Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 1281–1284

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

4-[(8-Alkyl-8-azabicyclo[3.2.1]octyl-3-yl)-3-arylanilino]-*N*,*N*-diethylbenzamides: High Affinity, Selective Ligands for the Delta Opioid Receptor Illustrate Factors Important to Antagonist Activity

James B. Thomas,[a] Robert N. Atkinson,[a] Richard B. Rothman,[b] Jason P. Burgess,[a] S. Wayne Mascarella,[a] Christina M. Dersch,[b] Heng Xu[b] and F. Ivy Carroll[a,*]

[a]*Chemistry and Life Sciences, Research Triangle Institute, Research Triangle Park, NC 27709, USA*
[b]*Clinical Psychopharmacology Section, NIDA Addiction Research Section, PO Box 5180, Building C, 5500 Nathan Shock Dr., Baltimore, MD 21224, USA*

Received 1 March 2000; accepted 31 March 2000

Abstract—The tropane derived compounds, 4-[(8-alkyl-8-azabicyclo[3.2.1]octyl-3-yl)-3-arylanilino]-*N*,*N*-diethylbenzamides (**5a–d**), were synthesized and found to have high affinity and selectivity for the δ receptor. Compounds **5a–d** are structurally similar to the full agonist (−)-RTI-5989-54 (**3**); yet, efficacy studies for compounds in this series (**5a–d**) reveal greatly diminished agonist activity as well as antagonism not found in piperidine-based compounds like **3**.

Introduction

Since the discovery of the nonpeptide δ opioid receptor selective compounds, BW373U86 (**1**)[1] and SNC-80 (**2**),[2] a great variety of new opioid ligands[3,4] have appeared utilizing the diethylbenzamide substructure to influence δ selectivity. This unique δ address moiety has been incorporated into both classical and nonclassical opioid ligands[5] in a search for analgesics possessing a reduced side-effect profile relative to the μ opioid analgesic morphine. Several groups, including our own,[3,6,7] have studied the effect on overall ligand activity produced by transposition of the internal nitrogen atom in compound **1** with the benzylic carbon as represented by compound **3**. These compounds were found to be δ receptor selective full agonists with the potency being directly affected by the 3-methyl group as well as its *cis* relative disposition to the 4-diaryl system. Recent reports of antagonist activity in compound **4** by Su et al.[8] demonstrated that alteration of the placement of the methyl groups in **1**, and consequently, the conformational disposition of the piperazine ring could convert agonists into antagonists.

Relative to the piperazine analogues **1** or **2**, the piperidine compound **3** is more conformationally flexible. The reports of the antagonistic activity of the more conformationally rigid **4** suggested that at least part of this effect could result from a decrease in conformational flexibility of **4**, and thus, antagonist activity might be elicited from piperidine compounds similar to **3** if the number of conformations available to **3** were restricted. To test this hypothesis, the tropane (bridged piperidine) derived compounds **5a–d** were prepared and tested in opioid binding and functional assays. In this communication we report that compounds of this nature demonstrate high degrees of δ selectivity and binding affinity as well as antagonist activity with potency modulated by the *N*-substituent.

Chemistry

Preparation of **5a–d** (Scheme 1) began with reductive amination of 3-tropanone (**6**) with 3-methoxyaniline using titanium (IV) isopropoxide[9] which gave **7** as a single epimer (*endo* based on the observations of Abdel-Magid et al.)[10] in 38% yield. This intermediate was then coupled to the butylated hydroxyanisole (BHA) ester of 4-fluorobenzoic acid to give **8** in 83% yield.[11] Removal of the BHA group was accomplished by *trans*-esterification

*Corresponding author. Tel.: +1-919–541-6679; fax: +1-919–541-8868; e-mail: fic@rti.org

