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Azepinone as a conformational constraint in the design of κ-opioid receptor agonists

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Abstract—A new class of κ-opioid receptor agonists is described. The design of these agents was based upon energy minimization and structural overlay studies of the generic azepin-2-one structure **3** with the crystal structure of arylacetamide κ agonist **1**, ICI 199441. The most active compound identified was ligand **4a** (K_i = 0.34 nM), which demonstrated potent antinociceptive activity after oral administration in rodents.

κ-Opioid receptors are one of three well-characterized opioid receptor types.[1] Centrally-acting κ-receptor agonists, for example, arylacetamides **1** (ICI 199441) and **2** (U50,488) display potent antinociceptive activity in vivo.[2] κ Agonists initially held great promise as analgesics putatively free of respiratory depression, constipation, and other undesirable side effects observed with clinically administered μ-opioid receptor agonists. However, early clinical trial data revealed that κ-receptor agonists were accompanied with their own set of central nervous system (CNS) liabilities, namely dysphoria and diuresis.[3] These side effects prevented their continued clinical development and commercialization as analgesics. Recently, κ-opioid receptors were identified in tissues outside the CNS.[4] It has been hypothesized that peripherally acting κ agonists may retain significant antinociceptive activity without their associated classical CNS side effects.[5] This hypothesis was supported in a recent clinical investigation of chronic pancreatitis patients that were treated with ADL 10-0101, a peripherally acting κ agonist and experienced pain relief without dysphoria.[6]

As part of an ongoing program aimed at discovering agonists that target peripheral κ receptors,[7] a novel series of constrained analogs **3** of ICI 199441 (**1**)[8] was synthesized and evaluated as potential κ agonists. The rationale for the selection of the azepinone (seven-membered ring) as the cyclic constraint was based upon energy minimization and structural overlay studies of **4a**,**b** with the crystal structure of **1**.[9] As illustrated in Figure 1, diastereomers **4a** (*syn*-isomer) and **4b** (*anti*-isomer) reveal a high degree of overlap with the backbone conformation of **1**, suggesting such compounds may be biologically active. This analysis presupposes that the crystal structure reflects, at least to some extent, the receptor bound bioactive conformation of **1**. It was not known a priori, which diastereomer (if either) would be the more active conformer. The details of the synthesis of **4a**,**b** and related analogs and their in vitro binding affinity and selectivity against the human κ-opioid receptor is the subject of this letter.[10]

The synthesis of the azepin-2-ones **4a**,**b** is presented in Scheme 1. Fukuyama sulfonylation (standard Schotten–Baumann conditions) of commercially available *S*-(+)-phenylglycine **5** followed by DCC coupling with pyrrolidine afforded sulfonamide **6** in 40% yield. This material was alkylated with a slight excess of allyl bromide using potassium carbonate as base in DMF

