DESIGN OF MULTIVALENT LIGANDS FOR THE TREATMENT OF PAIN WITHOUT TOLERANCE OR TOXICITIES OF CURRENT DRUGS

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NEW PARADIGMS FOR DRUG DESIGN

■ In Disease States Such as Chronic Pain, Cancer, Diabetes, Addiction, CNS Diseases, etc. There Are Changes in Gene Expression That Are Related to Treatment Attempts
  - Not Surprising Standard Therapies Are Ineffective

■ Drug Design Should Consider System Level Adaptations
  - This Involves Increased Complexity Involving Interactions at More Than One Target.

■ Advantages Can Be Taken of These Changes in Drug Design

I. The development of many degenerative diseases (Cancer, Diabetes, Prolonged and Neuropathic Pain, etc.) involves changes in the expressed genome.

II. These observations led us and others (e.g. Lipkowski, Portoghese, etc.) to try and develop multivalent ligands that would address directly the disease state.

III. Since multiple pharmacophores must be designed into a single molecule interacting with multiple receptors/enzymes/acceptors careful reexaminations of approaches to ligand design is required.

**LIGAND DESIGN FOR DISEASE**
ADVANTAGES OF PEPTIDES AS DRUGS

I. Most Hormones, Neurotransmitters, and Other Modulators of Biological Function in Multicellular Animal Life are Peptides and Proteins.

Nature Is Smart - There Must Be A Reason

II. Peptides and Proteins Can Adopt Many Conformations Via Low Energy Pathways- Many Bioactive Peptides and Proteins Must Adapt Several Conformations During Their Lifetime

III. Peptides and Proteins Can Adopt The Periodic Table and The Biosphere Into Their Structures

Only Organic Structure That Apparently Can

IV. Peptides, Properly Constructed, Can Exist Either in Aqueous or Membrane Environments
INTRODUCTION

- Pain is the most common and ubiquitous disease in the world.
- In the USA TODAY over 100 million people are suffering from prolonged pain. That translates into 1.5 billion people worldwide.
- There are no effective treatments for prolonged and neuropathic pain.
- Pharma is doing very little to deal with this tragic situation.

WHY? A New Paradigm is Needed
ENDOGENEOUS HUMAN OPIOID LIGANDS AND OPIOID TOXICITY

I. OPIOID LIGANDS ARE ACTIVE IN HUMANS EVERY DAY OF OUR LIVES. THEY ARE NONTOXIC.

II. THE ENDOGENEOUS OPIOID LIGANDS ARE PEPTIDES - A. ENDORPHINS; B. ENKEPHALINS; C. DYNORPHINS

III. THE ENDOGENEOUS OPIOID LIGANDS ARE RELATIVELY NON-SELECTIVE

IV. MOST OF OUR CURRENT DRUGS FOR PAIN ARE RECEPTOR/ENZYME SELECTIVE. MOST ARE QUITE TOXIC AND ARE NONPEPTIDES.

V. NATURE IS SMART (3.5 BILLION YEARS OF CHEMISTRY OF LIFE). WE SHOULD PAY MORE ATTENTION TO WHAT NATURE IS TELLING US.
WHAT WE NEED IN AN OPIOID DRUG FOR LONG TERM USE

I. Ligand With None of the Current Opioid Toxicities
   a. No Respiratory Depression
   b. No Inhibition of Gut Motility
   c. No Addiction Potential
   d. No Cardiac Toxicity
   e. No CNS Toxicity (e.g. Dysphoria)

II. Multiple Delivery Systems
   I. Depot, Slow Release, Oral, Transdermal, etc.

III. Penetrate Blood Brain Barrier (OR NOT)
   I. Central vs. Peripheral Action

EVIDENCE FOR UP REGULATION OF NEUROPEPTIDES AND THEIR RECEPTORS IN DEVELOPMENT OF PROLONGED AND NEUROPATHIC PAIN FROM GENOMICS AND PROTEOMICS

1. Antibodies to PEPTIDE NEUROTRANSMITTERS THAT Cause Pain Show Large Increases of Neurotransmitters Released in Pain Pathways.

