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Dimeric β-Turn Peptidomimetics as Ligands for the Neurotrophin Receptor TrkC

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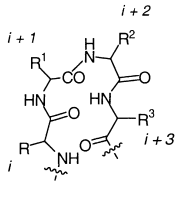
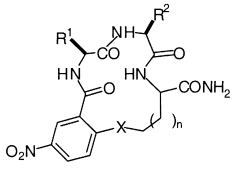
Abstract—Twelve dimeric peptidomimetics **1** were prepared via a divergent–convergent strategy. These peptidomimetics incorporated the same amino acids as $i+1$ and $i+2$ residues in key β-turns of the neurotrophin NT-3. Cytosensor microphysiometry was used to gauge the effects of the dimers **1** on cells that overexpress the NT-3 receptor, TrkC. Increases in extracellular acidification rates were observed for some monomers **3**, but the active dimers gave greater effects.

Many receptors, including single transmembrane tyrosine kinase receptors, are dimeric. They associate with protein dimers, and function via processes that involve receptor dimerization.[1] This situation presents significant challenges to medicinal chemists hoping to prepare small molecule agonists of these receptors.[2] Ideally, they must produce ligands that not only bind to a receptor protein molecule but also induce receptor dimerization.



β-turn structure



A
n = 0 - 3; X = O, S, NH

Work from these laboratories has shown that compounds of the type **A**[3] have several favorable attributes with respect to mimicry at hot-spots involved in protein–protein interactions. The amino acid side chains R^1 and R^2 can be selected to be the same as those in the $i+1$ and $i+2$ residues of a protein β-turn that is known to be involved in the target protein–protein interface. They tend to adopt type I β-turn conformations.[4] Moreover, the compounds can be prepared in parallel on a solid phase,[4] allowing for production of exploratory libraries. This approach has led to the discovery of one of the first small molecule mimics of the nerve growth factor (NGF).[5,6]

Nerve growth factor is the best known of a series of neurotrophins that also includes neurotrophin 3 (NT-3).[7] There is much sequence homology and structural similarity between NGF and NT-3, but the two proteins are known to preferentially associate with different single transmembrane tyrosine kinase receptors, TrkA and TrkC, respectively. These selectivity differences are partially, and perhaps primarily, due to the display of different amino acid side chains at some β-turn secondary structures that project prominently from the surface of NGF or NT-3 homodimers.

Both NGF and NT-3 are potential therapeutic targets for treatment of neurodegenerative diseases.[8] Preliminary work, the focus of a current patent application, has shown that some compounds of type **A** can be functional mimics of NT-3. The research presented in this Letter describes syntheses of molecules that have two type **A** fragments paired in dimeric arrangements (**1**; Scheme 1) designed to induce receptor dimerization. These dimers were tested for their ability to stimulate cells that

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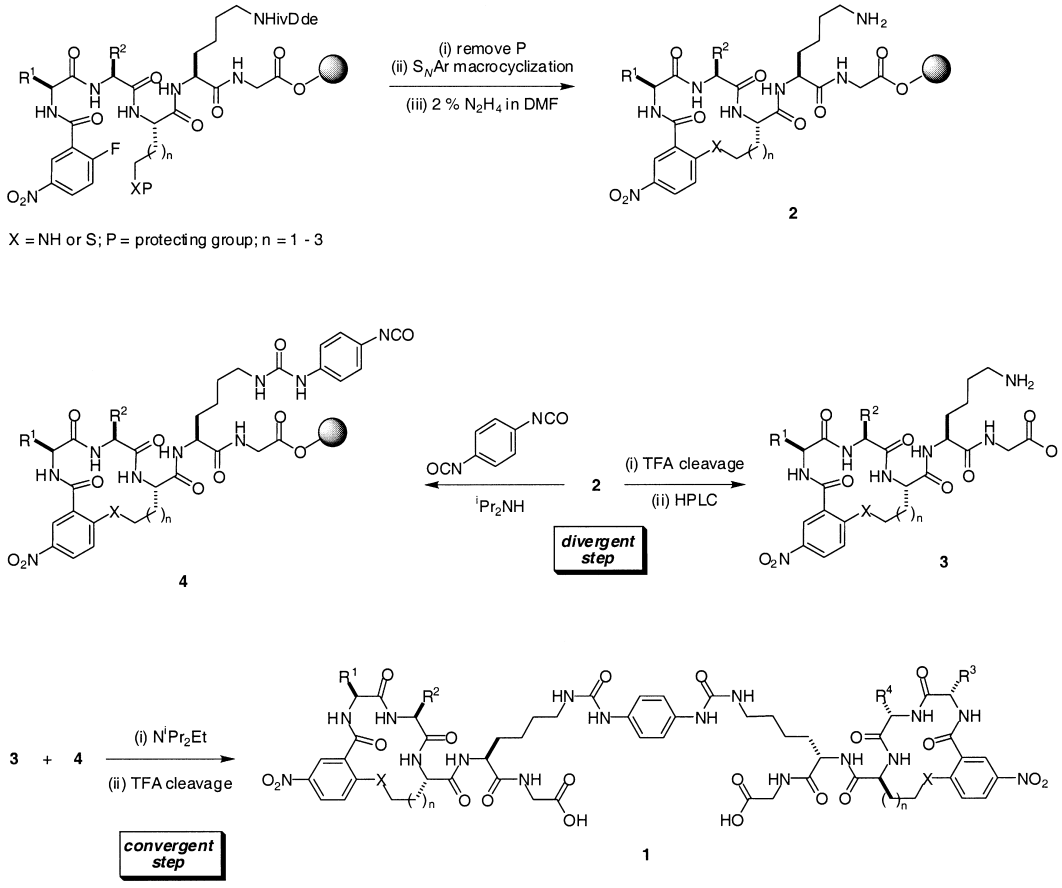