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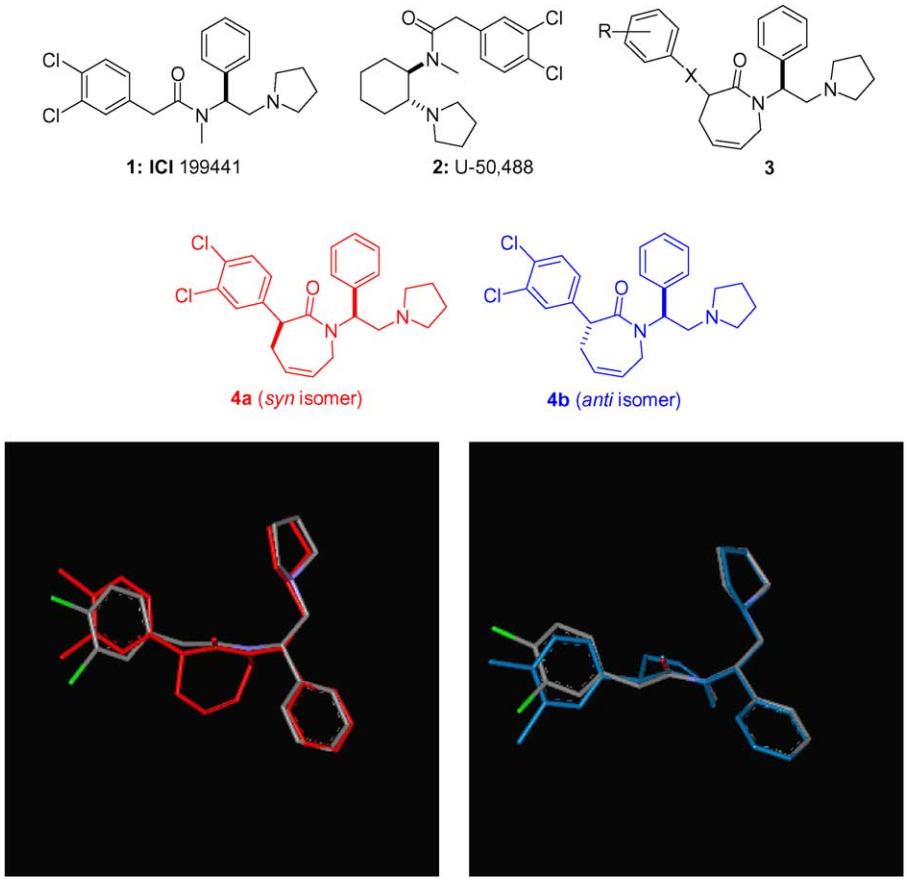
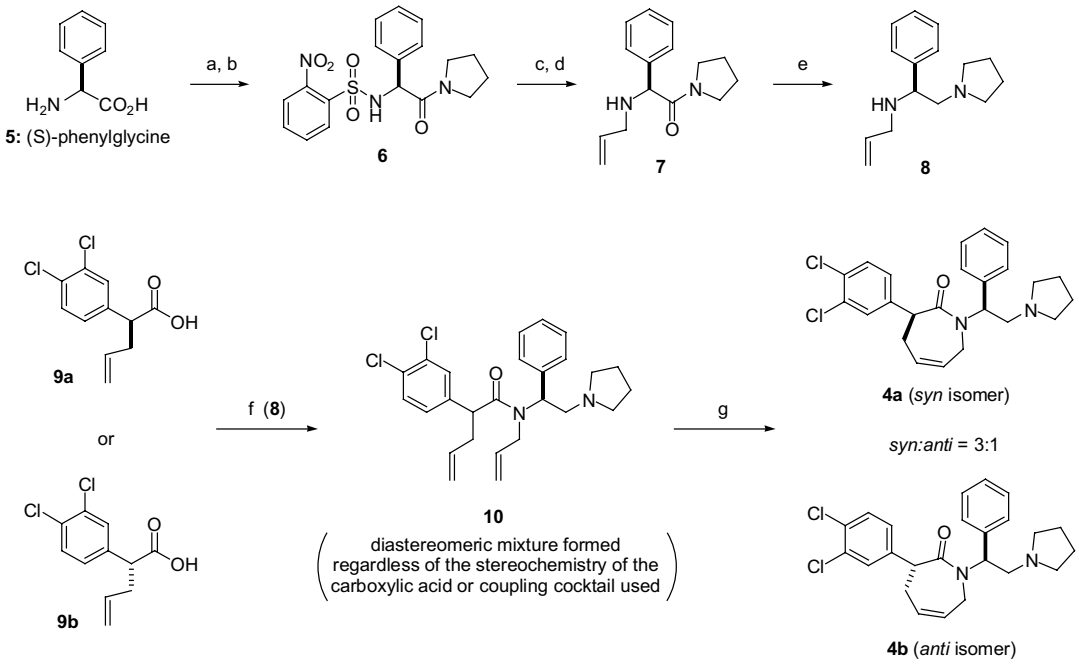
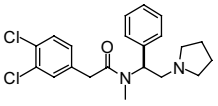
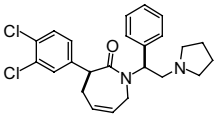
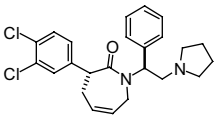
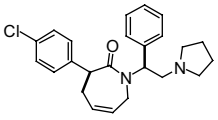
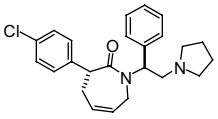
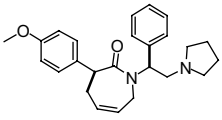
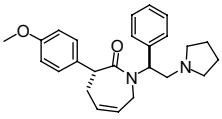
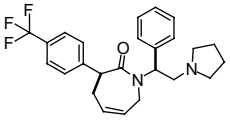
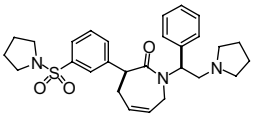
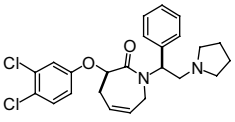


