ELSEVIER

monitor

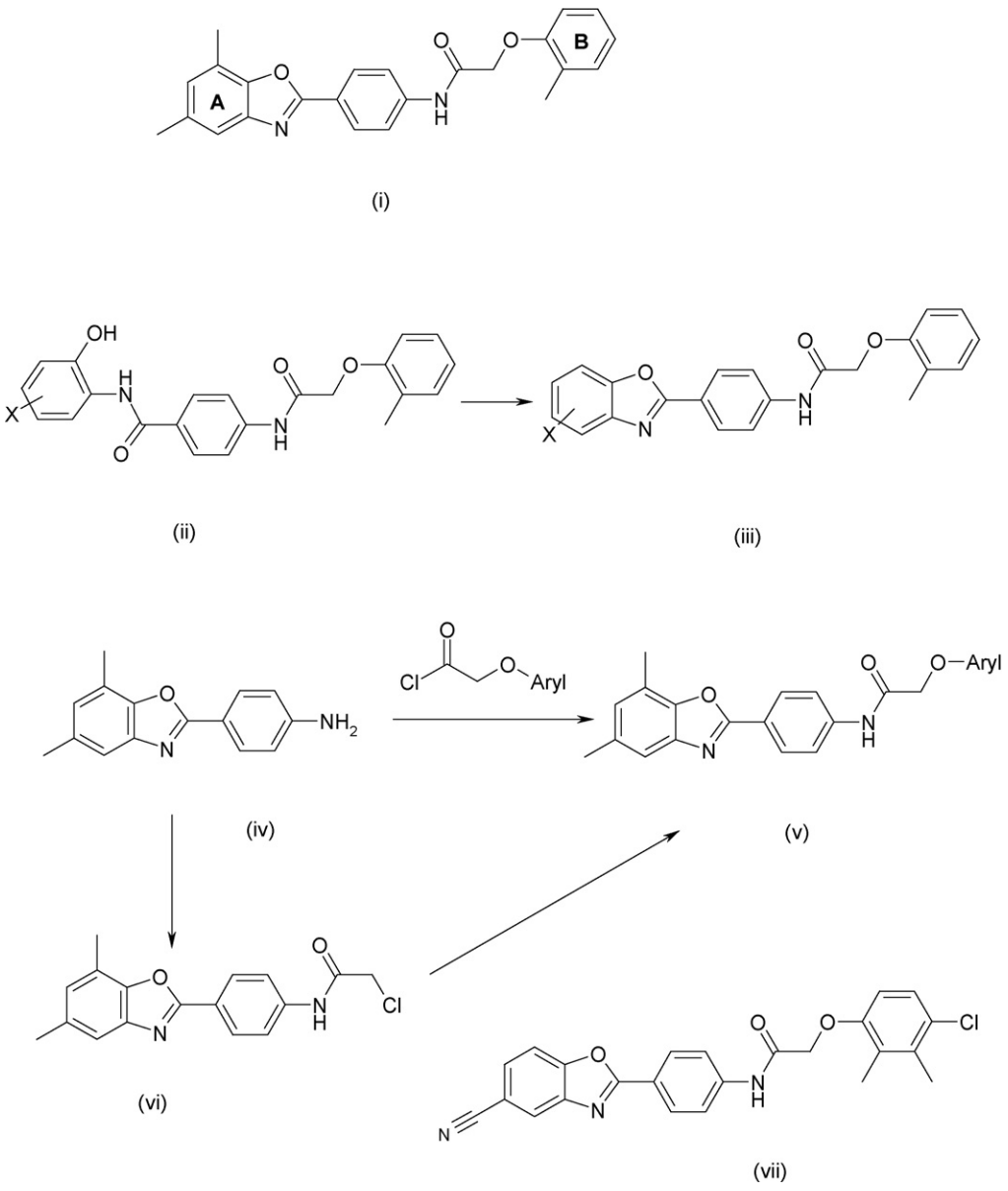
MOLECULES

Following up high-throughput screening with parallel chemistry for the generation of actives against biological targets