0960-894X/00/$ - see front matter
PII: S0960-894X(00)00209-2

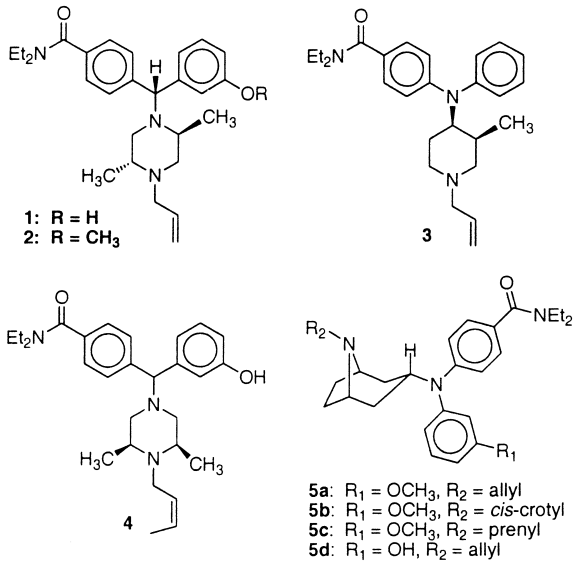


Chart 1.

with refluxing sodium methoxide in toluene/*N*-methylpyrrolidinone followed by saponification of the methyl ester. The zwitterionic intermediate was isolated as a slurry of its HCl salt and converted without purification into the diethylamide using benzotriazol-1-yl-oxy-tris-(dimethylamino) phosphonium hexafluorophosphate (BOP a.k.a. Castro's reagent), diethylamine, and triethylamine in a tetrahydrofuran (THF) slurry to give **9** in 78% yield. Conversion to the appropriately *N*-substituted analogue was accomplished by treating **9** with phenyl chloroformate followed by hydrolysis of the resulting carbamate with potassium hydroxide in isopropyl alcohol. *N*-Alkylation with the appropriate alkyl bromide then gave **5a**–**c**. Compound **5d** was prepared from **5a** in 77% yield by treatment with boron tribromide.

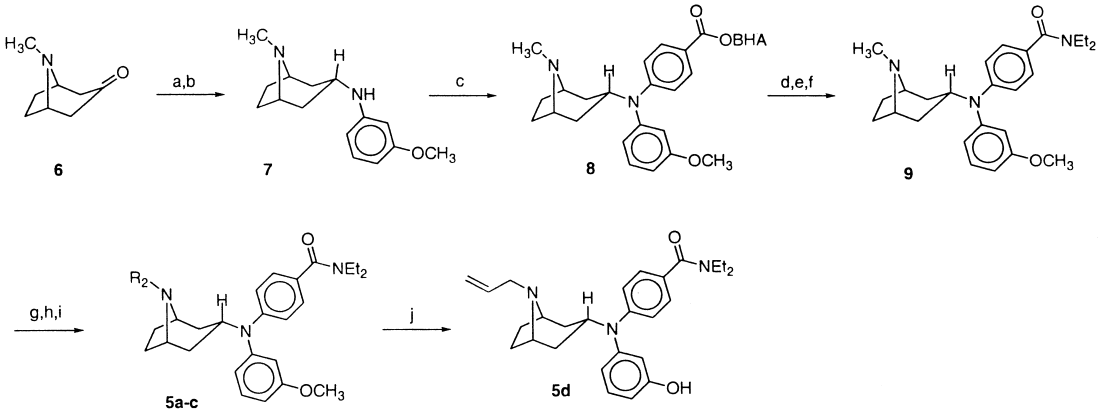
Biological

The binding affinities of **5a**–**d** and the reference compounds SNC-80 (**2**) and (−)-RTI-5989-54 (**3**) for the μ, δ, and κ opioid receptors were determined using competitive binding assays following previously reported procedures[6,7] (Table 1). Determination of agonism was accomplished by measuring the stimulation of binding of the GTP analogue [^{35}S]GTP-γ-S in guinea pig caudate elicited by the test compounds (Table 2).[6,7] The receptor responsible for any observed agonist activity was determined by measuring stimulation in the presence of selective antagonists. Measures of antagonism for the three opioid receptors were obtained by monitoring the test compounds ability to inhibit stimulation produced by the selective agonists DAMGO (μ), SNC-80 (δ), and U69,593 (κ) (Table 3).[6,7]

