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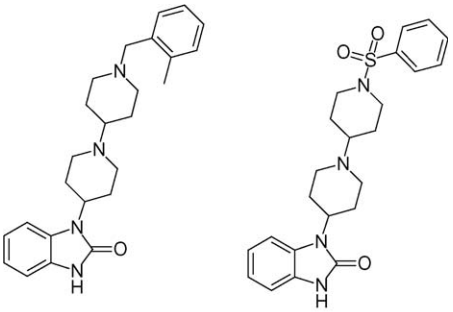
MOLECULES

Parallel chemistry libraries for the discovery of biologically active substances

Synthesis and SAR of analogues of the M1 allosteric agonist TBPB

The GPCR family A contains, as members, muscarinic acetylcholine receptors. These receptors mediate the metabotropic actions of the neurotransmitter acetylcholine. Five distinct subtypes of muscarinic acetylcholine receptors (M1–M5) have been cloned and sequenced [1]. Muscarinic acetylcholine receptors regulate cholinergic signaling and they play a crucial role in a wide variety of CNS and peripheral functions, for example, memory and attention mechanisms and nociception [2]. Thus, agents that can selectively modulate the activity of specific muscarinic acetylcholine receptors have therapeutic potential in multiple pathological states, including Alzheimer's disease and schizophrenia [1]. There are, however, barriers to drug discovery in this area. Because of the high sequence conservation within the orthosteric binding site of the five muscarinic acetylcholine receptor subtypes, to date it has been difficult to develop subtype selective ligands. Recently [3], work has been disclosed on TBPB (i), which is a potent, centrally active and highly selective M1 allosteric agonist. TBPB (i), however, represented an un-optimized screening lead with unwanted antagonist activity at the dopamine D2 receptor (IC_{50} = 5.1 μM). Further work focused on incorporating alternative benzyl moieties to afford compounds with equivalent M1 efficacy and muscarinic acetylcholine receptor selectivity, but no D2 inhibitory activity. More recent work [4] describes the synthesis, SAR and pharmacological profile of new TBPB analogs in which the benzyl moiety was replaced with either an amide, sulphonamide or urea linkage to determine the effects on muscarinic acetylcholine receptor activation through capping of the distal nitrogen atom and altering the basicity, and topology of TBPB (i). In this newer work, several small libraries of amide, sulphonamide and urea derivatives were prepared, totaling 53 compounds made by parallel chemistry. This library was screened at 10 μM concentration, single point M1 followed by full concentration–response curves along with M2–M5 selectivity assays. Several active compounds were obtained from this screening effort. One of the most potent compounds was (ii) which possessed an EC_{50} for M1 activation of 1.4 μM and complete selectivity for M1 versus M2–M5. This work is also consistent with that based on other allosteric ligands, namely that these data suggest that the allosteric binding site for TBPB and related analogues is shallow. Further work and refinements to the TBPB scaffold are warranted to broaden our understanding of SAR in these series of compounds.



(i) (ii)

Synthesis and biological evaluation of a focused library of beauveriolides

A crucial stage in the formation of vascular plaques is lipid droplet accumulation in macrophages. The formation of plaques limits blood flow and can result in rupture of blood vessels, ultimately leading to the development of atherosclerosis in the arterial wall. Therefore, inhibitors of lipid droplet accumulation in macrophages would be beneficial for the treatment of atherosclerosis [5] The beauveriolides, a family of cyclic depsipeptides isolated from a culture broth of Beauveria sp. FO-6979, can act as inhibitors of lipid droplet accumulation in mouse macrophages. For example, beauveriolide I (iii) was found to reduce the number and size of lipid droplets in mouse macrophages in a dose-dependant manner, without cytotoxicity and to inhibit cholesteryl ester synthesis with an IC_{50} value of 0.78 μM [6]. Building on previous work [7] that established a method for combinatorial synthesis of beauveriolide analogues using a 2-chlorotrityl chloride linker, and evaluation of their inhibitory activity against cholesteryl ester synthesis in mouse macrophages [6], the present work under discussion [8] attempts to understand the effects of substituents on the L-Ala and D-Leu parts in (iii) and also the evaluation of their acyl-CoA:cholesterol acyltransferase inhibitory activity in cell- and enzyme-based assays. The authors [8] designed a 48-member library of beauveriolides consisting of amino acid building blocks. The synthesis of final library members proceeded as follows: cyclic products of general structure (iv) were prepared by coupling of a first amino acid onto 2-chlorotrityl chloride resin. Next, deprotection of the Fmoc group protecting the amino acid nitrogen, followed by standard iterative peptide coupling/deprotection with further amino acid building blocks, afforded a tripeptide. After subsequent deprotection from solid phase using 4 M HCl, followed by solution phase cyclisation, depsipeptide of general structure (iv) was obtained. The library members thus realised were screened for their effect on acyl-CoA:cholesterol acyltransferase activity by measuring the cholesteryl ester synthesis of lipid droplets in a cell-based assay using mouse macrophages and in an enzyme assay using mouse liver microsomes. Several active compounds were obtained. One of the most potent was (v) which possessed an IC_{50}

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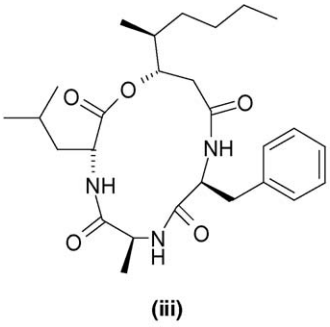
of 20 nM in the cell-based assay for cholesteryl ester synthesis of lipid droplet accumulation in macrophages. This work is of interest because the disclosed library work has aided our understanding of SAR in this series of compounds in a time-efficient manner. Further work in this area is warranted to provide compounds with drug-like properties.



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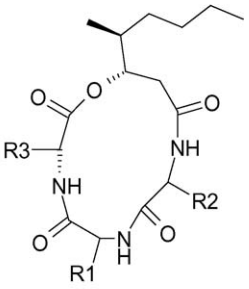

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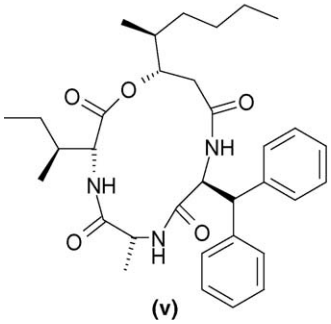

(v)

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