2-Arylbenzoxazoles as new cholesteryl ester transfer protein inhibitors

The treatment of coronary heart disease utilizing 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 which have the potential for raising high-density lipoprotein cholesterol (HDL-C) in plasma through the knowledge that there is an inverse relationship between HDL-C and the risk of coronary heart disease [1]. Current therapies, such as fibrates or niacin, exert only a moderate effect on increasing HDL-C levels while displaying considerable side effects [2]. Thus, there is a need in the medical community 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 brought to the fore. CETP facilitates the transfer of cholesterol ester from HDL to LDL and VLDL in exchange for triglycerides [3] and additionally, CETP inhibition leads to increased HDL-C in humans. HDL particles can accept cholesterol from peripheral tissues, so CETP inhibitors could promote reverse cholesterol transport and, therefore, be antiatherogenic [4]. Recent work has led to the identification and optimization of a novel series of 2-arylbenzoxazole-based CETP inhibitors [5]. In this work, a screening campaign was carried out on the BMS compound collection using a fluorescence assay. Potent compounds thus obtained were then evaluated for activity in a human whole plasma assay (WPA) and the 2-arylbenzoxazole hit (**i**) was obtained as a starting point for further investigation. Compound (**i**) possessed an IC_{50} in the WPA assay of 10 μM. The modular structure of this chemotype was well suited for rapid exploration of its SAR through the application of parallel synthesis. The SAR 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 (**i**). The synthetic strategy to study various A ring substitutions involved the formation of two amide bonds to deliver compounds of generic structure (**ii**) which then underwent a microwave-assisted acid-promoted cyclization of the anilide–alcohol (**ii**) using *p*-toluenesulfonic acid to deliver compounds of general structure (**iii**). The average purity (by HPLC-UV) of the final products was 94%. The synthetic approach utilized for variation of the B-ring proceeded via formation of aniline (**iv**) and then exploration of the B-ring through the parallel synthesis of aryloxyacetyl chlorides, to provide the desired products of generic structure (**v**). Alternatively, and to improve the range of diversity contained within the final compounds, aniline (**iv**) was acylated with chloroacetyl chloride to give (**vi**). This compound then underwent a displacement of the chlorine of (**vi**) with phenols using parallel synthesis to give the desired products of generic structure (**v**) in 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 compounds tested was (**vii**) which possessed an IC_{50} of 0.91 μM in the WPA assay. This work is of interest because it has utilized parallel synthesis to rapidly generate several libraries of singletons and to elucidate SAR in the series under discussion. Further work in this area is warranted with a view to improve the compounds CETP inhibition profile, for example in the WPA assay.

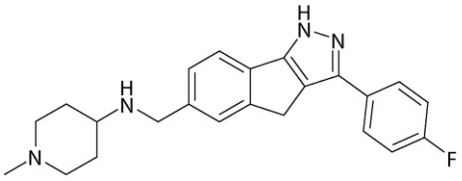
1359-6446/06/$ - see front matter © 2008 Elsevier Ltd. All rights reserved. doi:10.1016/j.drudis.2008.06.009



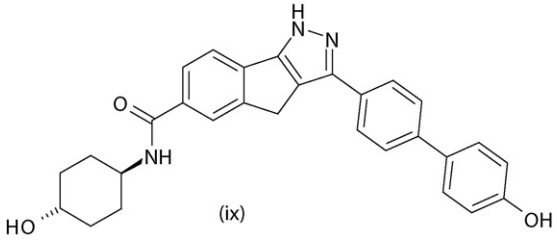
1,4-Dihydroindeno[1,2-*c*]pyrazoles as potent and selective checkpoint kinase 1 inhibitors

