterial drug target, glutamine synthetase. Based on one of the hits from virtual screening, a small library of 15 analogues was synthesised producing four compounds that inhibited glutamine synthetase.[14]

The related 3C and 3C-like proteinase ($3C^{pro}$ and $3CL^{pro}$) of picornaviruses and coronaviruses, respectively, are good drug targets. As part of an effort to generate broad-spectrum inhibitors of these enzymes, a library of inhibitors based on a halopyridinyl ester was prepared. Three of the compounds inhibited the cleavage activity of HAV $3C^{pro}$ with K_{ic}s of 120–240 nM - among the most potent non-peptidic inhibitors reported to date against a $3C^{pro}$.[15]

Vancomycin is mainly used as an antibacterial agent of last resort, but recently vancomycin-resistant bacterial strains have been emerging. A recent study aimed to create new anti-drug-resistance antibacterials which can be synthesised in a few steps from inexpensive starting materials. Sulpha derivatives that inhibit dihydropteroate synthase (DHPS) in the folate pathway would act as folate metabolite-mimics and inhibit bacterial folate metabolism. Screening of sulphonanilide libraries led to the discovery of benzenesulphonanilides with potent anti-methicillin-resistant *Staphylococcus aureus* (MRSA)/vancomycin-resistant *Enterococcus* (VRE) activity.[16]

Synthesis and in vitro evaluation of a library of modified endomorphin 1 peptides has been prepared. The N- and C-termini of Endo-1 were modified by lipoamino acids (Laa) and/or sugars to overcome metabolic instability and poor membrane permeability. Analogues were assessed for μ-opioid receptor affinity, inhibition of cAMP accumulation, enzymatic stability, and permeability across Caco-2 cell monolayers.[17]

An immobilised Staurosporine aglycone isostere, where one of the indole nitrogen atoms was replaced by carbon, has been sequentially functionalised to generate compounds that inhibit TrkA kinase. In the first phase, initial screening of a library of C13-hydroxymethyl-7-oxo-indenopyrrolocarbazoles resulted in several potent compounds, one of which was further optimised to generate the corresponding carbamates on solid phase.[18]

Insulin-like growth factor receptor (IGF-1R) is a growth factor receptor tyrosine kinase that acts as a critical mediator of cell proliferation and survival. This receptor is overexpressed or activated in tumour cells and is emerging as a novel target in cancer therapy. A novel series of submicromolar IGF-1R inhibitors based on an isoquinolinedione template has been reported. Chemical triage and parallel synthesis incorporating focused library arrays were instrumental in moving this discovery forward.[19]

Synthesis, biological evaluation, and SAR dependencies for a library of novel aryl and heteroaryl substituted *N*-[3-(4-phenylpiperazin-1-yl)propyl]-1,2,4-oxadiazole-5-carboxamide inhibitors of GSK-3β kinase have been described. The inhibitory activity of the synthesised compounds is highly dependent on the character of substituents in the phenyl ring and the nature of terminal heterocyclic fragment of the core molecular scaffold.[20]

The molecular duplication of non-nucleoside reverse transcriptase inhibitor (NNRTI) *O*-(2-phthalimidoethyl)–*N*-arylthiocarbamates has led to the identification of symmetric formimidoester disulphides as a novel class of potent NNRTIs. The lead compound prevented wild-type HIV-1 multiplication in MT-4 cell culture with an EC_{50} value of 0.35 μM. To perform a structure–activity relationship study, 40 analogues of the lead compound were prepared by an unprecedented one-pot method of solution-phase parallel synthesis.[21]

The cyclodepsipeptide Jaspamide is an interesting marine metabolite, that possesses a potent inhibitory activity against breast and prostate cancer, as a consequence of its ability to disrupt actin cytoskeleton dynamics. A recent report describes the synthetic modification of the natural metabolite, generating small arrays of unnatural variants.[22]

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Further reading

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