Results and Discussion

The radioligand binding data for the compounds **5a**–**d** along with comparative data for SNC-80 (**2**) and the piperidine derivative **3** are shown in Table 1. As is apparent, the tropane analogues **5a**–**d** all display excellent affinity for the δ opioid receptor versus the μ or κ receptors and possess slightly higher affinity for δ relative to the piperidine **3** but less affinity than the piperazine **2**. This translates into higher δ versus μ selectivity for all the tropane derivatives except **5c** relative to the piperidine derivative **3**. Moreover, unlike the piperidine compounds in general, the selectivity for δ over μ receptors evident in the tropane analogues, **5a** and **5b**, rivals that found in SNC-80 (**2**). Compound **5d** demonstrates that analogues possessing an aryl hydroxy substituent produce higher affinity and lower selectivity relative to the corresponding methyl ether derivatives like **5a**. Similar behavior was found for transitions between **1** and **2**; however, in this series, the methyl ether compounds are generally more selective.[2]

In terms of δ versus κ selectivity, the tropane compounds appear to possess values quite similar to those obtained for the piperidine compound **3** with the exception of the hydroxy substituted compound **5d**. Indeed, comparison of the selectivity observed for this compound to that of its methoxy analogue **5a** illustrates that a 5.5-fold loss in δ selectivity results from removal of the methyl in compound **5a**. Inspection of the binding data for the κ



Scheme 1. (a) Ti(Oi-Pr)$_4$, 3-methoxyaniline; (b) $NaBH_4$, EtOH; (c) *n*-BuLi, THF, HMPA then 1-(2,6-di-*tert*-butyl-4-methoxy)-4-fluorobenzoate; (d) *N*-methylpyrrolidinone, $NaOCH_3$, toluene; (e) EtOH, H_2O; (f) Et_2NH, BOP, Et_3N (g) PhOCOCl; (h) KOH, i-PrOH, H_2O; (i) R-Br, EtOH, K_2CO_3; (j) BBr_3.

Table 1. Radioligand binding results at the μ, δ, and κ opioid receptors[a]

Compound	K_i (nM ± S.D.)				
	μ [^{3}H]DAMGO[b]	δ [^{3}H]DADLE[c]	κ[^{3}H]U69,593[d]	μ/δ	κ/δ
2, SNC-80	1614 ± 131	1.57 ± 0.19	3535 ± 1841	1028	2251
3, (−)-RTI5989-54	2623 ± 307	5.85 ± 0.31	1448 ± 196	448	247
5a	2547 ± 344	3.28 ± 0.31	1076 ± 84	776	328
5b	>3400	3.7 ± 0.16	1166 ± 99	>918	355
5c	1105 ± 114	3.51 ± 0.32	788 ± 45	315	224
5d	686 ± 94	1.26 ± 0.08	73 ± 7.7	544	58

[a]Mu and δ binding was performed using rat brain membranes, and κ assays were performed using guinea pig brain membranes.
[b][^{3}H]DAMGO [(D-Ala^2,MePhe^4,Gly-ol^5)enkephalin]. Tritiated ligand selective for μ opioid receptor.
[c][^{3}H]DADLE [(D-Ala^2,D-Leu^5)enkephalin]. Tritiated ligand selective for δ opioid receptor.
[d][^{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.

Table 2. % Stimulation of [^{35}S]GTP-γ-S binding by DAMGO, SNC-80, U69,593, and compounds **5a**–**d** (10 μM) in guinea pig caudate membranes

	Unblocked condition% stimulation (mean ± S.D.)	μ Condition% stimulation (mean ± S.D.)[a]	δ Condition% stimulation (mean ± S.D.)[b]	κ Condition% stimulation (mean ± S.D.)[c]
DAMGO	115 ± 10	90 ± 10	8.6 ± 1.2	6 ± 5
SNC-80 (**2**)	102 ± 7	1.9 ± 1.7	105 ± 2	4 ± 3
U69,593	148 ± 7	34 ± 5	30 ± 3	103 ± 25
5a	46 ± 3	0	41 ± 4	0
5b	37 ± 8	0	33 ± 2	0
5c	29 ± 5	0	28 ± 13	0
5d	79 ± 9	44 ± 2	40 ± 3	47 ± 18

[a]The μ-selective condition used 20 nM naltrindole (NTI, antagonist selective for δ opioid receptor) and 6 nM nor-binaltorphimine (nor-BNI, antagonist selective for κ opioid receptor).
[b]The δ-selective condition used 6000 nM CTAP (antagonist selective for μ opioid receptor) and 6 nM nor-BNI.
[c]The κ-selective condition used 6000 nM CTAP and 20 nM NTI.