2. Antibodies to RECEPTORS Show Increased Concentrations of Receptors in Spinal Pain Pathways.

3. Prolonged Treatment of Naive Animals with Morphine Leads to Development of Neuropathic Pain – Also 1 and 2.

4. In Some Cases Antagonists for These New Receptors Can Block or Partially Block This Pain.
Sustained morphine exposure increases expression of pain transmitters (ex. Substance P) in the spinal dorsal horn, leading to induce unexpected hyperalgesia and/or allodynia.
HYPOTHESIS

Molecules with high affinity and antagonist actions at CCK or NK-1 receptors, and high affinity and agonist actions at opioid receptors

- Have enhanced activity in neuropathic pain states and in acute pain states
- Have applicability for treatment of chronic pain without the development of tolerance
- Have utility in individuals tolerant to the pain-relieving actions of opiates
ADVANTAGES OF MULTIVALENCY IN DESIGNING DRUGS FOR DISEASE

I. Most Non-Pathogenic Diseases Are the Result of Multiple Changes in Expressed Genome.

II. Drugs Addressing Only One of These Changes Inadequate.

III. Multiple Drugs Have Different Metabolisms – Dosaging Difficult.

IV. Single Ligand Which Interacts with Multiple Receptor – One Metabolism.

V. Single Multivalent Ligand – Synergy Especially If Targets Are In Proximity

PEPTIDES ARE THE BEST SCAFFOLD FOR DOING THIS
DIFFICULTIES IN DESIGNING MULTIVALENT LIGANDS FOR MULTIPLE BIOLOGICAL TARGETS IN A SINGLE BIOACTIVE MOLECULE

1. Each pharmacophore for each target (receptor, enzyme, cytokine, etc.) requires a unique chemical structure (signature).

2. The binding pocket for each target has a unique three dimensional structure. Each pharmacophore must be represented within a single structure.

3. Undesirable steric and stereoelectronic properties of other pharmacophore(s) must not interfere with properties of other pharmacophores OR of the receptor/enzyme/acceptor target.

4. Issues of potential for overlapping pharmacophores in design Vs. biocompatible linkers to separate pharmacophores before design and synthesis begins.

5. Complexities of synthetic chemistry and structural chemistry can provide major problems and roadblocks.
Biological rationale for Opioid/NK1 combination

**NK1 Antagonist Activity Acts as a Counter for Opioid-Induced Systemic Change**

- Substance P (SP) plays a major role in opioid signal transmission
- Sustained opioid exposure increases expression of SP and NK1R in the spinal dorsal horn
- Previous articles reported that co-administration of opioid agonist and NK1 antagonist led to:
  - Enhanced analgesic efficacy
  - Prevention of opioid-induced tolerance
- NK1-KO mice do not develop opioid-induced tolerance and do not show rewarding for opiates

**Anatomical Overlaps of Peptides and Receptors in Superficial Dorsal Horn**

- Fused DML can work at the same site of action

**Opioid agonist + Neurokinin1 antagonist**

- Counteract against multiple systemic changes due to prolonged pain
- No or reduced opioid-induced tolerance expected
- No or reduced physical dependence expected
I. Combination of Two Pharmacophores Through Amino Acid Moiety Serving as an Address Moiety for BOTH Pharmacophores

H-Tyr-DAla-Gly-Phe-Phe-Pro-Leu-Trp-O-3,5-Bn(CF$_3$)$_2$

**Opioid Pharmacophore**

**Address Amino Acid**

**NK-1 pharmacophore**

Synthesis of Novel Ligands: Combination of Solid Phase Peptide Synthesis (SPPS) and Solution Phase Synthesis

**Loading**
- Fmoc-Trp(Boc)-OH, DIEA, DCM

**SPPS**
- (i) 20% piperidine in DMF
- (ii) Fmoc-AA-OH or Boc-Tyr(′Bu)-OH, HCTU, DIEA, DMF

**Cleavage**
- 1% TFA

**Amidation in solution phase**
- HATU, HOAt, DMF

**Deprotection**
- 82.5% TFA, 5% H₂O, 5% PhOH, 5% Thioanisole, 2.5% Ethanedithiol

**2-Chlorotrityl resin**
- Cl

**Boc-Tyr(′Bu)-D-Ala-Gly-Phe-Xxx-Pro-Leu-Trp(Boc)-O-H**

**Boc-Tyr(′Bu)-D-Ala-Gly-Phe-Xxx-Pro-Leu-Trp(Boc)-OH**

**H-Tyr-D-Ala-Gly-Phe-Xxx-Pro-Leu-Trp**
MODIFICATION AT C-TERMINAL

TY005: Tyr-DAla-Gly-Phe-Met-Pro-Leu-Trp-0-3,5-Bn(CF$_3$)$_2$
TY027: Tyr-DAla-Gly-Phe-Met-Pro-Leu-Trp-NH-3,5-Bn(CF$_3$)$_2$
TY025: Tyr-DAla-Gly-Phe-Met-Pro-Leu-Trp-NH-Bn

