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Amino acid conjugates as κ opioid receptor agonists

Virendra Kumar,* Deqi Guo, Jeffrey D. Daubert, Joel A. Cassel, Robert N. DeHaven, Erik Mansson, Diane L. DeHaven-Hudkins and Alan L. Maycock

Adolor Corporation, 700 Pennsylvania Drive, Exton, PA 19341, USA

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Abstract—A novel series of kappa (κ) opioid receptor agonists were synthesized by incorporating the key structural features of known κ opioid agonists while replacing the aryl acetamide portion with substituted amino acid conjugates. Compounds **3j** (K_i = 6.7 nM), **3k** (K_i = 3.6 nM), **3l** (K_i = 4.6 nM), **3m** (K_i = 0.83 nM) and **3o** (K_i = 2 nM) possessed potent affinities for the κ opioid receptor in vitro with reasonable selectivity over other opioid receptors.

During the past decade efforts have been devoted to the investigation of kappa (κ) opioid receptor agonists as analgesics lacking side effects such as sedation, dysphoria and diuresis.[1–3] A number of groups have taken diversified approaches to achieve this goal, and the key structural features or 'primary pharmacophore' common to the aryl acetamide series of κ opioid receptor agonists that have emerged, are summarized in Figure 1.[3–5] The amide and tertiary amine nitrogens are separated by a two-carbon chain and acetamides are formed from aryl acetic acids, illustrated by ICI 199441 (**1**). Most of the efforts in the literature have focused on modification of the substituents at these two-carbon chains. The acetamide fragment of aryl acetamides was not altered except for aryl and heteroaryl groups since it was assumed that any substantial changes in this linkage would reduce agonist activity at the κ opioid receptor.[1,3,6] However, thiourea derivatives, bearing only the diamine segment of the κ opioid agonist 'primary pharmacophore' and represented by compound **2**, not only retain κ opioid receptor activity but also selectivity over other opioid receptors.[6,7] This finding provided avenues for the modification of the acetamide portion of κ opioid receptor agonists.

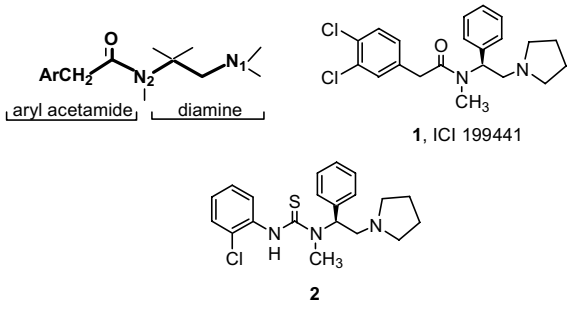

Figure 1.

In a continuing effort of our research group to develop new classes of κ opioid receptor agonists that lack the above mentioned central nervous system (CNS) liabilities, we have synthesized an exploratory series of amino acid conjugates of general structure **3** (Fig. 2). The amino acid derivatives were selected to decrease the lipophilic character of centrally acting κ opioid agonists such as ICI 199441 (**1**), thus rendering compounds with limited access to the CNS.[8,9] These compounds were prepared by incorporating the 'primary pharmacophore'

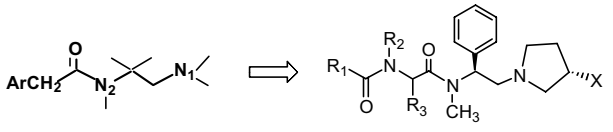

Figure 2.

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* Corresponding author. Tel.: +1 484 595 1060; fax: +1 484 595 1573; e-mail: vkumar@adolor.com

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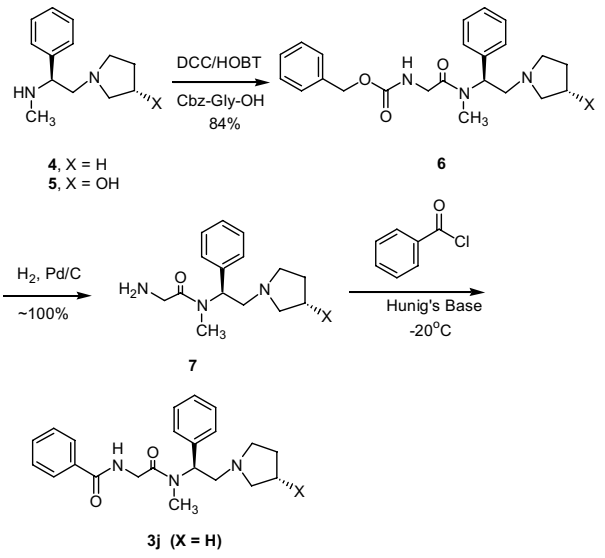
common to known κ opioid agonists but the aryl acetamide portion was replaced with substituted amino acid conjugates. The initial goal of this research was to establish potent κ opioid receptor affinity, followed by receptor selectivity and an improvement in the peripheral index in vivo as a means of limiting access to the CNS.[8,10] We describe here the syntheses and in vitro binding affinities of these novel κ opioid agonists.

