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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

Keywords: κ Opioid receptor agonists; Dynorphin A; Chimeric receptors.
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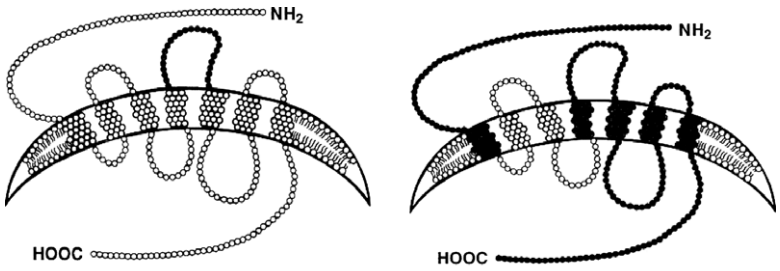


Figure 1. Schematic representation of chimeric receptors.

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

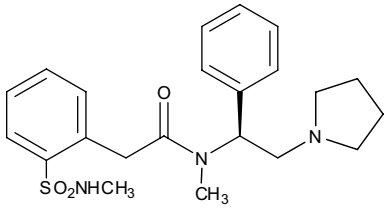
Competitors of [^{3}H]diprenorphine binding from a variety of structural classes bound to these chimeras with affinities similar to those with which they bound to the μ opioid receptor. In contrast, dynorphin A analogs bound to the chimeras with the affinities close to those with which they bound to 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



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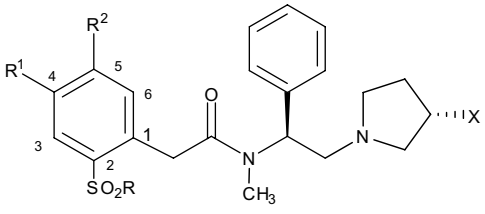
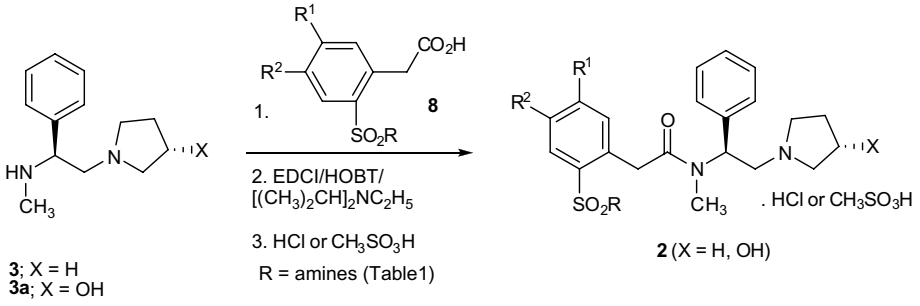


2 (X = H, OH)

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.

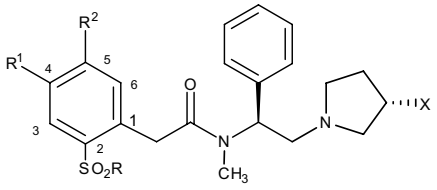
phenyl ring in providing the regioselective syntheses of the arylacetamide portion of the target compounds.

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.

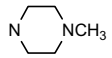
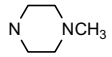


Scheme 1. General synthesis of targets (example).

Table 1. Opioid and chimeric receptor binding



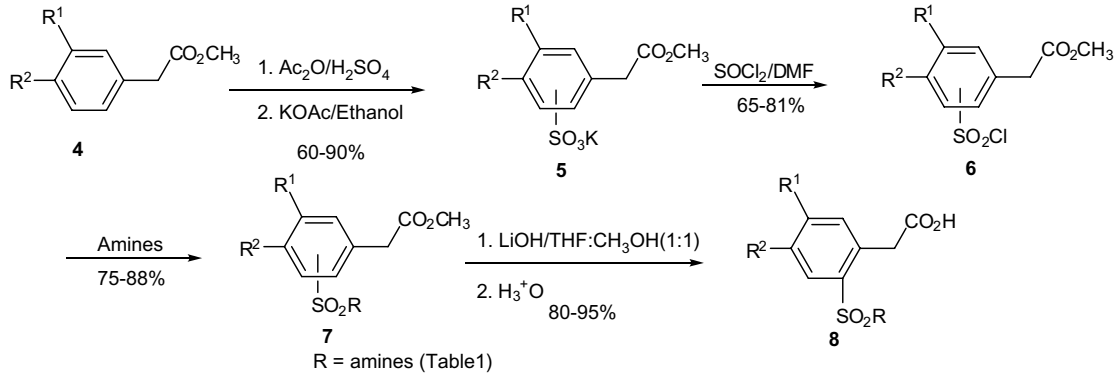
2 (X = H, OH)

