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Identification of potent and highly selective chiral tri-amine and tetra-amine μ opioid receptors ligands: An example of lead optimization using mixture-based libraries

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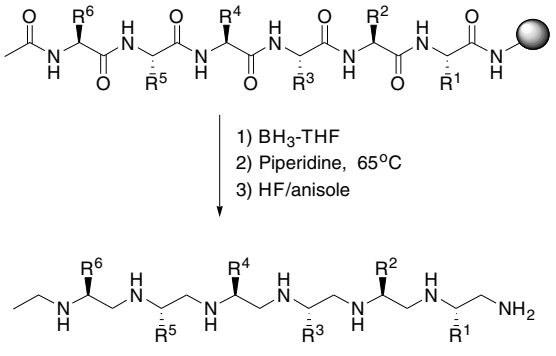
Abstract—The generation of chiral polyamine libraries has been successfully accomplished in our laboratory following exhaustive reduction of resin-bound peptides. Herein, we report the synthesis and screening results of a positional scanning mixture-based library of chiral hepta-amines in a radioreceptor assay for the opioid receptor. The positional scanning hepta-amine library was generated by the exhaustive reduction of a library of 34,012,070 hexapeptides. Following screening of the entire library, combinations of the most active functionalities found at each position were used to synthesize and screen 40 individual hepta-amines and served as starting 'hits' for further SAR studies. The individual compounds showed IC_{50} values ranging from 14 to 345 nM. As might be anticipated by the known studies of μ opiate antagonists, the identified active hepta-amines possessed aromatic rings derived from phenylalanine and tyrosine amino acid side chains. Following SAR studies, a truncation analog, reduced and permethylated YYF-NH_2, was found to be highly active (0.5 nM) as a selective μ antagonist in the guinea pig ileum bioassay.

Solid-phase parallel synthesis is used worldwide to generate libraries of small organic compounds for the acceleration of the drug discovery process.[1–14] Due to the well-understood chemistry and excellent synthetic purity and yields obtained during the solid-phase synthesis of peptides, our primary efforts have been directed toward the synthesis and design of acyclic and heterocyclic compounds using resin-bound amino acids, peptides, and peptidomimetics as starting materials.[15–24] The chemistry of amino acids,[22] their activation, protection, and deprotection are well documented, and they are commercially available in enantiomerically pure forms. Post-synthetic chemical modification of peptide libraries using the 'libraries from libraries' approach[16] enables the generation of peptidomimetic libraries and low molecular weight, small molecule organic libraries. A range of peptide and peptidomimetic libraries have been modified using a variety of chemical reagents (acylation, alkylation, reduction, etc.) to generate an ever-expanding range of chemical diversities having strikingly different physicochemical properties relative to their starting libraries.[16,18,25] Peptide libraries have been successfully modified by exhaustive alkylation of their amide bonds to yield peptidomimetics and/or by reduction of the backbone amide carbonyl groups to yield polyamines. The amine functionality is a critical factor in hydrogen bonding and folding of enzymes. Amines are also key



Scheme 1.

Keywords: Polyamines; Amide reduction; Libraries; Positional scanning libraries; μ Opiate receptors.
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components of ligands having specificity for the opioid receptors μ, γ, and κ.

A variety of mixture-based combinatorial libraries made up of different numbers of polyamines, as well as large arrays of individual polyamines, have been synthesized in our laboratory.[26,23] Those libraries have been screened internally and in collaboration with outside collaborators.[27–29] Examples of reported activities include antimalarial, antitubercular, HIV inhibitory, and antitumoral activities.[30] As introduced by our laboratory, the exhaustive reduction of peptides and chiral polyamides on solid-supports has been utilized in a wide range of synthetic procedures. Typical reaction conditions for the solid-phase reduction of polyamides consist of a 72 h treatment of resin-bound peptides with