Figure 1. Overlays of **4a** and **4b** with **1** showing nearly coincident backbone conformations.



Scheme 1. Preparation of azepinone-based κ-opioid receptor agonists **4a**,**b**. Reagents and conditions: (a) 1 M aq NaOH (1.1 equiv), **5** (1.0 equiv), 25 °C, 30 min; then 6 M aq NaOH, 1.05 equiv (2-NO_2)$PhSO_2Cl$, THF, 0–25 °C, 20 h (85%); (b) sulfonamide intermediate (1 equiv), HOBT (1.1 equiv), pyrrolidine (1.1 equiv), THF; then 1 M solution DCC in DCM (1.1 equiv), 0–25 °C, 16 h (91%); (c) **6** (1 equiv), K_2CO_3 (2 equiv), allyl-Br (1.3 equiv), DMF, 25 °C, 16 h (96%); (d) allyl sulfonamide intermediate (1 equiv), K_2CO_3 (3 equiv), PhSH (1.4 equiv), DMF, 25 °C, 16 h (85%); (e) **7** (1 equiv), 1 M solution LAH in THF (1 equiv), 0–25 °C, 16 h (86%); (f) **8** (1 equiv), **9a** (or **9b** or racemate; 1 equiv), 2-Cl-1-methylpyridinium iodide (2 equiv), TEA (3 equiv), DCM, 25 °C, 44 h (93%); (g) **10** (1 equiv), DCM, 1 M HCl in Et_2O (4 equiv), 25 °C, 30 min; solvents removed in vacuo and HCl salt dried in vacuo, 60 °C, 3 h; then Grubb's catalyst (0.05 equiv), DCM, 40 °C, 16 h (**4a**: 30%; **4b**: 13%).

Table 1. Azepinone in vitro binding and selectivity data[17]

Compound	Structure	K_i (nM)		
		κ	μ	δ
1		0.043	53	24
4a		0.34	1000	370
4b		17	[a]	[a]
11a		2.0	[a]	[a]
11b		35	[a]	[a]
12a		22	[a]	[a]
12b		800	[a]	[a]
13		0.72	[a]	1000
14		1900	[a]	[a]
15		2.6	[a]	[a]

For the assay description, see Ref. 19. K_i, values are the geometric means computed from at least three separate determinations.

[a] Inhibition (<60%) at 10 μM screening concentration; estimated K_i > 5000 nM.

at room temperature and subsequently deprotected with benzenethiol to produce amide **7**. Treatment of **7** with LAH in THF gave the key chiral diamine **8** (~65% overall yield from **6**).[11] Coupling of diamine **8** using Mukaiyama's reagent to the optically pure 2-(3′,4′-dichlorophenyl)pent-4-enoic acid **9a** (Pharmacore, Inc.) gave the penultimate diene intermediate **10**. Ring closing metathesis (RCM) of **10** with either Grubbs' first or second generation catalysts proved unsuccessful until the tertiary pyrrolidine amine nitrogen was converted to its hydrochloride salt. Most surprisingly, a 3:1 mixture of ring closed products **4a** and **4b** (vida infra) was obtained in 60% yield.[12a] These products were separated by silica gel chromatography and gave very distinct ^{1}H NMR spectra.[12b] The chemistry was repeated using antipode **9b**. However, this also gave the identical 3:1 ratio of cyclized products **4a**,**b**. Apparently, racemization of **9a** and **9b** occurred before coupling with the hindered chiral diamine **8**.[13] This effect is reminiscent of that seen in kinetic resolutions.[14] A wide range of coupling reagents and reaction conditions were investigated including DCC, oxalyl chloride, Mukaiyama's reagent, TBTU, PyBrOP, and Goodman's DEPBT;[15] however, in every case a 3:1 ratio of RCM products was obtained. Since racemization could not be circumvented, all subsequent couplings were carried out using the requisite *racemic* 2-aryl-pent-4-enoic acids or 2-aryloxy-pent-4-enoic acid[16] and Mukaiyama's reagent to generate a separable 3:1 mixture of the azepin-2-ones **11**–**15** (Table 1). With respect to assigning relative stereochemistry, the X-ray crystallographic analysis of a major RCM product was secured (compound **14**; Fig. 2), confirming the *syn*-stereochemistry.[12] In conjunction with ^{1}H NMR spectral analysis that clearly distinguished between the *syn*- and *anti*-diastereomers, the *syn*-stereochemistry was confidently assigned to the major product isolated from all the RCM reactions.