Compound	R^1R^2	R	X	Yield (%)	κ K_i (nM)	μ K_i (nM)	δ K_i (nM)	κ-Mediated [^{35}S]GTPγS EC_{50} (nM)	Chimera 3 K_i (nM)	Chimera 4 K_i (nM)
		ICI 199441 (**1**)			0.044	53	24	0.30	14	1.0
		Dynorphin A (**2**)			0.21	9.4	5.9	1.1	0.31	0.18
1	H	$NHCH_3$	H		8.4	>1000	>1000	11	400	110
2a	OCH_3	H at 2-Position	H	65	2.8	>1000	500	6.0	300	120
2b	OCH_3	$N(CH_3)_2$	H	55	0.50	42	56	0.26	3.2	2.5
2c	OCH_3	$N(CH_3)_2$	OH	45	1.6	25	9.3	0.24	6.4	2.8
2d	OCH_3	$N(CH_3)CH_2Ph$	H	46	0.68	21	1.5	0.54	3.3	4.8
2e	OCH_3		H	67	0.31	14	5.4	0.11	1.5	1.2
2f	OCH_3		OH	49	1.1	5.9	0.85	0.12	38	8.3
2g	OCH_3		H	62	1.3	52	23	0.39	4.8	6.8
2h	OCH_3		OH	74	3.8	27	4.4	0.71	ND	ND
2i	OCH_3		H	75	0.55	15	9.4	0.17	1.3	2.2
2j	OCH_3		OH	77	1.4	9.0	1.8	0.34	ND	ND
2k	$O–CH_2–O$	H at 2-Position	H	70	0.70	450	580	0.43	220	68
2l	$O–CH_2–O$	$N(CH_3)_2$	H	48	0.22	47	110	0.10	5.2	0.71
2m	$O–CH_2–O$	$N(CH_3)_2$	OH	41	0.54	34	6.2	0.11	4.5	0.50
2n	$O–CH_2–O$	$N(CH_3)CH_2Ph$	H	82	0.19	6.5	3.4	0.12	5.5	1.7

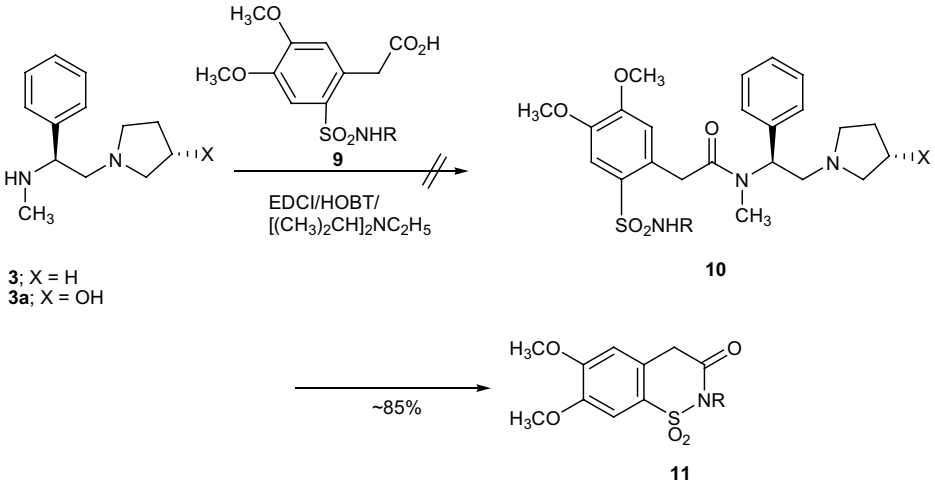
Notes: A series of concentrations of each compound was tested for its ability to inhibit [^{3}H]-diprenorphine binding to κ, μ, δ, and chimeric receptors and K_i values determined as described in Refs. 14,15 The values shown are geometric means of at least 3 determinations. The ability of compounds to stimulate κ opioid receptor-mediated [^{35}S]GTPγS was determined as described in Ref. 16. ND = not done.

The sulfamoyl acids (**8**) were prepared by a slight modification of published method.[13] The preparation of these acids is described in Scheme 2. The regioselectivity of sufamoyl substituents was directed by the electronic and steric properties of the methylphenylacetates (**4**). The mixtures of the ester **7** were readily separated by column chromatography and each was characterized before hydrolysis to give 2-sulfamoyl acids **8**.

Our attempts to prepare the 4,5-dimethoxy substituted analog of compound **1** failed due to the presence of a reactive proton on the sulfamoyl nitrogen. Although the 2-sulfamoyl phenylacetic acids (**9**) were readily synthesized, the condensation reactions with diamine **3** or **3a** failed to give the desired products **10**. In the presence of condensing agents such as EDCI, these acids underwent intramolecular cyclization to give the benzothiaz-



Scheme 2. General method for preparing sulfamoyl aryl acetic acids.