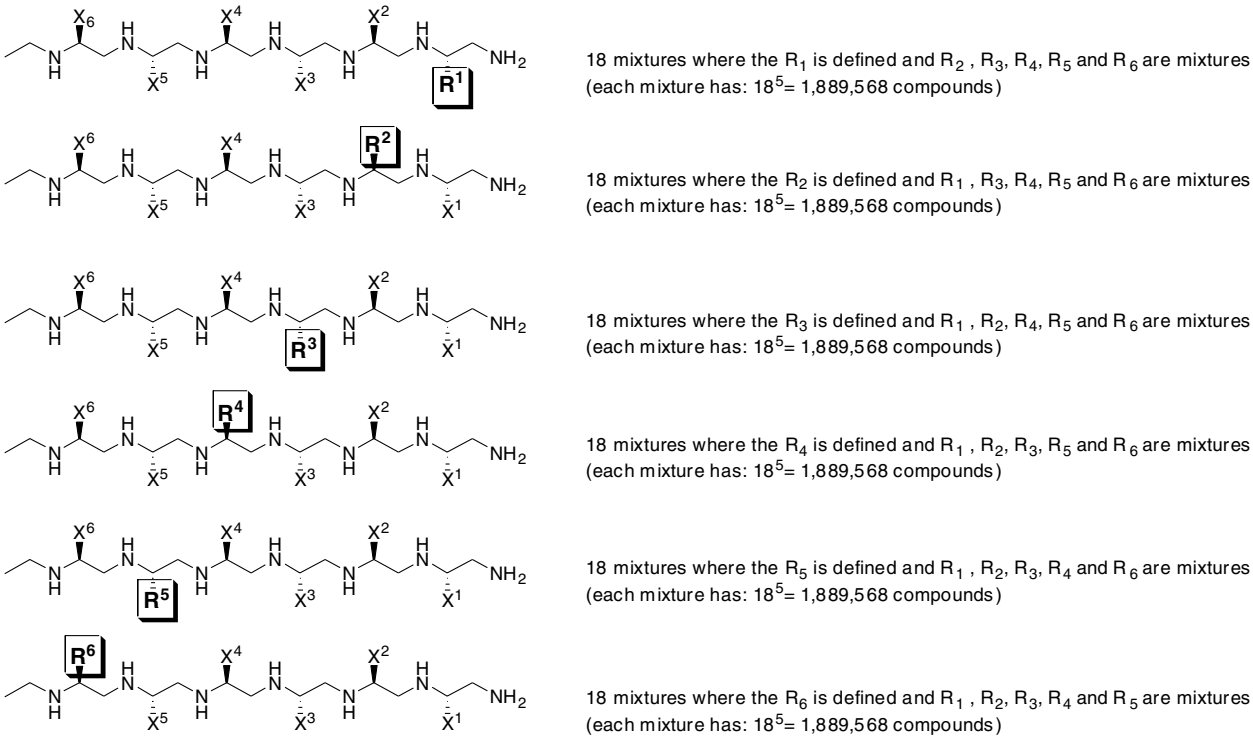


Figure 1. Positional scanning synthesis of the hepta-amine library: Synthesis of six sublibraries each containing the same $18^6 = 34{,}012{,}170$ compounds organized in different formats.

