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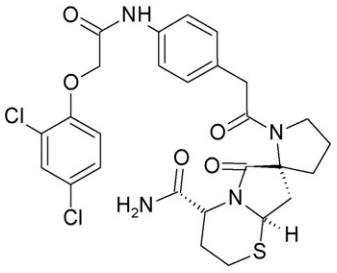
Targeted library design for the generation of actives against biological targets Peptidomimetic inhibitors of myeloid differentiation factor 88

The nuclear transcription factor-κB (NF-κB[a]) is known to be involved in a host of disease states, such as inflammation, immunity and apoptosis. In response to damaging stimuli, NF-κB activation is observed in most cells involved in the immune response, such as neutrophils, macrophages and lymphocytes. Thus, the immediate and transitory activation of NF-κB is important to the normal physiological response to pathogenic damage. NF-κB recognizes consensus sequences for the enhancers of genes coding for pro-inflammatory cytokines (e.g. TNF, IL-1, IL-2, IL-6 and IL-11), chemokines (e.g. IL-8, RANTES and MIP-1R), adhesive molecules (ICAM-1, VCAM-1, E-selectin), and enzymes producing inflammatory mediators (iNOS and COX2) [1]. Dysregulation of this mechanism through persistent and excessive activation, is associated with chronic inflammatory diseases [2]. The adaptor protein, MyD88, is believed to play a critical role in transduction events triggered by the majority of toll-like receptor (TLR) and IL-1 and IL-18 receptors, the result of a postulated common transduction mechanism in the IL-1R/TLR superfamily [3] Thus, inhibition of adaptor proteins such as MyD88 which is involved in the activation of NF-κB, is expected to be more effective than inhibition of individual ligand activities, due to the mechanistic sharing of a common transduction pathway. The recent identification of a critical sub-region of MyD88 TIR domain provides a starting point for peptidomimetic ligand design and, thus, SAR exploration [4]. Recent work has capitalized on this observation with the intention of identifying mimetics of this particular portion of MyD88, which could interfere with its homodimerization [5], thus preventing recruitment of MyD88 to each of the IL-1R/TLR receptors and, so, interfering with their signaling. The approach taken by these researchers to construct mimetics of the consensus peptide of MyD88 TIR domain was to subdivide the region into three distinct portions, consisting of a charged portion (Arg-Asp amino acids), a hydrophobic portion (Val-Leu amino acids), and a β-turn portion (Leu-Pro-Gly-Thr amino acids). When combining all the building blocks, a 4368 direct and 234 retro-inverse peptidomimetic library was possible. However, for practical reasons, not all combinations were synthesized, but rather a diverse set of 83 compounds was actually prepared on solid-phase using a polymer supported (aminomethyl)polystyrene (Rink amide) resin. Chosen compounds for synthesis all met the 'rule of five' [6]. Those compounds synthesized next underwent biological screening. The ability of the peptidomimetics to inhibit protein–protein interaction was assessed by a yeast 2-hybrid assay and further validated in a mammalian cell system (HeLa cells) by evaluating the inhibition of NF-κB activation, a transcription factor downstream of the MyD88 signaling pathway that allows production of essential effector molecules for immune and inflammatory responses. From this library, a number of active compounds were identified. One of the most potent compounds tested was (i), which gave a 26% inhibition (expressed as the percentage inhibition at 100 μM) in the NF-κB assay. This work is of interest because it utilized parallel synthesis to generate peptidomimetic compounds rapidly and to elucidate SAR in the series under discussion. In particular, the design and synthesis of a peptidomimetic library derived from the heptapeptide Ac-RDVLPGT-NH_2, belonging to the Toll/IL-1 receptor (TIR) domain of the adaptor protein MyD88, was undertaken. Further work in this area is warranted with a view further to optimize potency and, in addition, to use more sensitive functional assays, to address the effect of compounds on cells of the immune system.



