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OPTICALLY PURE (-)-4-[(N-ALLYL-3-METHYL-4-PIPERIDINYL)PHENYLAMINO]-*N,N*-DIETHYLBENZAMIDE DISPLAYS SELECTIVE BINDING AND FULL AGONIST ACTIVITY FOR THE δ OPIOID RECEPTOR

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Abstract: The optical isomers of 4-[(N-allyl-3-methyl-4-piperidinyl)phenylamino]-*N,N*-diethylbenzamide (**3**) have been prepared and tested in both binding and functional assays. The data show that (-)-**3** is responsible for the δ opioid activity demonstrated by the racemic material. This compound displays a binding affinity of 5.5 nM for the δ opioid receptor as well as a 470-fold δ versus μ selectivity. Importantly, (-)-**3** is a full agonist at the δ receptor in comparison with SNC-80 (**2**). Taken together, the data suggest that (-)-**3** behaves more like the prototypical δ agonists, BW373U86 or SNC-80, and less like the peptidomimetic compound SL-3111 (**5**).

Over the past decade, much effort has been expended to develop opioid analgesics that operate via the δ opioid receptor.[1] The principle reason driving this search is the undesirable side-effect profile associated with μ opioid analgesics that include addiction, respiratory depression, and a negative impact on gut motility. The discovery of the δ opioid receptor selective compounds, BW373U86 (**1**)[2] and SNC-80 (**2**),[3] represented a major breakthrough in this area. The atypical structural motif exhibited by these compounds relative to more classical opioid ligands[4] opened the door for additional research efforts based upon this novel scaffold. Transposition of the internal nitrogen atom in compounds **1** or **2** with the benzylic carbon, yielding compounds of structure **3**, converts the piperazine into a piperidine, a structural unit more familiar to opioid alkaloids. We recently reported that **3**, as a racemic mixture, showed good binding affinity and selectivity towards the δ opioid receptor but demonstrated a lack of full efficacy relative to **1** or **2**.[5] Though its binding data and structure suggested that (±)-**3** might behave like **2**, its lack of maximal stimulation implied that (±)-**3** might be more like the peptide agonist cyclic [penacillamine,[2] penacillamine[5]]enkephalin (DPDPE) or its non-peptide surrogate SL-3111 (**5**).[6] Since the very different pharmacology displayed by these compounds relative to **2** has been attributed to occupation of different receptor domains,[5,7] it is important to establish to which group the nitrogen transposed compounds **3** belong. In this communication, we present the syntheses of (+)- and (-)-**3** and report their opioid binding and functional properties. The results show that (-)-**3** possesses a significant increase in δ opioid potency and selectivity relative to (±)-**3**, with agonist efficacy very close to that of the full agonist SNC-80 (**2**). Thus, the opioid properties of (-)-**3** are more like those of compounds **1** and **2** than those of SL-3111 (**5**).

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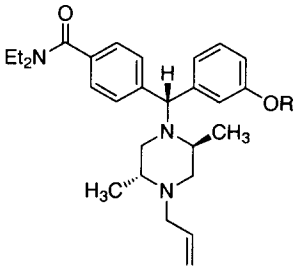
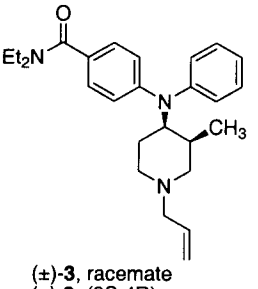
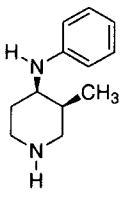


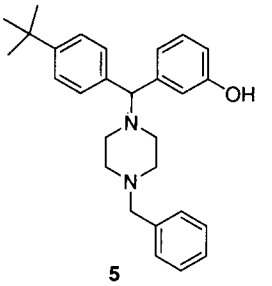
1, R = H
2, R = CH_3



(±)-**3**, racemate
(–)-**3**, (3S,4R)
(+)-**3**, (3R,4S)



(–)-**4**, (3S,4R)
(+)-**4**, (3R,4S)



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Results and Discussion

Compounds (-)-**3** and (+)-**3** were synthesized in optically pure form using a procedure analogous to that used for (±)-**3**[5] starting from optically pure piperidines (-)-**4** and (+)-**4** which were readily available via the methods of Rice[8] and Janssen.[9] Since the absolute stereochemistry of (+)- and (-)-**4** has been established, and the stereocenters present in (-)-**4** and (+)-**4** are not altered during their transformation to (-)-**3** and (+)-**3**, the absolute stereochemistry of (-)-**3** and (+)-**3** is also established. The biologically active isomer (-)-**3** is the (3S,4R)-isomer and has the structure depicted.