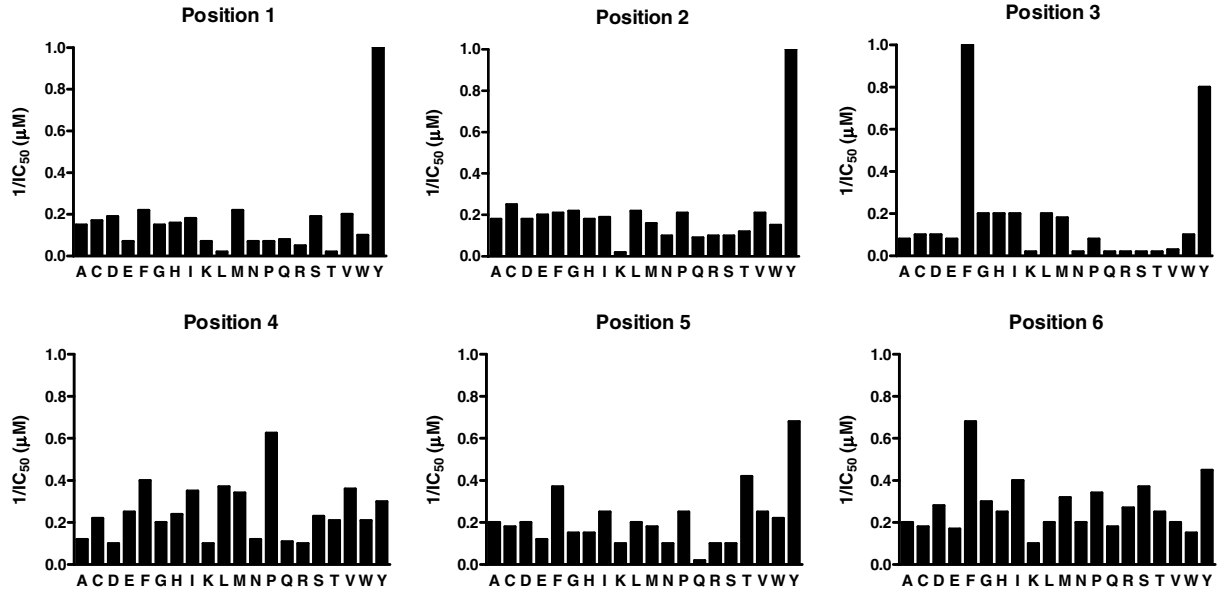
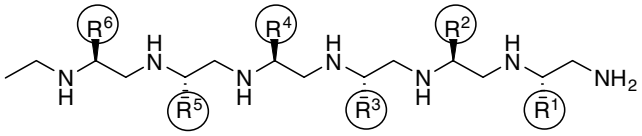


Figure 2. Screening of reduced positional scanning hepta-amine library in a μ receptor assay.

BH_3–THF at 65 °C. The resulting resin-bound borane-amine complexes are then disproportionated by overnight treatment with neat piperidine at 65 °C. Detailed experimental procedures have been previously reported.[15,23] We also reported the generation of chiral acyl-polyamines, poly-*N*-acylamines, polyureas, polythioureas libraries and a variety of diazacyclic compounds using resin-bound chiral polyamines as templates.[15–24]

The opioid receptors represent an important and a convenient system to investigate the power of combinatorial libraries to identify distinctly different ligands for related receptors: μ, δ and κ.[27,29,31–39] All three receptors have recently been cloned and they belong to the seven-trans-membrane G-protein-coupled family of receptors and have approximately 60% amino acid sequence homology. Screening of the same combinatorial library in separate assays selective for each of the three receptors provides not only new ligands for these receptors but also yields insights into the ability of combinatorial libraries to discriminate between closely related receptors. Herein, we report the synthesis and screening results of a positional scanning mixture-based library of 34,012,070 chiral hepta-amines in a radioreceptor assay for the μ opioid receptor. The hepta-amine library was generated following exhaustive reduction of an hexapeptide library synthe-

Table 1.



No	R^6	R^5	R^4	R^3	R^2	R^1	IC_{50} (nM)
1	Y	Y	F	P	T	M	14
2	Y	Y	F	P	T	P	15
3	Y	Y	F	P	Y	P	18
4	Y	Y	F	P	T	F	19
5	Y	Y	F	P	Y	S	19
6	Y	Y	F	P	T	S	19
7	Y	Y	F	P	T	Y	20
8	Y	Y	F	P	Y	Y	27
9	Y	Y	F	P	Y	F	27
10	Y	Y	Y	P	T	S	32
11	Y	Y	F	P	Y	M	34
12	Y	Y	Y	P	T	P	41
13	Y	Y	F	F	Y	P	48
14	Y	Y	Y	P	T	Y	61
15	Y	Y	F	F	Y	S	66
16	Y	Y	F	F	T	Y	78
17	Y	Y	Y	P	Y	P	79
18	Y	Y	F	F	T	P	82
19	Y	Y	Y	P	Y	F	83
20	Y	Y	Y	P	Y	Y	87
21	Y	Y	Y	P	Y	S	89
22	Y	Y	F	F	T	S	90
23	Y	Y	F	F	Y	Y	95
24	Y	Y	F	F	T	F	108
25	Y	Y	Y	P	T	M	112
26	Y	Y	Y	P	Y	M	113
27	Y	Y	F	F	T	M	115
28	Y	Y	F	F	Y	M	126
29	Y	Y	Y	P	T	F	128
30	Y	Y	Y	F	Y	P	148
31	Y	Y	Y	F	T	P	155
32	Y	Y	Y	F	T	S	163
33	Y	Y	F	F	Y	F	176
34	Y	Y	Y	F	T	M	181
35	Y	Y	Y	F	T	Y	200
36	Y	Y	Y	F	Y	F	258
37	Y	Y	Y	F	Y	M	260
38	Y	Y	Y	F	Y	Y	261
39	Y	Y	Y	F	T	Y	273
40	Y	Y	Y	F	Y	S	345