Scheme 1.

overexpress TrkC, using cytosensor microphysiometry,[9] and the data obtained from those studies are also outlined.

Scheme 1 shows the 'divergent–convergent' approach used to obtain the target dimers **1**. Six supported, cyclic peptidomimetics **2** were prepared on Wang resin using the procedures reported previously,[4] but with the following critical modification. A lysine residue having an ivDde-protected amine side chain[10] was incorporated such that it becomes an exocyclic residue after the S_NAr macrocyclization. The amine side chain of this residue was then unmasked using a dilute hydrazine solution; the ivDde group {ivDde = l-(4,4-dimethyl-2,6-dioxocyclohex-1-yldiene)-3-methylbutyl} was selected because it could be removed under these conditions without cleaving the peptidomimetic from the resin. Each batch of each supported peptide was then divided into two equal portions. One portion of each supported peptidomimetic was cleaved from the resin and purified giving six amines **3**. The other portions of supported peptide were treated with 1,4-diisocyanatobenzene to give the supported isocyanates **4**. A sample of each amine **3** in solution was then reacted with a sample of each supported isocyanate **4**, to give 12 different supported dimers. Finally, cleavage from the resin gave the target dimers **1**. Tables 1 and 2 summarize the molecular characteristics of the monomers and the dimers, respectively.[11]

Divergent–convergent strategies of the type described above enable libraries to be coupled with themselves. This combinatorial technique, 'reactions of libraries with libraries',[12,13] is distinct from those which involve non-combinatorial transformation of one library into another. The numerical advantage is such that when a library of n compounds is reacted with itself, $n(n+1)/2$ dimers can be formed.[14] For instance, 5050 dimers could be obtained from only 100 monomers.

The cytosensor microphysiometer is designed to measure pH changes caused by metabolic stimulation of cells in a low-buffered media. Any ligand that binds to a cell surface receptor and initiates cell signaling ultimately causes the cell to extrude protons. Thus increases in extracellular acidification rates (ECARs) can be measured to gauge a signaling process caused by binding to cell surface receptors. Moreover, stimulation of different receptor types on a cell surface results in

Table 1. Molecular characteristics of monomers **3**

Compound	n	X	R^1	R^2
3a	2	NH	H	CH_2CONH_2
3b	2	NH	CH_2CONH_2	H
3c	2	NH	$CH(CH_3)OH$	H
3d	1	S	H	CH_2CONH_2
3e	1	S	CH_2CONH_2	H
3f	1	S	$CH(CH_3)OH$	H

different rates of change of ECARs. Plots of ECAR as a function of time can therefore reveal slopes characteristic of stimulation of certain receptor types.

Two cell lines were used in these experiments: transfectants that overexpress the TrkC receptor, 293-TrkC cells, and the same cell line not overexpressing TrkC, 293-2.2. The basal ECARs measured by the instrument were normalized prior to exposure to the test compounds and plotted as a function of time. As a positive control, both cell types were exposed to recombinant human (rh) NT-3 (50 ng/mL). The 293-TrkC cells showed a slow increase of ECAR, and the response continues to increase for an extended period of time, typically longer than the duration of exposure to the compound (Fig. 1a). The ECAR reaches a maximum at approximately 3 h after the exposure while the control cells (293-2.2) showed very little increase in ECAR during the entire experiment (Fig. 1b). Slow increase then slow decline of ECARs is characteristic of the neurotrophin Trk receptors.[15,16]

Some of the NT-3 mimics added to the 293-TrkC cells caused changes in ECAR that were similar to those caused by rhNT-3. Conversely, they showed no response or a very small ECAR increase with the control cell line (293-2.2). The percent increase in the ECAR of the positive mimics of NT-3 is shown in Fig. 2. Of the monomers, **3b** and **3c** gave a moderate response, the rest showed insignificant increases in ECAR. Three of the bivalent ligands were found to increase ECAR. All three positive dimers **1aa**, **1ac** and **1cc** contained a monomer sequence TG and/or GN. All the active compounds are 15-membered ring amines ($n=2$, X = NH). Dimers **1aa**, **1ac** and **1cc** were also tested using cells that overexpress the NGF receptor, Trk-A, and none increased the ECARs above the control untreated cells (data not shown).