The radioligand binding data for (±)-**3** and optically pure compounds (-)-**3** and (+)-**3** along with comparative data for BW373U86 (**1**) and SNC-80 (**2**) are shown in Table 1. Comparison of the binding data for

Table 1. Radioligand Binding Results at the μ, δ, and κ Opioid Receptors for Optically Pure 4-[(N-Allyl-3-methyl-4-piperidinyl)phenylamino]-*N,N*-diethylbenzamides

	K_i (nM±SD)			
Compd	μ [^{3}H]DAMGO[a]	δ [^{3}H]DADLE[b]	κ [^{3}H]U69,593[c]	μ/δ
1, BW373U86[d]	36 ± 3.4	0.91 ± 0.05	NA	40
2, SNC-80	1614 ± 131	1.57.± 0.19	3535 ± 184.1	1030
3, racemic	1212 ± 132	11.9 ± 0.9	3284 ± 299	102
(+)-**3**, 3R,4S	>10000	139 ± 11.4	3722 ± 556	>72
(-)-**3**, 3S,4R	2623 ± 307	5.58 ± 0.31	1448 ± 196	470

[a] [^{3}H]DAMGO [(D-Ala^2,MePhe^4,Gly-ol^5)enkephalin]. Tritiated ligand selective for μ opioid receptor. [b] [^{3}H]-DADLE [(D-Ala^2,D-Leu^5)enkephalin]. Tritiated ligand selective for δ opioid receptor. [c] [^{3}H]U69,593 {[^{3}H]-(5α,7α,8β)-(-)-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]benzeneacetamide}. Tritiated ligand selective for κ opioid receptor. [d] Data taken from reference 3.

(±)-**3** and its optical isomers reveals that the high δ opioid binding affinity as well as the δ receptor selectivity of the racemic mixture is due to (-)-**3**. The (-)-isomer (-)-**3** is twice as potent as the racemic mixture (±)-**3**, whereas (+)-**3** is 12-fold less potent than (±)-**3** resulting in a 25-fold separation of binding affinity between the (-) and (+) enantiomers. In terms of opioid receptor selectivity, (-)-**3** demonstrates a nearly fivefold increase in δ versus μ selectivity compared with the racemic material. This increased δ selectivity is due in part to the increase in δ

receptor affinity and partly to a threefold decrease in affinity for the μ receptor. Relative to the racemic mixture or the (+)-**3** isomer, (-)-**3** shows a significantly improved δ opioid binding affinity and δ selectivity, a behavior common to BW373U86 as well as other opioid ligands.[1,2]

In terms of its observed δ opioid [^{35}S]GTPγS functional activity, the K_d for (-)-**3** is roughly half of that found for the racemic mixture as might be expected while the opposite enantiomer showed no stimulation of [^{35}S]GTPγS binding in the brain tissue preparation (Table 2). Compound (-)-**3** showed functional activity only in

Table 2. Functional K_d and E_{max} Values of DAMGO, SNC-80, U69,593, and the Enantiomers of 4-[(N-Allyl-3-methyl-4-piperidinyl)phenylamino]-*N*,*N*-diethylbenzamide Using GTPγS Binding Assays in Guinea Pig Caudate Membranes