sized from 18 of the 20 proteogenic amino acids (cysteine and tryptophan were excluded due to side products) (Scheme 1).

Positional scanning synthetic combinatorial libraries (PS-SCLs) are composed of one sublibrary for each variable position.[13,29,40–43] In the case of single position defined PS-SCLs, each compound present in a given mixture has a common individual building block at a given position, while the remaining positions are composed of mixtures of all of the building blocks used to prepare the library; a common single building block thus defines each relevant mixture. The sublibraries for each position represent the same collection of individual compounds, and they differ only by the location of the defined position. The screening data permit the identification of key functionalities at each diversity position. It is important to note, however, that the activity found for a mixture is due to the presence of specific active compound(s) within the mixture, and not the individual functionalities as separate independent entities. The combination of all positional

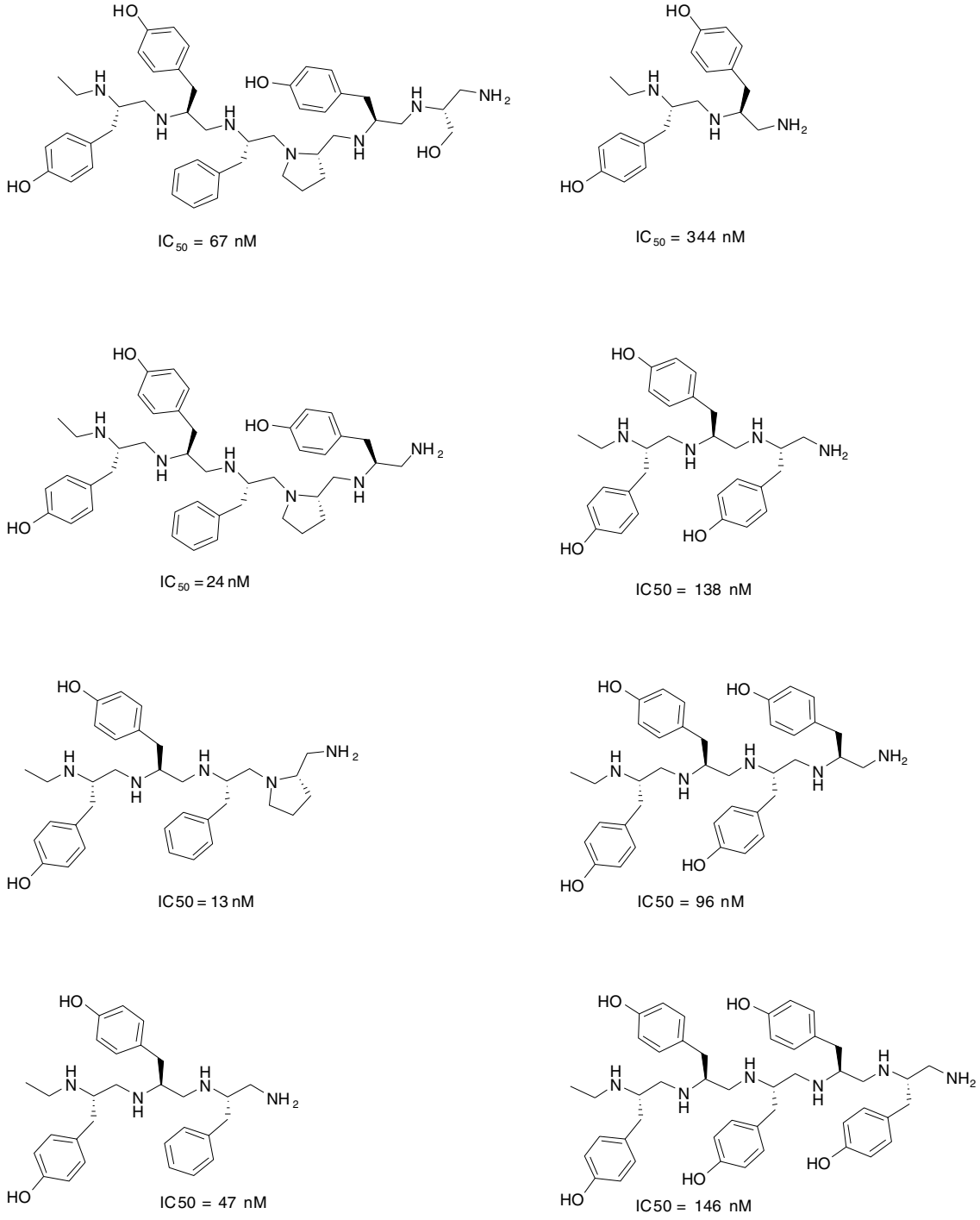


Figure 3.

functional groups identified as key elements leads to the identification of individual active compound(s) (Fig. 1).

The library was screened in a μ opioid receptor specific assay. Preparation of rat brain homogenates and the receptor binding assay were carried out as described in

