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Use of receptor chimeras to identify small molecules with high affinity for the dynorphin A binding domain of the κ opioid receptor

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Abstract—A series of 2-substituted sulfamoyl arylacetamides of general structure **2** were prepared as potent κ opioid receptor agonists and the affinities of these compounds for opioid and chimeric receptors were compared with those of dynorphin A. Compounds **2e** and **2i** were identified as non-peptide small molecules that bound to chimeras 3 and 4 with high affinities similar to dynorphin A, resulting in K_i values of 1.5 and 1.2 nM and 1.3 and 2.2 nM, respectively.

The identification of the mu (μ), delta (δ), and kappa (κ) subtypes of the opioid receptor led to the suggestions that agonists selective for receptor subtypes might be effective analgesics with fewer serious side effects.[1] Even though the arylacetamide series of κ opioid receptor agonists lack μ opioid receptor-mediated side effects, the utility of these agonists as antinociceptive agents is limited due to side effects such as dysphoria, diuresis, and psychotomimesis.[2–4] In clinical trials, the naturally occurring peptide κ opioid receptor agonist, dynorphin A, mediates analgesia without dysphoria, diuresis, and psychosis, indicating that the antinociceptive effects of κ opioid receptor agonists could be dissociated from their side effects.[5,6] A metabolically stable analog of dynorphin A, E2078, is an effective analgesic in post-surgical patients at doses that produce no side effects.[7] This exemplifies that there are opportunities for identifying metabolically stable small peptides or small molecule κ opioid receptor agonists as effective analgesics that lack the side effect profile of the arylacetamides.

This distinction in the side effect profiles of arylacetamides and dynorphin A could in part be related to the different binding regions for the κ opioid receptor[8,9] which were observed through the use of chimeric receptors composed of sequences derived from κ and μ opioid receptors. These different modes of binding have led to a hypothesis that different domain selectivity of agonists that bind to the κ receptor might be related to different patterns of side effects.[9] Therefore, in an effort to discover small molecule κ opioid receptor agonists that have a therapeutic profile similar to that of dynorphin A and related compounds, we have recently described[10] the design and construction of two μ/κ chimeric receptors composed primarily of amino acid residues derived from the μ opioid receptor for the screening of compounds.

The chimeric receptors used in this study include one of the chimeric receptors used in the earlier study (designated chimera 3)[10] and another chimeric receptor (designated chimera 4). These receptors are depicted in Figure 1 in which filled circles represent amino acids derived from the κ opioid receptor and open circles represent amino acids derived from the μ opioid receptor. For chimera 3, the 25 amino acids of the putative second extracellular loop of the μ opioid receptor were replaced with the 28 amino acids (8 identical) of the putative second extracellular loop of the κ opioid receptor. The chimera 4 construct was made using a synthetic oligonucleotide corresponding to the Bcl1–Sty1 region (343 bp) of the human κ opioid receptor in which amino acid numbers 86–178 were replaced with the corresponding amino acids of the human μ opioid receptor. This construct is a human κ opioid receptor where the first and second intracellular loops, the first extracellular loop, and the second and third transmembrane regions were replaced

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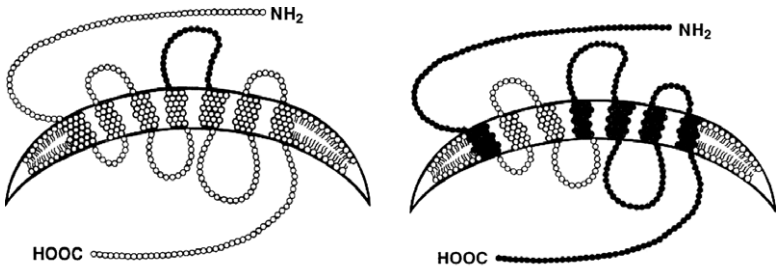


Figure 1. Schematic representation of chimeric receptors.

with the corresponding regions from the human μ opioid receptor. DNA sequencing was used to verify each construct.

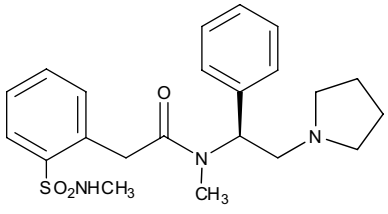
Competitors of [^{3}H]diprenorphine binding from a variety of structural classes bound to these chimeras with affinities similar to those with which they bound to the μ opioid receptor. In contrast, dynorphin A analogs bound to the chimeras with the affinities close to those with which they bound to the κ opioid receptor. Pharmacological characterization of [^{35}S]GTPγS binding mediated by chimera 3 showed that it behaved as if it were a μ opioid receptor with high affinity for dynorphin A analogs.[10] These two chimeric receptors were used to screen for compounds that bind to the κ opioid receptor in a dynorphin-like fashion. The compounds will be used to test the hypothesis that binding domain selectivity can be used as a guide in identifying κ opioid receptor selective agonists as analgesics with reduced side effect profiles.

Our initial approach was to introduce 4,5-dimethoxy or 4,5-methylenedioxy groups in compound **1**, and vary only the amine portion of the 2-sulfamoyl group and the substitution in the 3 position of the pyrrolidine. We have synthesized a novel series of 2-substituted sulfamoyl arylacetamides of general structure **2** as potent κ opioid receptor agonists and compared the affinities of these compounds for μ, δ, κ opioid and chimeric receptors with those of dynorphin A. Once a compound from this series having a profile similar to that of dynorphin A is identified, in vivo testing in various analgesic models will be performed, not only to evaluate the analgesic properties, but, more importantly, to assess the side effect profiles.

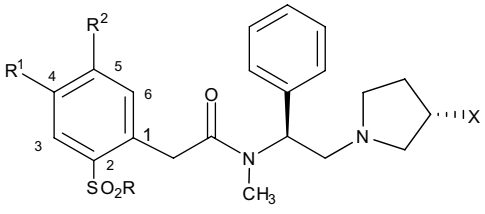
The diamines **3** and **3a** (Scheme 1) were prepared according to published methods from (*S*)-phenylglycine.[11,12] As mentioned earlier, the 4,5-dimethoxy and 4,5-methylenedioxy substituted phenylacetic acids were selected to take advantage of the electron rich

Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln
1 8 17

Dynorphin A



1



2 (X = H, OH)

After screening of non-peptide κ opioid receptor agonists of different templates (Upjohn, Glaxo, ICI, and Dupont), compound **1** of the ICI template, having a 2-sulfamoyl substitution in the phenylacetamide moiety, was identified as a lead because it bound to chimera 3 and chimera 4 receptors with K_i values of 400 nM and 110 nM, respectively, while having a K_i value >1000 nM at the μ opioid receptor. Another observation was made during the evaluation of these compounds that phenylacetic acids with electron rich groups such as methoxy or dimethoxy tended to have higher affinities for the chimeras than unsubstituted compounds.

phenyl ring in providing the regioselective syntheses of the arylacetamide portion of the target compounds.

A general synthetic pathway was designed for the condensation of diamines **3** and **3a** with the sulfamoyl phenylacetic acid **8** in the presence of EDCI/HOBT/Hunig's base (Scheme 1). The desired compounds **2** were purified by chromatographic methods and converted to either hydrochloric or methanesulfonic acid salt for the final isolation. The yields of these compounds are shown in Table 1.