	Unblocked Condition (nM ± Sd)	Blocked with 20 nM NTI[d]	Blocked with 6 nM nor-BNI[e]	Blocked with 6000 nM CTAP[f]
DAMGO,[a] K_d	592 ± 105	1850 ± 287	509 ± 111	
E_{max}	123 ± 6	124 ± 6	135 ± 7	No stimulation
SNC-80,[b] K_d	317 ± 54		629 ± 71	673 ± 108
E_{max}	142 ± 6	No stimulation	143 ± 4	131 ± 5
U69,593,[c] K_d	684 ± 74	1980 ± 269	4894 ± 2172	2142 ± 223
E_{max}	177 ± 5	178 ± 8	58 ± 11	167 ± 6
(±)-**3**, K_d	3500 ± 500	No stimulation	3722 ± 1094	4667 ± 1937
E_{max}	63 ± 5		60 ± 7	55 ± 9
(3S,4R)-(-)-**3**, K_d	1782 ± 459	No stimulation	2952 ± 440	3604 ± 837
E_{max}	126 ± 5		91 ± 5	85 ± 8
(3R,4S)-(+)-**3**, K_d	No stimulation			
DPDPE, K_d	577 ± 150			
E_{max}	51.4 ± 2.8			

[a] DAMGO [(D-Ala^2,MePhe^4,Gly-ol^5)enkephalin]. Agonist selective for μ opioid receptor. [b] SNC-80 ([(+)-4-[(αR)-α-(2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide). Agonist selective for δ opioid receptor. [c] U69,593 [(5α,7α,8β)-(-)-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]benzeneacetamide]. Agonist selective for κ opioid receptor. [d] Naltrindole (NTI). Antagonist selective for δ opioid receptor. [e] nor-Binaltorphimine (nor-BNI). Antagonist selective for κ opioid receptor. [f] CTAP. Antagonist selective for μ opioid receptor.

the absence of the δ receptor selective antagonist naltrindole as was observed for the racemic material implying that the observed stimulation arises from activation of δ receptors. Importantly, (-)-**3** displayed a much improved maximal stimulation relative to (±)-**3** or (+)-**3** with an E_{max} virtually indistinguishable from that displayed by the full agonist SNC-80 (**2**). Thus, the partial agonist character shown by the (+) and (-) mixture likely results from the presence of the inactive (+)-enantiomer in the mixture.

It has been suggested that the piperazines **1** and **2** interact with different domains of the δ receptor relative to DPDPE or SL-3111. As was discussed earlier, data obtained from testing of racemic **3** were not sufficient for

assigning their potential site of receptor interaction. However, comparison of the information obtained for (+)-**3** and (-)-**3** demonstrates that (-)-**3** does not behave like the peptide ligand or its small-molecule mimic. The principal difference observed from the available data is that (+)-**3** and (-)-**3** maintain distinct differences in activity between binding and functional assays relative to (±)-**3**. For example, (-)-**3** shows greater affinity and selectivity than (+)-**3**, as well as being more potent and selective than the racemate, while (+)-**3** is far less potent than (-)-**3**, and both less potent and less selective than the racemate. Particularly important to the present discussion is the observation that this same trend is preserved in the functional assay. However, as was recently disclosed for the peptidomimetic SL-3111 (**5**), this behavior is observed only in the binding assay. In the functional assay, the enantiomers of SL-3111 are both significantly less potent than the racemate, and neither of the enantiomers shows much difference in activity. Furthermore, both enantiomers lose their high δ selectivity. Taken together with the full efficacy observed for (-)-**3** and not for DPDPE, the data suggest a closer relationship between **1** or **2** and **3** compared with DPDPE or SL-3111.

Conclusions

The synthesis and evaluation of the optical isomers of 4-[(N-allyl-3-methyl-4-piperidinyl)phenylamino]-*N,N*-diethylbenzamide (**3**) in both opioid binding and [^{35}S]GTPγS functional assays indicate that one enantiomer (-)-**3** is responsible for the activity demonstrated by the racemic material. The high binding affinity, selectivity, and full agonist activity displayed by (-)-**3** compare favorably with that of the prototypical δ opioid ligands BW373U86 and SNC-80. A comparison of binding and functional behavior of (±)-**3** and its enantiomers to the same behavior for SL-3111 and its enantiomers suggests that (-)-**3** behaves more like the prototypical δ agonists BW373U86 or SNC-80 and less like the peptidomimetic compound SL-3111 (**5**).

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