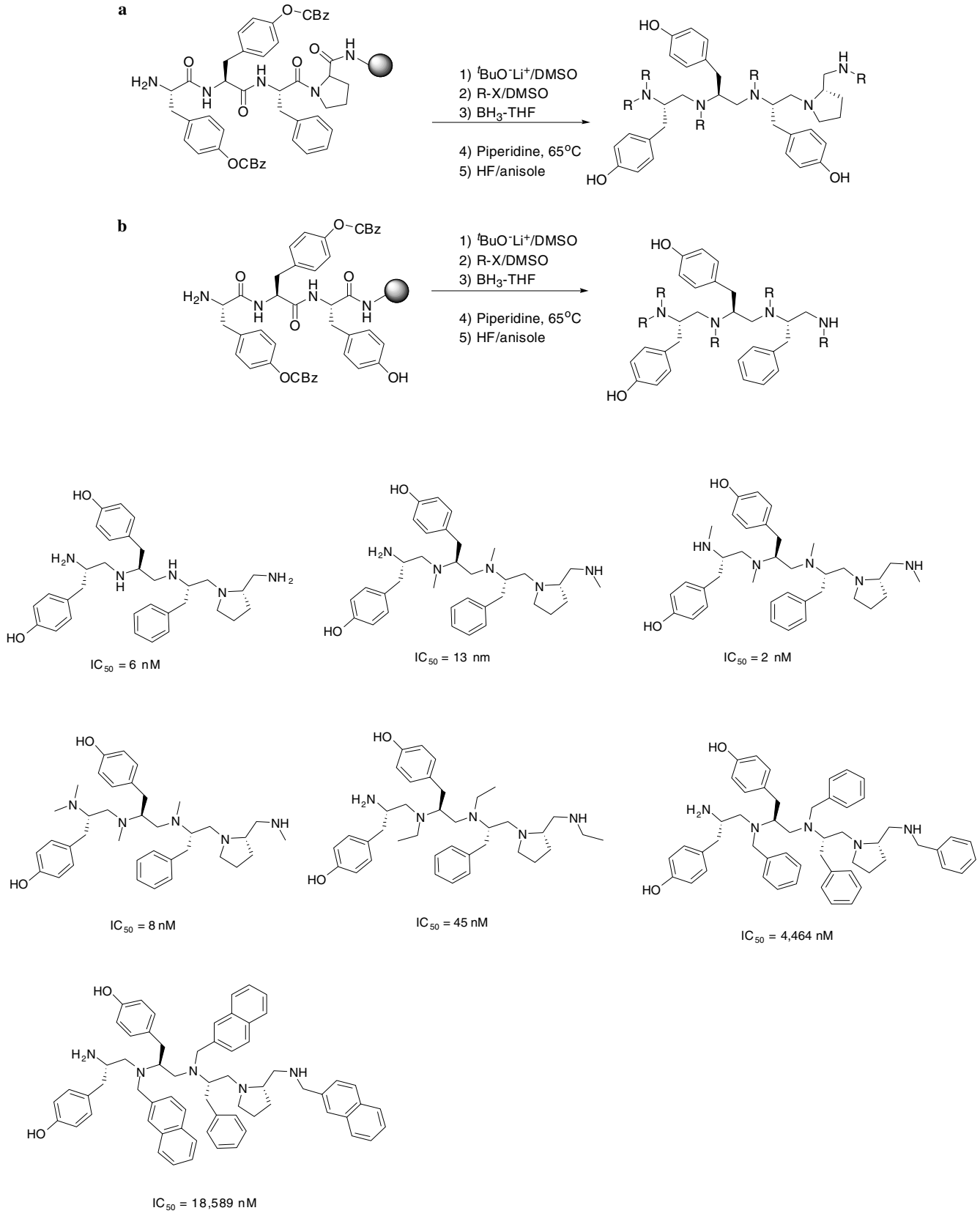


Figure 4.

previous communications.[27,29,33,35,36,44] Each tube in the screening assay contained 0.5 mL of membrane suspension (0.1–0.2 mg protein), 3 nM [^{3}H]-labeled DAMGO (specific activity 36 Ci/mmol), and 50 μL amine mixture (0.08 mg/mL) in 50 mM Tris–HCl buffer (pH 7.4). The final volume was 0.65 mL. Guinea pig ileum (GPI) and mouse vas deferens (MVD) bioassays were preformed as reported previously. Following the screening of the hepta-amine library, a cutoff IC_{50} value of 3–4 μM was chosen for all sublibraries (Fig. 2). Thirteen mixtures were selected. One mixture for position 1 (R^1) corresponding to tyrosine, one mixture for position 2 (R^2) corresponding to tyrosine, two mixtures for position 3 (R^3) corresponding to phenylalanine and tyrosine, two mixtures for position 4 (R^4) corresponding to proline and phenylalanine, two mixtures for position 5 (R^5) corresponding to tyrosine and threonine, and five mixtures for position 6 (R^6) corresponding to phenylalanine, methionine, proline, serine, and tyrosine were found to have the desired activity. It generated 40 possible combinations ($1 \times 1 \times 2 \times 2 \times 2 \times 5$) representing the functionalities found at each position for each of the most active mixtures. Forty individual hepta-amines were synthesized and screened. The individual compounds showed IC_{50} values ranging in activity from 14 nM to 345 nM (Table 1).

Interestingly, all of the active hepta-amines were found to possess at least two hydroxyphenyl groups derived from tyrosine amino acid side chains. This was the starting point for our structure–activity relationship (SAR) studies.