• Modification at C-terminal:
  – Critical activity difference at rat NK1 receptor
  – Less substance P antagonist activity difference at guinea pig ileum (Species difference)
  – Act as an “address region” for opioid activities

• Expected activities in human
  – Opioid activity: TY025 > TY027 > TY005
  – SP antagonist activity: TY025 = TY027 > TY005
IN VITRO ASSAYS FOR MU/DELTA NK1 AGONIST ANTAGONIST LIGANDS

TY027: Tyr-DAla-Gly-Phe-Met-Pro-Leu-Trp-N-3,5-Bn(CF3)2

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<td>Morphine</td>
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Yamamoto et al., 2007

Largent-Milnes et al., BJP, 161, 2010

This work was supported by grants from the USDHS, National Institute on Drug Abuse, DA-13449 and DA-06284.
TY027 CROSSES THE BBB

$K_{in} (\text{ul/min/g})$

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<td>Insulin</td>
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<td>TY027</td>
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Ratio of TY027 in Brain (R<sub>Br</sub> ± SEM)

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<tr>
<td>Sucrose</td>
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<tr>
<td>TY027</td>
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* indicates statistical significance.
NMR STRUCTURES OF PEPTIDES

H-Tyr-DAla-Gly-Phe-Met-Pro-Leu-Trp-R

Aligned with backbone atoms of residue 5-8

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<td>res 2-5 (type IV), res 6-9 (Type IV)</td>
<td>Helical</td>
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- Different secondary structure might affect on the NK1 antagonist activities
Collaborative Efforts by Opioid and NK1 Pharmacophores

One Methyl Can Change the Mind of a Molecule & One Fluorine Atom May Make a Molecule Patient Friendly

AKG117: H-Tyr-D-Ala-Gly-NMePhe-Pro-Leu-Trp-NHBn(3',5'-(CF₃)₂)
AKG116: H-Dmt-D-Ala-Gly-Phe-Pro-Leu-Trp-NHBn(3',5'-(CF₃)₂)
AKG115: H-Dmt-D-Ala-Gly-NMePhe-Pro-Leu-Trp-NHBn(3',5'-(CF₃)₂)
AKG127: H-Dmt-D-Ala-Gly-Phe(pF)-Pro-Leu-Trp-NHBn(3',5'-(CF₃)₂)
AKG128: H-Dmt-D-Ala-Gly-NMePhe(pF)-Pro-Leu-Trp-NHBn(3',5'-(CF₃)₂)

Table 1a. Binding affinity results at opioid and NK1 receptors

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<td>AKG117</td>
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<td>-7.05 ± 0.04</td>
<td>207</td>
<td>-6.35 ± 0.13</td>
<td>1/5</td>
<td>3.35 ± 0.74</td>
<td>61.1 ± 2.0</td>
<td>1/18</td>
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<td>-8.63 ± 0.04</td>
<td>1</td>
<td>-8.66 ± 0.03</td>
<td>1/1</td>
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<td>26.9 ± 1.98</td>
<td>1/19</td>
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<td>-7.92 ± 0.07</td>
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<td>2.23 ± 0.07</td>
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<tr>
<td>AKG127</td>
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<td>-7.18 ± 0.04</td>
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<td>0.88 ± 0.07</td>
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<td>2.62 ± 0.51</td>
<td>33.8 ± 6.17</td>
<td>1/13</td>
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Other halogens (i.e. Cl, Br, I) derivatives of Phe have not shown good results.
INTRAVENOUS (I.V.) TY 027 ATTENUATES NERVE-INJURY INDUCED TACTILE AND THERMAL SENSITIVITIES
Chronic TY027 Does Not Result in Antinociceptive Tolerance & Paradoxical Hypersensitivity

**Sham-operated rats**

Nociception

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<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>15</th>
<th>vehicle</th>
<th>TY027 (i.th.)</th>
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Paw Withdrawal Latency (s ± SEM)

The effect of TY027 was not dose dependent after repeated exposure.

The effect of TY027 at Day 7 was not significantly different from Day 1.

**SNL rats**

Thermal Hyperalgesia

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<th>6</th>
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Paw Withdrawal Latency (s ± SEM)

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**SNL rats**

Tactile Allodynia

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Paw Withdrawal Threshold (g ± SEM)

No development of allodynic tolerance observed.