(i)

Acylguanidine inhibitors of β-secretase: optimisation of substituents extending into the S_1 and S_3 substrate binding pockets

Alzheimer's disease (AD), a progressive neurodegenerative disease, is the leading cause of dementia. Estimates vary between 5% and 50% that early onset AD cases result from a variety of genetic mutations. The remainder may therefore be classed as sporadic with an increasing risk of incidence with age [7]. β-Amyloid plaques are a recognizable feature of AD and are formed by the aggregation of amyloid fibrils, which, in turn, are formed from the neurotoxic amyloid β-peptide (β-amyloid, $Aβ_{40,42}$). Proteolytic cleavage of amyloid precursor protein (APP) by β-secretase provides the membrane-bound β-C-terminal fragment, which is itself cleaved by γ-secretase to generate Aβ [8] BACE-1 inhibitors may, therefore, decrease levels of Aβ and have

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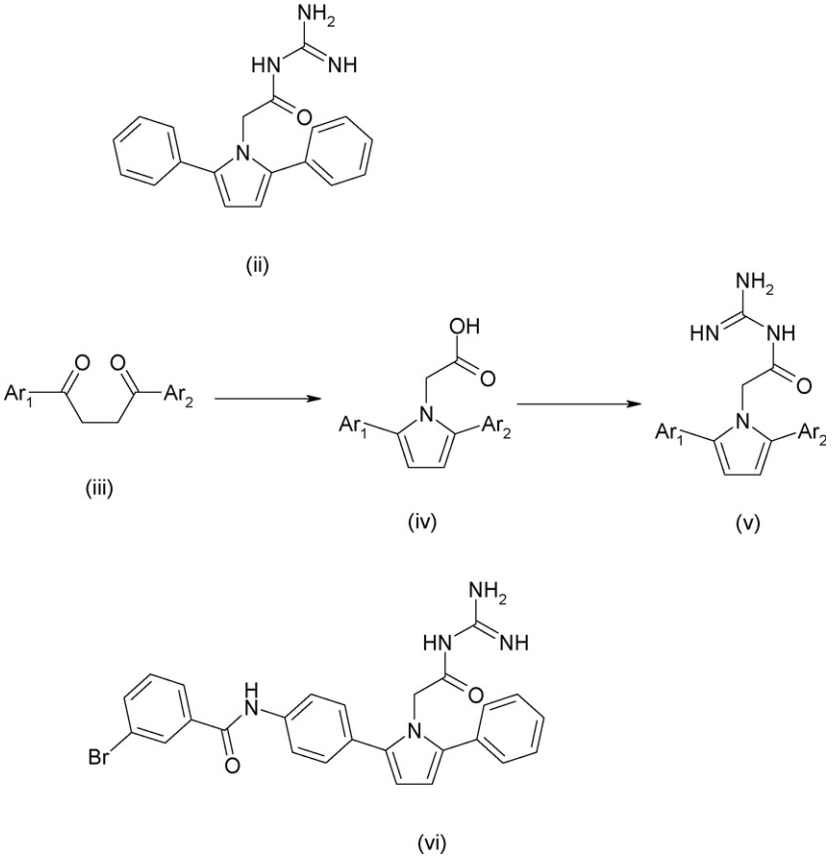
therapeutic benefit in the treatment of AD. A recent high throughput screening campaign identified *N*-[2-(2,5-diphenyl-pyrrol-1-yl)- acetyl]guanidine (ii) as an inhibitor of BACE-1 with low micromolar activity (BACE-1 IC_{50} = 3.7 μM, K_d = 2.8 μM) and this compound also inhibited Aβ formation in a cellular assay with an IC_{50} of 8.9 μM [9]. More recent work [10] has undertaken a hit-to-lead optimization of this series of BACE-1 inhibitors. The synthesis of target inhibitors proceeded via the synthesis of 1,4-diarylbutane-1,4-diones (iii), prepared in a one-step cross-coupling reaction, using the procedure of Kulinkovich [11]. The crude diketones were coupled with glycine in an acid-catalyzed condensation to give (iv), which were subsequently treated with a carbodiimide-mediated coupling of the resultant pyrrole acetic acids with guanidine hydrochloride to give the target acylguanidines of general structure (v). A library of 156 singletons was, thus, synthesized in this way and all compounds produced were assayed for BACE-1 inhibition in a fluorescence resonance energy transfer (FRET) assay at a concentration of 10 μM. One of the most potent compounds tested was (vi), which possessed an IC_{50} against BACE-1 of 0.6 μM. Future studies on this series may focus on optimization of potency via improvements in interactions with the substrate pocket.



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