The synthesis of amino acid conjugates utilized the previously reported diamine intermediates, (*S*)-**4**[11] and (*S*,*S*)-**5**.[12] A general synthetic methodology was developed for the preparation of these compounds and is described in Scheme 1. Condensation of diamines with N-carbobenzyloxy (Cbz)-protected amino acids gave the intermediate **6**. Removal of the Cbz protecting group by catalytic hydrogenation followed by acylation of the free amine (**7**) with aliphatic or aromatic acid chlorides in the presence of Hunig's base gave the desired compounds, for example, compound **2j** (R^1 = Ph, R^2 = H, R^3 = H and X = H). This method was used to prepare other amino acid conjugates of diamines **4** and **5** (Table 1).

The new compounds were evaluated in vitro for opioid receptor binding affinity. Determinations of affinities for κ, mu (μ) and delta (δ) opioid receptors were conducted by displacement of bound [^{3}H]diprenorphine from membranes prepared from cells expressing the cloned human opioid receptors. Methodological details have been previously described in the literature.[13,14] The selectivity for the κ opioid receptor versus the μ and δ opioid receptors was defined by the ratio of the K_i values and the [^{35}S]GTPγS functional binding assay[15] was used to determine κ agonist activity of the lead compounds (Table 1).

Condensation of (*R*,*S*)-*N*-Cbz-phenylglycine with diamine **4** resulted in two diastereomeric compounds **3b** and **3c**, which were readily separated. The absolute stereochemistry of these compounds was not assigned. The



Scheme 1.

diastereomer 1 (**3b**) bound to the κ opioid receptor with an affinity sixfold higher than the diastereomer 2 (**3c**) with K_i values of 138 and 856 nM, respectively, indicating the chiral preference of the receptor in this part of the molecule. The corresponding free amino compound **3a**, as a mixture of diastereomers was, only half as active in the binding assay with a K_i value of 258 nM. The diastereomers of the amine **3a** were not separated, but it could be anticipated that one of the diastereomers of the free amine would have a comparable binding potency to **3b**. Extension of the chain at R^3 with a (*S*)-benzyl group (**3d**, K_i = 150 nM) or substitution with a smaller group at R^1 (**3e**, K_i = 178 nM) did not lead to compounds with improved κ opioid receptor affinity. Replacement of the benzyloxy group at R^1 with a phenyl group gave the benzoyl compound **3f**, which showed a modest improvement in binding affinity with a K_i value of 99 nM.

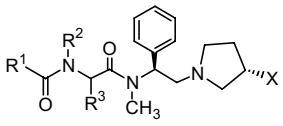
It is evident that retaining a phenyl or benzyl group at the R^3 position of structure **3** would not allow for an increase in κ opioid receptor affinity, probably because of the intolerance for sterically bulky groups by the receptor in this position. To circumvent this problem, we prepared the next series of compounds with no substitutions at this position (R^3 = H).

Therefore, compound **3g** containing a benzyloxy group at the R^1 position demonstrated an increased affinity for the κ opioid receptor (K_i = 84 nM) when compared to compound **3b** (Table 1). However, a significant change in the κ binding affinity was observed when the benzyloxy group at R^1 was replaced with a benzyl group. The K_i value (16.3 nM) at the κ opioid receptor of the resulting compound, **3i**, was 5- to 10-fold lower than previous compounds. Shortening the chain further to a phenyl group gave compound **3j** with a K_i value of 6.7 nM for the κ opioid receptor and 61- and 50-fold selectivity, respectively, for κ over μ and δ opioid receptors.