No paradoxical allodynia induced.

**Sham-operated and SNL rats**

Paradoxical Allodynia

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<td>SNL</td>
<td>3.1 Q. 6</td>
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Paw Withdrawal Threshold (g ± SEM)
TY027 showed better side-effect profile when compared with morphine:

- No motor impairment observed
- Does not produce conditioned place preference, a model of reward

Largent-Milnes, T., Yamamoto T. et al. *manuscript under preparation*
TY027 DOES NOT SHOW CONDITION PLACE PREFERENCE

**A) i.p.**

**B) Systematic**

- Baseline
- Vehicle (5μl, i.c.v.)
- MS (10μg/5μl, i.c.v.)
- TY027 (20μg/5μl, i.c.v.)

- Baseline
- Vehicle (1ml/kg, i.v.)
- MS (3mg/kg, i.v.)
- TY027 (6mg/kg, i.v.)

* Indicates significance at p < 0.05
TY027 DOES NOT ALTER GI TRANSIT
CONCLUSIONS

I. Multivalent ligands can be designed with high potency at multiple receptors. Now done with 5 different combinations.

II. Mixture of agonist and antagonist pharmacophores are possible.

III. Peptide ligands and peptido-non peptide ligands can be design which cross the blood brain barrier.

IV. Multivalent opioid ligands can be created that have activity in prolonged and neuropathic pain where morphine is ineffective.

V. Opioid ligands can be created that do not lead to development of tolerance and motor impairment and diminished or no opioid toxicities or development of addiction.

Question: What are biochemical signaling mechanism responsible for above properties?
ACKNOWLEDGEMENTS

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Professor Josephine Lai
Professor Henry I. Yamamura
Professor Todd Vanderah
Professor John Streicher
Shou-Wu Ma
Professor Eva Varga
David S. Herman
Dr. Talley Largent-Milnes
Peg Davis
Professor Richard Egleton
DEVELOPMENT OF NOVEL ENKEPHALIN ANALOGUES

(n=0, 1; R= Ph, H; opioid pharmacophore = Tyr-DAla-Gly-Phe-, Dmt-DAla-Gly-Phe-, etc)

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<td>Phe(p-\text{Cl})</td>
<td>Fen</td>
<td>4.46</td>
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<tr>
<td>8</td>
<td>Dmt</td>
<td>DTic</td>
<td>Gly</td>
<td>Phe(p-F)</td>
<td>Fen</td>
<td>4.02</td>
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<table>
<thead>
<tr>
<th>Affinity ((K_i) in nM)</th>
<th>GTP binding</th>
<th>Opioid (nM)</th>
</tr>
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<tbody>
<tr>
<td>hDOR (\text{EC}_{50})</td>
<td>rMOR (\text{EC}_{50})</td>
<td>rMOR (\text{EC}_{50})</td>
</tr>
<tr>
<td>hDOR</td>
<td>rMOR</td>
<td>hDOR</td>
</tr>
<tr>
<td>-----</td>
<td>-----</td>
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</tr>
<tr>
<td>1</td>
<td>0.36</td>
<td>0.38</td>
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<tr>
<td>2</td>
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<td>3</td>
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<tr>
<td>7</td>
<td>0.11</td>
<td>0.15</td>
</tr>
<tr>
<td>8</td>
<td>0.48</td>
<td>0.35</td>
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</table>

**New drug-design concept:** Designed Multiple Ligands

- Modulate multiple targets simultaneously by a single molecule
- Our DML design: fused DML for **opioid agonist & neurokinin 1 antagonist**

Cocktail of two molecules

Merged

Fused

Linked
**Blood brain barrier permeability of TY027**

**BBB permeability**
*In situ* perfusion rat model

<table>
<thead>
<tr>
<th>Ratio of TY027 In Brain (RBr ± SEM)</th>
<th>Sucrose</th>
<th>TY027</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

A peptide was radiolabeled with I^{125} and used for the experiment to detect the trace of I^{125} in a brain.