As part of our initial SAR studies, a series of derivatives were synthesized in which the polyamine chain was shortened, while maintaining the focus on the aromatic amino acids. Six polyamines were synthesized starting from hepta-amines (derived from hexapeptides) to triamines (derived from dipeptides) (Fig. 3). All compounds showed $^3H_2$-DAMGO inhibition with IC_{50} activities varied from 15 nM to 344 nM. Since the hydroxyphenyl group derived from the side chain of tyrosine was present in all of the active compounds, we synthesized and screened tetra-amine, penta-amine, and hexa-amine analogs all derived from their corresponding tetra-, penta-, and hexa-tyrosines. The three compounds showed inhibition activities with IC_{50} values ranging from 96 nM to 146 nM (Fig. 3).

We then focused our studies on the truncation analogs, reduced YYFP-NH_2, which was found to be as active (13 nM) as the most active hepta-amines and was found to be a selective μ antagonist in the guinea pig ileum bioassay. We then decided to modify the polyamine backbone in such a manner as to retain the diversity derived from the amino acid side chains, while altering the polyamine character. A series of derivatives were then synthesized according to the strategy described in Scheme 2a, in which the backbone nitrogens of the compound reduced YYFP were per-alkylated with either methyl, ethyl, allyl, benzyl, or naphthylmethyl groups using the relevant alkyl halide (Fig. 4). The permethylated analogs were found to be the most active penta-amines with activities ranging from 2 nM to 13 nM.