The results of the in vitro binding assay for compounds **4a**,**b**, **11**–**15** and reference compound **1** are presented in Table 1. Azepin-2-one **4a** possessed subnanomolar affinity for the κ-opioid receptor (K_i = 0.3 nM), comparing favorably to the acyclic arylacetamide **1** (K_i = 01.04 nM).[17] This result supports the hypothesis put forth by the modeling overlay studies. The μ/κ selectivity for **4a** and **1** are likewise comparable with each being on the order of ca. 1000-fold. The *anti*-diastereomer **4b** was 50-fold less potent than the *syn*-diastereomer **4a**. This trend, *syn*- more active than the *anti*-diastereomer, was consistently observed throughout the azepin-2-one series (compare **11a** to **11b** and **12a** to **12b**) and may arise from an unfavorable non-bonded interaction between the

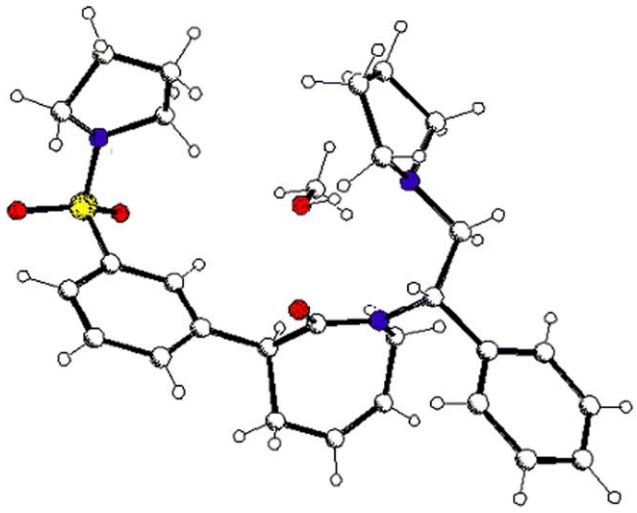


Figure 2. Crystal structure of **14** showing *syn*-stereochemistry.

hydrocarbon ring atoms in the *anti*- versus the *syn*-ligands in the active site of the receptor. Another discernible SAR trend was the preference for lipophilic substituents present in the aromatic group attached to the azepinone ring. For example, **4a** (3,4-dichlorophenyl), **11a** (4-chlorophenyl), and **13** (4-trifluoromethylphenyl) possessed potent κ binding ranging from 0.34 to 2 nM. This is in contrast to the more hydrophilic aryl ring substituents, for example, **12a** (4-methoxyphenyl) and **14** (3-pyrollidinylsulfonylphenyl) that were 10- to 1000-fold less active. This same preference toward lipophilic aryl ring substitution was also observed in the acyclic series.[7] Lastly, the 3,4-dichlorophenoxy-substituted azepin-2-one **15** also demonstrated significant potency and high selectivity for the κ receptor (K_i = 2.6 nM, >10,000 selectivity versus μ and δ). This again underscores the preference for a lipophilic aryl substituent and indicates that the receptor's binding pocket is able to accommodate an extended aryl ring unit.[18]