**Intravenous** (静脈内投与)  
**Tactile Allodynia** (異痛症)

<table>
<thead>
<tr>
<th>Paw Withdrawal Threshold (g ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL</td>
</tr>
<tr>
<td>Post-SNL BL</td>
</tr>
<tr>
<td>vehicle</td>
</tr>
<tr>
<td>10.0 mg/kg</td>
</tr>
</tbody>
</table>

- TY027 crosses the Blood Brain Barrier
- TY027 is systemically effective in several types of neuropathic pain models

Largent-Milnes, T., Yamamoto T. et al. *manuscript under preparation*
TY005 works as an opioid agonist and NK1 antagonist in vivo

Paw flick latency test in rat

Effect on substance P induced flinching in rat

Analgesic effect of TY005 is reversed by pretreatment of δ/μ-opioid antagonists

TY005 dose-dependently reduces Substance P induced flinching

New Paradigms For Cancer

- Determine a molecular signature that discriminates target from normal cells
- Synthesize a construct that will selectively interact with target cells
- Have the construct carry an imaging agent (for diagnostics) or a therapeutic (theragnostics).
- Therapeutic can be cell-specific (magic bullet) or a tissue disruptor (smart bomb).
Receptor Visualization
Reagents - Solid-phase

Melanotrophic peptide-conjugated beads for microscopic visualization and characterization of melanoma melanotropin receptors

Shubh D. Sharma*, Jinwen Jiang†, Mac E. Hadley†, David L. Bentley‡, and Victor J. Hruby*§

**Fig. 1.** Binding of MSH beads with melanoma cells. (A) Scanning electron micrograph showing MSH microspheres bound to C-8161 human amelanotic melanoma cells. (Bar = 5 μm.) (B) Light microscopic visualization of specific binding of B16 mouse melanoma cells to MSH microspheres. (Bar = 100 μm.) (C) Clustering of microspheres observed during binding to B-16 mouse melanoma cells. (Bar = 5 μm.) (D) Human amelanotic melanoma cells (V3). (Bar = 5 μm.)

**Fig. 2.** Negative control experiments to determine specificity of binding of melanoma cells to melanotropin-conjugated beads. (A) Black ade of binding of human A375P melanoma cells with MSH beads (microspheres) due to pretreatment of cells with an unconjugated (free) MSH ligand, [Nle-4, d-Phe-7]-a-MSH. (Bar = 10 μm.) (B) Naive (no MSH present) microspheres fail to bind to B-16 mouse melanoma cells. (Bar = 10 μm.) (C) Substance P-conjugated beads (microspheres) do not bind to B16 mouse melanoma cells. (Bar = 100 μm.) (D) MCF-7 breast cancer cells do not bind to MSH-conjugated microspheres. (Bar = 10 μm.)
Linkers

Length

Flexibility/rigidity

Density

Hetero Bivalent Ligands

Legend:

- ♦ = MSH-7
- □ = NDP-α-MSH
- ★ = CCK-6
- ○ = DELTORPHIN-II
- - = [Pro-Gly]_{3} linker, 18 atoms, semi-rigid
- ~ = PEGO_{20} linker, 20 atoms, flexible

MSH-7: Ac-Ser-Glu-Nle-His-DPhe-Arg-Trp

CCK-6: Nle-Gly-Trp-Nle-Asp-Phe-NH_{2}

Delt-II: H-Tyr-DAla-Phe-Glu-Val-Val-Gly-NH_{2}

hMC4R - CCK-2R

δOR - hMC4R
Synthesis Of Labeled Multivalent Ligands

1) Alloc removal Pd(0)
2) DOTA-NHS ester

1) PEGO attachment
2) Fmoc/tBu SPPS

1) TFA cleavage
2) LuCl₃, 0.1 mM Amm. acetate

Hetero Bivalent Ligands Evaluation

H-Delt(II)-pego-[PGPGPG]ₙ-pego-MSH(7)-NH₂

δ-OR > hMC4R

δ-OR

50x

2 nM

hMC4R < CCK-2R

hMC4R

CCK-2R
Hetero-Bivalent Ligand (0.8 nM) Labeling of HEK Cells

MC4R Only       MC4R & CCKR

3 minutes

Increase Gain
Hetero-Bivalent Ligand (0.1 nM) Labeling of HEK Cells

MC4R & CCKR

CCK-R Only

3 minutes

Increase Gain
DESIGNED DENDRIMER STRUCTURES
EFFECTS OF VALENCY ON AFFINITY

Multimeric ligands were tested using Time Resolved Fluorescence. Ligands were competed against Eu-NDP-a-MSH.