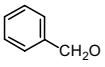
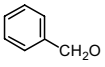
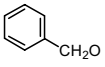
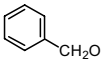
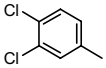
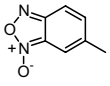
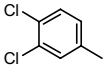
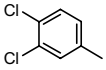
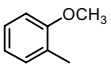
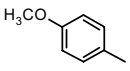
Enhancement in the binding affinity of κ opioid receptor agonists has been reported with substitutions on the aromatic rings of aryl acetamides.[1,3] Therefore, the replacement of phenyl of **3j** with 3,4-di-chlorophenyl (**3k**) and benzofuroxan (**3l**) furnished compounds, which exhibited potent κ opioid receptor affinity with K_i values of 3.6 and 4.6 nM, respectively. It has been shown in the literature that substitution of the pyrrolidine with 3-(*S*)-hydroxypyrrolidine often resulted in compounds with improved κ opioid receptor binding affinity,[1,3] thus compound **3m** showed a fourfold higher affinity (K_i = 0.83 nM) for the κ opioid receptor than **3k**. However, the κ opioid receptor selectivity of **3m** over the δ opioid receptor was somewhat compromised. A limited number of compounds, **3j–o**, were assessed in [^{35}S]GTPγS binding assay and were full agonists at the κ opioid receptor (Table 1).

A very limited structure–activity relationship was explored around the lead compound **3m**. Substitution of a methyl group at the R^2 position of **3m** resulted in compound **3n**, which had not only a 24-fold weaker affinity

Table 1. In vitro binding to cloned human opioid receptors



3
a-p

Compound	R^1	R^2	R^3	X	Yield[a] (%)	K_i (nM) [^{3}H]diprenorphine κ (95% CI)	Selectivity for κ over μ (fold)	Selectivity for κ over δ (fold)	[^{35}S]GTPγS EC_{50} (nM)[b]
3a	—	H_2	*(R,S)* 	H	71	258 (104–674)	146	>100	ND
3b (Diastereomer 1)		H	*(R or S)* 	H	11	138 (27–690)	22	27	ND
3c (Diastereomer 2)		H	*(R or S)*	H	11	856 (401–1826)	4	>100	ND
3d		H	*(S)* 	H	78	150 (86–259)	13	5	ND
3e	OCH_3	H	*(S)* 	H	57	178 (83–382)	>100	>100	ND
3f		H	*(S)* 	H	73	99 (39–252)	>100	13	ND
3g		H	H	H	84	84 (31–223)	11	2	ND
3i		H	H	H	44	16.3 (14–19)	>100	49	ND
3j		H	H	H	68	6.7 (2.7–17)	61	50	3.85
3k		H	H	H	59	3.6 (1.4–9.2)	34	24	2.25
3l		H	H	H	58	4.6 (1.5–14)	55	11	4.37
3m		H	H	OH	30	0.83 (0.69–0.99)	160	11	0.76
3n		CH_3	H	OH	75	20 (17–24)	>100	1.5	41.42
3o		H	H	OH	34	2 (1.9–2.2)	134	25	1.98
3p		H	H	OH	48	48 (39–57)	39	>100	ND
ICI 199441 (**1**)						0.043 (0.027–0.21)	1241	567	

ND = not determined.

[a] The compounds were isolated as either hydrochloride or methane sulfonic acid salts and characterized by MS, NMR, mp and elemental analyses.

[b] Geometric means computed from two to three separate determinations.

for the κ opioid receptor but a further lowering of selectivity with respect to the δ opioid receptor. The κ opioid receptor affinity was also reduced when the 3,4-dichloro groups of **3m** were replaced with a methoxy in compounds **3o** and **3p**. But, the 2-methoxy compound, **3o**, was 24 times more potent in receptor binding than the corresponding 4-methoxy, **3p**, indicating receptor sensitivity to steric bulk rather than electronic effects.

In summary, we were successful in replacing the aryl acetamide portion of κ opioid receptor agonists, such as ICI 199441 (**1**), with amino acid conjugates, resulting in compounds with high affinity for the κ opioid receptor. Compounds **3j** (K_i = 6.7 nM), **3k** (K_i = 3.6 nM), **3l** (K_i = 4.6 nM), **3m** (K_i = 0.83 nM) and **3o** (K_i = 2 nM) emerged as leads of this series and were full agonist at the κ opioid receptor. Even though most of the new compounds were less potent in κ opioid receptor binding affinity and were less selective to other opioid receptors when compared to ICI 199441 (**1**), these compounds may have an advantage by limited access to the CNS due to reduce lipophilicity. Further synthetic efforts will be directed at improving the opioid receptor selectivity and extending our observations to in vivo evaluation of these compounds in various antinociceptive models and assessing the CNS liabilities.

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