In further studies, compound **4a** was evaluated in several in vivo animal models of antinociception (Table 2). Compound **4a** displayed potent antinociceptive activity in the mouse and rat after oral administration. The ED_{50} values of inhibition of acetic acid-induced writhing in mice and inhibition of formalin-induced flinching in rats after oral administration were 1.6 and 2.7 mg/kg, respectively. There was a clear separation between antinociceptive activity and sedation and ataxia in both the mice and rats following oral administration of **4a**, with a 30-fold separation between the ED_{50} values for sedation and the ED_{50} values for antinociception. Separation of these in vivo activities is one indicator of peripheral selectivity, suggesting that the azepinone class of κ agonists may represent a good starting point for the further design and optimization of peripherally acting agents.

In summary, the azepin-2-one was hypothesized as a conformational constraint for κ-opioid ligands based on near coincident backbone overlay of **4a**,**b** with the crystal structure of the archetypal arylacetamide κ agonist **1**. The hypothesis was confirmed upon the synthesis and evaluation of the series **4a**,**b**, and **11**–**15**, demonstrating potent, selective κ-receptor binding affinity. Structure–activity relationships in vitro clearly indicated a predilection for *syn*- versus *anti*-stereochemistry and a preference for lipophilic aromatic substituents. κ Agonist **4a** (K_i = 0.3 nM) was orally active in rodent models of antinociception and may be regarded as possessing some measure of peripheral selectivity as evidenced by the ca. 30-fold separation observed between antinociception and sedation and ataxia.

Table 2. Antinociceptive and sedative effects of **4a** upon oral administration

Mouse		Rat	
Acetic acid-induced writhing (ED_{50} (mg/kg))	Sedation (ED_{50} (mg/kg))	Formalin-induced flinching (ED_{50} (mg/kg))	Rotarod (% inhibition)[c]
1.6 (1.1–2.4)[a,b]	48 (25.3–90.7)	2.7 (1.2–4.5)	54% @ 100 mg/kg

For the assay descriptions, see Ref. 20.

[a] Values in parentheses are the 95% confidence intervals.

[b] The oral ED_{50} of **1** in writhing = 0.17 mg/kg (0.05–0.52) and the platform sedation ED_{50} = 7.5 mg/kg (3.6–18.3).

[c] Value represents the percent decrease in rotarod performance after drug treatment based on the comparison of baseline and post-treatment rotarod latencies.

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11. To ensure chiral integrity, diamine **8** was acylated with 2-naphthoyl chloride. The corresponding amide was then subjected to chiral HPLC analysis (Chiralpak AD) and was determined to be 96% ee, indicating that no significant racemization had occurred at the carbon atom bearing the phenyl ring.
12. (a) All new compounds showed physical and spectroscopic properties consistent with their structures; (b) For **4a**: ^{1}H NMR $CDCl_3$ (400 MHz) δ 11.95–11.89 (1H, br s), 7.71–7.70 (1H, d, J = 1.8 Hz), 7.61–7.59 (1H, dd, J = 1.8, 8.3 Hz), 7.42–7.40 (1H, d, J = 8.3 Hz), 7.39–7.34 (3H, m), 7.27–7.23 (2H, m), 6.42–6.38 (1H, dd, J = 3.0, 12.5 Hz), 5.60–5.55 (1H, m), 5.28–5.20 (2H, m), 4.96–4.85 (1H, dd, J = 3.3, 13.6 Hz), 4.13–4.05 (1H, m), 3.96–3.88 (2H, m), 3.24–3.14 (2H, m), 2.95–2.68 (3H, m), 2.41–2.36 (1H, m), 2.35–2.29 (1H, m), 2.24–2.14 (1H, m), 2.31–1.95 (2H, m). For **4b**: ^{1}H NMR $CDCl_3$ (400 MHz) δ 12.40–12.34 (1H, s), 7.48–7.46 (2H, m), 7.42–7.35 (5H, m), 7.19–7.16 (1H, dd, J = 1.9, 8.3 Hz), 5.77–5.67 (2H, m), 5.39–5.36 (1H, t, J = 6.4 Hz), 4.82–4.78 (1H, d, J = 8.1 Hz), 4.42–4.38 (1H, dd, J = 3.4, 12.8 Hz), 4.28–4.23 (1H, m), 3.80–3.59