A stratagem used in the battle against cancer is the application of treatments with DNA-damaging effects. The effectiveness of these cytotoxic treatments, such as ionizing or UV radiation and chemotherapy, is reduced because of severe side effects and drug resistance developed by patients. A solution to these problems may lie in sensitization to DNA-damaging treatments to make them more potent and/or more selective toward tumor cells. Recently, there have been studies on the role of checkpoint kinase 1 (CHK-1) in the cell cycle in response to genotoxic stresses. These studies demonstrated that inhibition of CHK-1 offers a mechanism for sensitizing various DNA-damaging therapies [6]. Human CHK-1 is a serine/threonine kinase that, upon DNA damage, is phosphorylated by the upstream kinases ATR and/or ATM. CHK-1 subsequently phosphorylates Cdc25A and induces its degradation. This results in the 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 [7]. As tumor cells can arrest at different cell cycle checkpoints mediated by CHK-1, they have a chance to repair themselves. Thus, the inhibition of CHK-1 to remove S and G2/M checkpoints will cause tumor cells to undergo premature mitotic entry, leading to cell death. Tumor cells and normal cells differ in that tumor 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 sensitizing DNA-damaging treatments of cancer using CHK-1 inhibitors. A recent publication [8] reports on the discovery of 1,4-dihydroindeno[1,2-*c*]pyrazoles as a new class of CHK-1 kinase inhibitors. The program utilized the screening hit (**viii**) as a starting point for further design. This compound possessed an IC_{50} of 510 nM against CHK-1. An X-ray cocrystal structure of (**viii**) in the CHK-1 active site indicated to the project team that they should focus their efforts on elucidating SAR around the fluoro group because this group 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 (**ix**) which possessed an IC_{50} of 6.2 nM. Compounds active in the enzymatic inhibition assay, such as (**ix**), 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 sensitize cells to a 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 function 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 an inhibitor's ability to sensitize doxorubicin is through abrogation of the G2/M checkpoint. 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 (**ix**) 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). This work is of interest because of the support provided by X-ray crystallography in the hit-to-lead phase, and the discovery of several biaryl phenol inhibitors that exhibit good profiles in both cell proliferation and cell cycle assays. Further work in this area is warranted to improve the properties of this series of inhibitors.



(viii)



(ix)

1 Kannel, W.B. (1995) Range of serum cholesterol values in the population developing coronary artery disease. *Am. J. Cardiol.* 76, 69c–77c

2 Barter, P. *et al.* (2003) High density lipoproteins (HDLs) and atherosclerosis; the unanswered questions. *Atherosclerosis* 168, 195–211

3 Barter, P.J. *et al.* (2003) Cholesteryl ester transfer protein. A novel target for raising HDL and inhibiting. *Atheroscler. Arterioscler. Thromb. Vasc. Biol.* 23, 160–167

4 Linsel-Nitschke, P. and Tall, A.R. (2005) HDL as a target in the treatment of atherosclerotic cardiovascular disease. *Nat. Rev. Drug Discov.* 4, 193–205

5 Harikrishnan, L.S. *et al.* (2008) 2-Arylbenzoxazoles as novel cholesteryl ester transfer protein inhibitors: optimization via array synthesis. *Bioorg. Med. Chem. Lett.* 18, 2640–2644

6 Tao, Z.-F. and Lin, N.H. (2006) Chk1 inhibitors for novel cancer treatment. *Anticancer Agents Med. Chem.* 6, 377–388

7 Uto, K. *et al.* (2004) Chk1, but not Chk2, inhibits Cdc25 phosphatases by a novel common mechanism. *EMBO J.* 23, 3386–3396

8 Tong, Y. *et al.* (2007) Discovery of 1,4-dihydroindeno[1,2-*c*]pyrazoles as a novel class of potent and selective checkpoint kinase 1 inhibitors. *Bioorg. Med. Chem.* 15, 2759–2767

Paul Edwards
mepauledwards@fsmail.net