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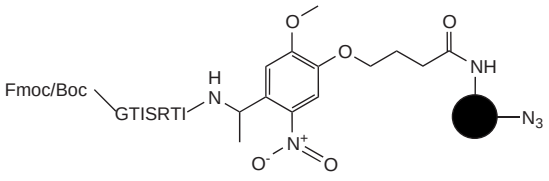
MOLECULES

Metalloproteinase inhibitors

The design, preparation and screening of chemical libraries in solution or on solid phase has been used for the rapid identification of proteinase inhibitors [1]. The use from the combinatorial chemistry perspective is in the synthesis of large numbers of compounds that can be screened simultaneously. In one-bead assays, the selection process to distinguish hits from non-hits is a difficult and laborious task, rarely performed in a quantitative and reproducible manner. To overcome these problems, an automatic sorting machine was developed to sort spherical beads according to their fluorescent intensity. This instrument is now available commercially (Union Biometrica, USA). A one-bead-two-compound library of phosphinic peptides was constructed in order to investigate the reliabilty of this novel, fully automated bead sorting apparatus in selection of beads containing potent inhibitors [2]. A solid phase library on PEGA1900 (Polymer laboratories, UK) was synthesised as a one-bead-two-compound library producing a putative 160,000 phosphinic peptide inhibitors. The PEGA1900 resin was initially derivatized to provide two orthogonally protected reaction sites (**i**). The combinatorial library was generated from one reaction site whilst the other enabled the introduction of an internally quenched fluorogenic substrate. The resulting beads each carried two distinct compounds: (a) the target for molecular interaction, a randomly synthesised library member X3X2-GΨ [PO2HCH2]LX2'X3' and with N-type derivatives of acetyl, H, or H-D-Ala linked to the support via a mass/ionisation spacer [GTISRTI], and a photolabile linker; (b) an internally quenched fluorogenic substrate AY(NO2)GPLGLYARK(Abz)G (-: scissile bond) attached directly to the functional group of the resin. Library synthesis on PEGA1900 provided, after cleavage of compounds from the solid support, phosphinic acids, which were incubated with the 65 kDa MMP-9, which penetrated into the PEGA polymer and cleaved the quenched fluorogenic substrate in beads containing no inhibitory compound. Pools of approximately 10,000 beads were sorted by the beadsorter based on the quantitative measurement of fluorescence intensity on the beads. The whole library was sorted in a few hours. Of those beads found to contain active constituents, ten sequences found to be active were selectived for re-synthesis on PEGA 800 resin using a Rink amide linker for evaluation as MMP-9 inhibitors in solution. One of the most active compounds was (**ii**), which possessed a Ki for MMP-9 of 1.4 nM. These results demonstrate that potent MMP inhibitors can be identified from a one-bead-two-compound library of phosphinic peptides using an automated sorting method. The use of automated sorting lends itself to sequential incubations with one enzyme to up-concentrate the most active inhibitors. As an alternative to incubation of a one-bead-two-compounds inhibitor library with just a single MMP, sequential incubations with different related proteinases can be used in order to search for selective inhibitors. Further work in this area is warranted.



(i)

$AGPLG\Psi[PO_2HCH_2]LYARG\text{-}NH_2$

(ii)

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Neuroprotective K+ channel inhibitor

Potassium (K+) channels play a pivotal role in electrical activity of all excitable tissues. Abnormalities in K+ currents results in cardiovascular, neurological, renal and endocrine pathology [3]. Pharmacological modulation of specific K+ currents is considered to be a significant strategy for the treatment of a broad range of disorders [3]. However, most low molecular weight modulators of K+ channels to

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date affect these channels at high micromolar to millimolar concentrations and lack sufficient target specificity [3]. Given the diversity of K+ channels, development of more potent and specific K+ channel drugs is a high priority for molecular pharmacology. Hindering their discovery is the traditional low-throughput capacity of ion channel assays. An approach to increase the throughput for screening K+ channel modulators has been tried previously using yeast that functionally express mammalian K+ channels. Yeast require K+ and so cannot grow in low [K+] medium when their endogenous K+ transporters trk1 and trk2 are disrupted [4]. However, growth of Dtrk1trk2 knockout yeast in low [K+] medium can be rescued (genetically complemented) by mammalian Kir2.1 channel expression [5]. Because growth in low [K+] medium is dependant upon the activity of the mammalian channel, it might be possible to screen for Kir2.1 channel modulators by monitoring yeast growth [6]. Using this approach, the authors identified a novel K+ channel inhibitor from an initial screen of 10,000 compounds produced in a combinatorial chemistry library. The library compounds were screened as singletons in an assay based on growth of yeast that functionally expresses mammalian Kir2.1 channels. From this screening procedure, 42 potential inhibitors of Kir2.1 were identified. One compound, 3-bicyclo[2.2.1]hept-2-ylbenzene-1,2-diol was confirmed to inhibit K+ channels in patch-clamp measurements in mammalian calls with EC50 values of 60 and 1 μM for Kir2.1 and Kv2.12 channels, respectively. Therefore, yeast-based screening has identified a novel neuroprotective mammalian K+ channel inhibitor and this approach could prove useful in the search for further novel ion channels.

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