(4H, m), 2.91–2.81 (1H, m), 2.70–2.58 (2H, m), 2.45–2.39 (1H, dd, $J = 3.3$, 18.4 Hz), 2.24–2.12 (2H, m), 2.04–1.92 (2H, m). For **14**: tan solid; mp 97–99 °C (methanol); ^{1}H NMR $CDCl_3$ (400 MHz) δ 7.75 (1H, br s), 7.73 (1H, d, $J = 7.8$ Hz), 7.64 (1H, d, $J = 7.6$ Hz), 7.52 (1H, t, $J = 7.8$ Hz), 7.35–7.25 (5H, m), 6.04 (1H, br t, $J = 7.6$ Hz), 5.68 (1H, m), 5.43 (1H, m), 4.49 (1H, d, $J = 11.7$ Hz), 4.25 (1H, d, $J = 17.4$ Hz), 3.36 (1H, dd, $J = 7.6$, 17.40 Hz), 3.29–3.23 (4H, m), 3.03 (1H, t, $J = 10.7$ Hz), 2.84 (2H, m), 2.63 (2H, s), 2.50 (3H, m), 1.77–1.71 (8H, m); LCMS *m/z* 494.3 (M+1).

13. The ^{1}H NMRs of the intermediate dienes were rather complex, the presence of rotomers notwithstanding, indicating a mixture of diastereomers. In one case, the mixture of dienes was separated and the major diene diastereomer so obtained was subjected to the ring closing metathesis reaction, affording exclusively the *syn*-product (unpublished observation); hence, the metathesis reaction was racemization free.
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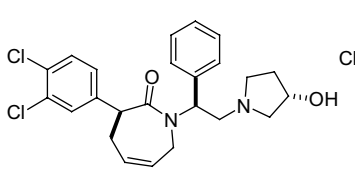
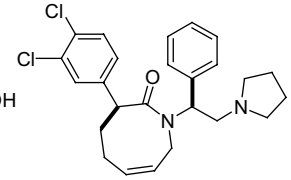
17. The azepin-2-ones were full agonists as determined in a [^{35}S]GTPγS functional binding assay (see Ref. 19). For **4a**: $EC_{50} = 10$ nM; for **4b**: $EC_{50} = 50$ nM.
18. (a) The 3-hydroxy analog **18** of **4a** was prepared. The hydroxy group is known to enhance receptor affinity for κ: Gottschlich, R.; Ackermann, K. A.; Barber, A.; Bartoszyk, G. D.; Greiner, H. E. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 677–682; (b) The RCM reaction proved to be a versatile reaction and was used to prepare higher order lactams, for example, **19**



18: K_i (κ) = 0.2 nM
(>100x selective versus μ and δ)



19: K_i (κ) = 12 nM
(>1000x selective versus μ and δ)

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20. In vivo test methods: (a) *Acetic acid-induced writhing*: Mice were treated with vehicle or compound **4a** and 15 min later injected with 0.6% acetic acid intraperitoneally. Five minutes after the administration of acetic acid, the number of writhes was counted for a 10 min period; (b) Horan, P.; de Costa, B. R.; Rice, K. C.; Porreca, F. J. *J. Pharmacol. Exp. Ther.* **1991**, *257*, 1154–1161. Briefly, mice were placed on a raised plastic platform ($11 \times 7.5 \times 3$ cm) and the latency to completely step off the platform was determined before (baseline sedation latency) and 30 min after drug treatment. Mice with baseline sedation latencies of 15 s or greater were not used. After the drug treatment, a 30 s cut-off was used for the latency to step off the platform; (c) *Formalin-induced flinching*: Late phase formalin-induced flinching was determined using the method of DeHaven-Hudkins, D. L.; Cortes Burgos, L.; Cassel, J. A.; Daubert, J. D.; DeHaven, R. N.; Mansson, E.; Nagasaka, H.; Yu, G.; Yaksh, T. *J. Pharmacol. Exp. Ther.* **1999**, *289*, 494–502.