Scheme 3. Condensation of sulfamoyl phenylacetic acids (**9**).

ones (**11**, R = CH_3) in excellent yields (>85%) and the diamines were recovered quantitatively (Scheme 3). To prevent the formation of benzothiazones, during these reactions, the sulfamoyl phenylacetic derivatives in Table 1 were prepared only from secondary amines lacking the reactive proton.

The new compounds were initially evaluated in vitro for opioid receptor binding affinities. Determinations of affinities for κ, μ, and δ opioid receptors were conducted by displacement of bound [^{3}H]diprenorphine from membranes prepared from cells expressing the cloned human opioid receptors using previously described methods.[14,15] Opioid receptor-mediated stimulation of [^{35}S]GTPγS binding[16] was used to determine functional activities of the lead compounds (Table 1). Compounds that showed at least 10-fold higher affinity for the κ opioid receptor than for the μ opioid receptor were evaluated for chimeric receptor affinities. The data are presented in Table 1.

The K_i values for binding to the κ opioid receptor for the new compounds ranged from 0.19 to 3.8 nM with varying affinities for μ and δ opioid receptors. Compounds containing 3′-*S*-hydroxypyrrolidine generally have been reported to show improved κ opioid receptor binding affinities.[17,18] However, the sulfamoyl series of compounds showed reduced κ opioid receptor binding affinities and gains in binding affinities for the μ and δ opioid receptors. These changes in binding affinity were more pronounced for the δ opioid receptor than for the other opioid receptors (Table 1).

The 4,5-dimethoxy- or 4,5-methylenedioxyphenylacetic acid derivatives (**2a** and **2k**), which lack the 2-sulfamoyl substitution, bind to the κ opioid receptor with K_i values of 2.8 nM and 0.70 nM and with good selectivity over the μ and δ opioid receptors (Table 1). However, **2a** and **2k** exhibited lower affinities for chimera 3 (K_i, 300 and 220 nM) and chimera 4 (K_i, 120 and 68 nM, Table 1). The introduction of a 2-dimethyl aminosulfamoyl group into **2a** and **2k** gave compounds **2b** and **2l**. The κ opioid receptor binding affinities were increased 6- and 3-fold, respectively, and there were substantial gains in the affinities for chimeras 3 and 4 yielding K_i values of 3.2 and 2.5 nM, respectively, for compound **2b** (Table 1). The selectivity with regard to the μ and δ opioid receptors was, however, reduced. Even though the chimeric receptor affinities of compounds **2b** and **2l** were approximately 10- to 4- fold lower than those of dynorphin A, these were the first non-peptide compounds of the series which bound to the chimeric receptor with

higher affinities than would be predicted by their affinities at the μ opioid receptor. This may indicate interactions in the chimeric binding with residues derived from the κ opioid receptor.

Further improvements in binding and selectivity were expected upon replacement of the pyrrolidine with 3′-*S*-hydroxypyrrolidine, which resulted in compounds **2c** and **2m**. But these and other compounds of this series did not improve the receptor affinities and in most instances the affinities were decreased relative to the corresponding pyrrolidine compounds (Table 1). This structural modification affected primarily binding to the δ opioid receptor. Thus, only a limited number of analogs having 3′-*S*-hydroxypyrrolidine were prepared.

Replacement of one of the methyl groups of **2b** with a bulky group such as benzyl (**2d**) had no significant effect on the binding affinities to either μ or κ opioid receptors. Again, the primary effect was on the reduction in affinity for the δ opioid receptor. Similarly, no changes in the chimeric receptor affinities were observed, indicating available space in the binding pocket for further structural modifications at this position. The cyclic amine derivatives of these sulfamoyl compounds, such as **2e**, bound to the opioid receptors with affinities that were comparable to dynorphin A bound (Table 1). The affinities for the chimeric receptors 3 and 4, however, were increased, yielding K_i values of 1.5 nM and 1.2 nM, respectively, compared to acyclic amines. Similarly the corresponding morpholine analog **2i** bound to chimeric receptors 3 and 4 with K_i values of 1.3 and 2.2 nM. As noted above, the corresponding 3′-*S*-hydroxypyrrolidine derivatives of these cyclic amines demonstrated less desirable receptor binding profiles. The decrease in binding affinities for chimeric receptors was observed for the *N*-methyl-piperazine analog (**2g**, Table 1) and the corresponding 3′-*S*-hydroxypyrrolidine derivatives (**2h**, Table 1), indicating the sensitivity of the receptors to those of the basic functionality in this part of the molecule. The chimeric receptor affinities of compounds having the 4,5-methylenedioxy group (**2k**–**2n**, Table 1) were comparable to the dimethoxy analogs.

In conclusion, compounds **2e** and **2i** have been identified as small molecules having in vitro profile comparable to that of dynorphin A. Future studies will be to evaluate these compounds in antinociceptive models and further optimization for chimeric receptor affinities will be undertaken.

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