As analogs of the permethylated reduced YYFP-NH_2, we synthesized and screened all possible permethyalted diastereomeres of reduced YYF-NH_2. Interestingly, as shown in Figure 5, the permethylated tetra-amine result-

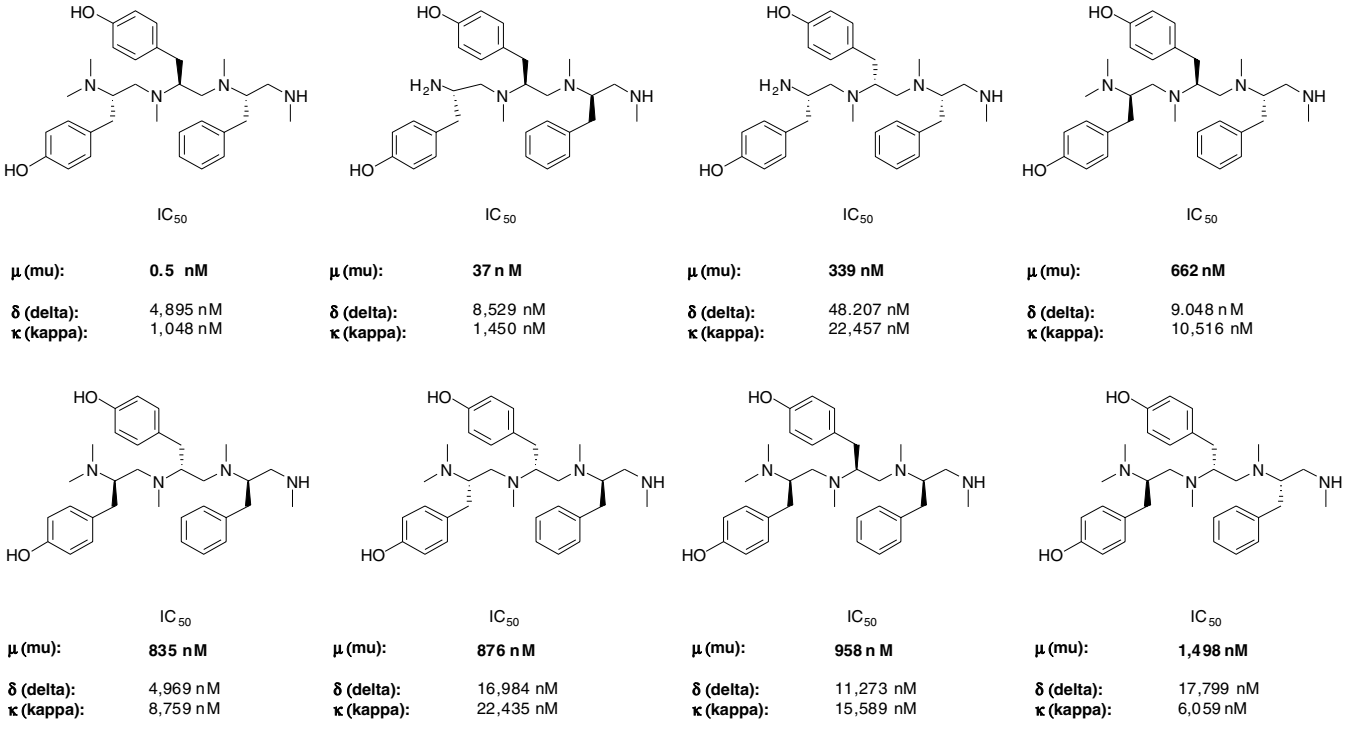


Figure 5.