Overlap of corresponding Mono Bi and Trimer compounds

$\log [\text{compound}]$

light units

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$ (nM)</th>
<th>Relative potency to Monovalent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monovalent on scaffold</td>
<td>4900 ± 760</td>
<td>-</td>
</tr>
<tr>
<td>Bivalent on scaffold</td>
<td>310 ± 73</td>
<td>16</td>
</tr>
<tr>
<td>Trimer</td>
<td>14 ± 1.5</td>
<td>350</td>
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</table>

*J. Med. Chem., 2011, 54, 7375-7384*
Imaging Agents

Cy5 Labeled δ-Opioid Receptor Antagonist

Fluorescence intensity values over whole tumor regions of interest (A&B) after 72 h of clearance of 100 ug and (C) after 24 h of clearance of 10 ug dose

(A) Intensity overlay of 72 h post 100 μg dose
(B) Intensity overlay of 24 h post 10 μg dose
Mouse treated with Rho-MT-II analogues targeting melanoma tumor

Two weeks after first injection of the Rho-MTII. The second treatment of Rho-MTII on the same mouse the tumor size is significant reduced compared to day 2.
Conclusions

- Multivalent ligands directed differentiation of cellular phenotypes (epitope combination approach)

- We can crosslink heterologous cell-surface receptors (FIRST such demonstration)

- Bivalent ligands comply with the predicted specificity (0.5 - 10 nM in bivalent binding mode vs 100 - 1600 nM in monovalent binding mode)

- Synthetic hetero-multivalency is in its infancy
24th American Peptide Symposium

ENABLING PEPTIDE RESEARCH
FROM BASIC SCIENCE TO DRUG DISCOVERY

Hyatt Regency
Grand Cypress Resort
Orlando, Florida
June 20-25, 2015

SYMPOSIUM CO-CHAIRS:
Ved Srivastava - GlaxoSmithKline - ved.x.srivastava@gsk.com
Andrei Yudin - University of Toronto - ayudin@chem.utoronto.ca

www.aps2015.org
ENDOGENOUS HUMAN OPIOID LIGANDS AND OPIOID TOXICITY

I. OPIOID LIGANDS ARE ACTIVE IN HUMANS EVERY DAY OF OUR LIVES. THEY ARE NONTOXIC.

II. THE ENDOGENOUS OPIOID LIGANDS ARE PEPTIDES - A. ENDORPHINS; B. ENKEPHALINS; C. DYNORPHINS

III. THE ENDOGENOUS OPIOID LIGANDS ARE RELATIVELY NON-SELECTIVE

IV. MOST OF OUR CURRENT DRUGS FOR PAIN ARE RECEPTOR/ENZYME SELECTIVE. MOST ARE QUITE TOXIC. ARE NONPEPTIDES.

V. NATURE IS SMART (3.5 BILLION YEARS OF CHEMISTRY OF LIFE). WE SHOULD PAY MORE ATTENTION TO WHAT NATURE IS TELLING US.
Our drug design concept for novel analgesics

- Merit of the fused DML: delivery & compliance
  - Easier to administer, better compliance
    - Reduce potential risk of drug-drug interaction
  - Higher local concentration is expected near synaptic cleft than co-administration of two drugs
    - Lower doses might be sufficient to show potent in vivo biological activity
    - Fewer side-effects expected

- Challenge: complexity
  - Design and optimization of the ligands is sometimes tough and very difficult
Our peptide-based DML Compounds

TY005: H-Tyr-DAla-Gly-Phe-Met-Pro-Leu-Trp-O-3,5-Bn(CF$_3$)$_2$
TY027: H-Tyr-DAla-Gly-Phe-Met-Pro-Leu-Trp-NH$_2$ 3,5-Bn(CF$_3$)$_2$

<table>
<thead>
<tr>
<th></th>
<th>Binding affinity</th>
<th>[${}^{35}$S]GTP$\gamma$S binding</th>
<th>MVD</th>
<th>GPI/LMMP</th>
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<tbody>
<tr>
<td></td>
<td>hDOR (Ki; nM)</td>
<td>rMOR (Ki; nM)</td>
<td>hNK1 (Ki; nM)</td>
<td>rNK1 (Ki; nM)</td>
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<td>hDOR</td>
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<td>rMOR</td>
<td>0.66</td>
<td>16</td>
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<td>7.3</td>
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Synaptic cleft

~20 nm

~2 nm

Pain signal

NK1 Receptor

Opioid Receptor

Opioid agonist pharmacophore

NK1 antagonist pharmacophore

TY027 ATTENUATES TACTILE AND THERMAL ALLODYNYIA