Several overall conclusions can be drawn from this study. First, active ligands for TrkC, as determined by cytosensor microphysiometry, can be identified from a very small library of carefully designed small molecule peptidomimetics. The data presented here suggest the divergent–convergent approach can generate dimers with improved activity relative to their monomer components. Other pharmacological data is necessary to confirm and quantitate the activities of these

Table 2. Molecular characteristics of dimers **1**

Compound	R^1	R^2	R^3	R^4
$n=2$, X = NH				
1aa	H	CH_2CONH_2	H	CH_2CONH_2
1ab	H	CH_2CONH_2	CH_2CONH_2	H
1ac	H	CH_2CONH_2	$CH(CH_3)OH$	H
1bb	CH_2CONH_2	H	CH_2CONH_2	H
1bc	CH_2CONH_2	H	$CH(CH_3)OH$	H
1cc	$CH(CH_3)OH$	H	$CH(CH_3)OH$	H
$n=1$, X = S				
1dd	H	CH_2CONH_2	H	CH_2CONH_2
1de	H	CH_2CONH_2	CH_2CONH_2	H
1df	H	CH_2CONH_2	$CH(CH_3)OH$	H
1ee	CH_2CONH_2	H	CH_2CONH_2	H
1ef	CH_2CONH_2	H	$CH(CH_3)OH$	H
1ff	$CH(CH_3)OH$	H	$CH(CH_3)OH$	H

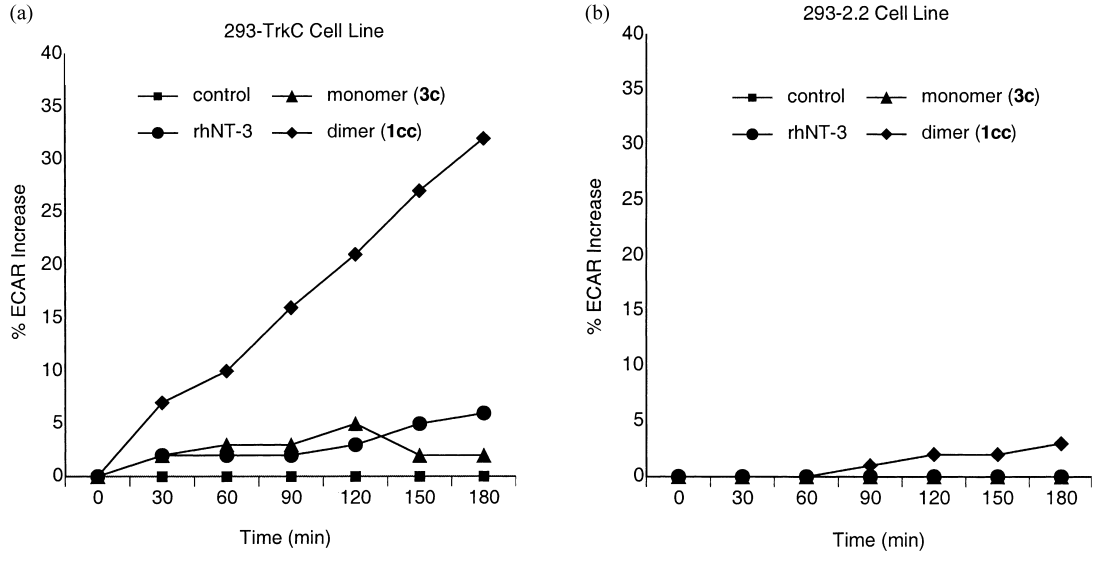

Figure 1.

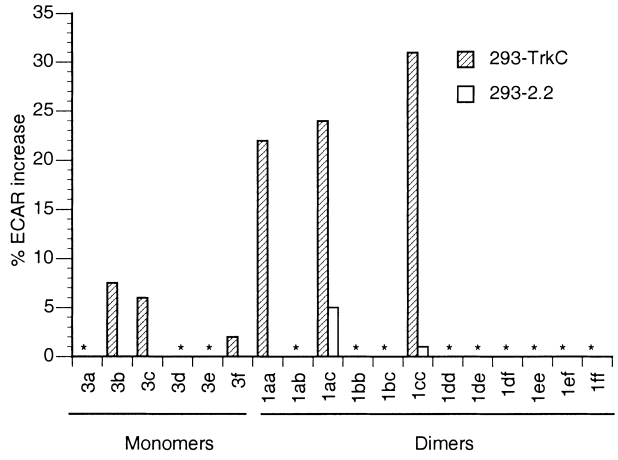

Figure 2.

compounds; experiments to do this are in progress. There are no small molecules that are known to interact with the TrkC receptor, so the data presented here represent an interesting new lead on how this may be done.

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