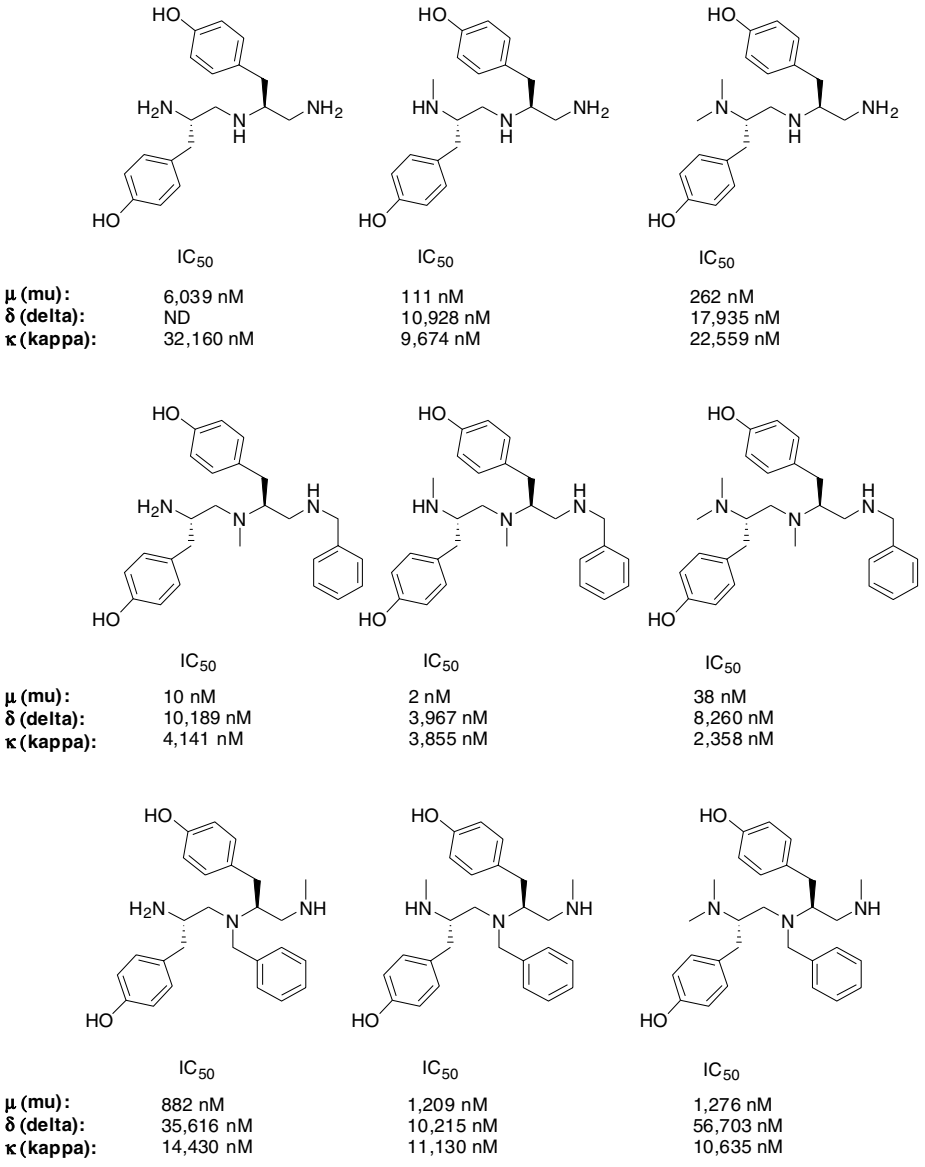


Figure 6.

ing from permethylated reduced YYF-NH_2 showed high activity IC_{50} = 0.5 nM (Fig. 5). The tetra-amines were tested in assays selective for σ and κ receptors using [3H]-DPDPE and [3H]-U69,593 as radioligands. All amines were found to bind poorly to both σ and κ receptors (Fig. 5). When examined in the GPI bioassay, the permethylated reduced YYF-NH_2 compound was found to be a highly specific, pure antagonist at the μ receptor.

An extra set of compounds derived from reduced and alkylated YY-NH_2 dipeptides were synthesized and screened in the μ, δ, and κ assays. Most of the compounds were found to bind selectively to the μ receptor (Fig. 6). The most active compound found was a tri-amine with an IC_{50} = 2 nM, in which one amine was selectively benzylated, while the remaining amines were methylated. The chemistry of peralkylation and selective alkylation of amide bonds was previously reported by our laboratory.[16,18,20,21] It is very important to mention that all the corresponding peptide homologs (non-reduced) of each active polyamine were inactive.

Mixture-based libraries are powerful tools for the identification of highly active compounds. In this paper, we have presented the identification of highly selective active compounds against the μ opioid receptor from a positional scanning hepta-amine library. These results provided useful information to initiate SAR studies toward the identification of potent and highly selective tri-amines and tetra-amines.

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