Table 3. % Inhibition of DAMGO, SNC-80, and U69,593 (10 μM)-stimulated [^{35}S]GTP-γ-S binding by compounds **5a**–**d** (10 μM) in guinea pig caudate membranes

Compound	% Inhibition of DAMGO-stimulated [^{35}S]GTP-γ-S binding	% Inhibition of SNC-80-stimulated [^{35}S]GTP-γ-S binding	% Inhibition of U69,593-stimulated [^{35}S]GTP-γ-S binding
5a	0	25 ± 1.1	0
5b	0	49 ± 0.4	0
5c	25 ± 3	69 ± 1	23 ± 0.6
5d	0	34 ± 6	0

receptor reveals that this change in selectivity results primarily from a 15-fold increase in κ affinity for **5d** versus **5a**. As mentioned previously, this is not unexpected since the methyl ethers in both the piperidine and piperazine series, like **3** and **2**, respectively, show greater selectivity as the ether compared with the phenol.

Inspection of Table 2 reveals that the tropane analogues **5a**–**c** all exhibit selective δ opioid stimulation of GTP binding but that this stimulation diminishes as the size of the *N*-substituent increased. Thus, the *N*-allyl analogue **5a** has 41% stimulation, whereas the *N*-prenyl analogue **5c** shows only 28% stimulation. Surprisingly, compound **5d**, which is highly selective for the δ receptor in the binding assay, produces stimulation for all opioid receptors in the GTP-γ-S functional assay (Table 2). Thus, changing a methoxy substituent (**5a**) to a hydroxy substituent (**5d**) dramatically impacts the behavior of **5d** such that stimulation is now observed for all opioid receptors. The partial agonist activity for compounds **5a**–**d** stands in contrast to the piperidine-based compound **3** which demonstrated full agonist activity in similar assays with an observed e_{max} equivalent to that found for **2**.[7] Thus, compared with similarly functionalized piperidine-based ligands, the more rigid tropane analogues demonstrate dramatically lower ability to stimulate GTP-γ-S binding.

In Table 3, the data listed show the antagonist activity of the tropane analogues **5a**–**d**. Across the series of methoxy substituted compounds **5a**–**c**, the antagonist activity is observed to increase with the size of the *N*-substituent. As noted above, it was across the same series (**5a**–**c**) that stimulation of GTP binding was found to decrease. Thus, taken together, the trend in the data clearly suggests that further manipulation of the *N*-substituent could lead to more potent δ antagonists with diminished agonist activity. The aryl hydroxy substituted compound **5d** demonstrates an even greater degree of antagonist activity than the similarly substituted methoxy derivative **5a**. This observation is in line with

expectations since typical opioid antagonists display greater activity with hydroxyl groups relative to methoxy groups.[12] The result from the present study suggests that further modification of *N*-substituents in the hydroxy series will lead to antagonists of greater activity than found in the methoxy series. For comparison, compound **3** does not inhibit SNC-80 stimulated GTP binding at a concentration of 10 μM.[6,7] Overall, the data available from this study reveal that reorganization of ring alkyl substituents and limiting conformational flexibility can minimize agonist activity and promote antagonist activity. Additional studies will have to be performed in order to determine what direct effect, if any, that the bridging ring may induce beyond increasing system rigidity. Especially critical for future studies will be determination of favored system conformations for the tropanes relative to the piperidines.

Conclusion

We have demonstrated that 4-[(8-alkyl-8-azabicyclo [3.2.1]octyl-3-yl)-3-arylanilino]-*N*,*N*-diethylbenzamides **5a–d** display high affinity binding and selectivity for the δ opioid receptor. Furthermore, the data obtained from this series shows that antagonist activity can be realized in the nitrogen-transposed compounds like **3**, if the 3-methyl group is removed and the system made more rigid by the addition of a bridging ring between the 2 and 6 positions. Accordingly, the hypothesis that the antagonism observed for **4** could result from limitations to the conformational flexibility found in compound **1** is supported. Further studies aimed at better understanding the conformational preferences of compounds **5a–d** are currently underway and will be reported in due course.

Acknowledgements

This research was supported by the National Institute on Drug Abuse, Grant DA09045.

References

1. Chang, K. J.; Rigdon, G. C.; Howard, J. L.; McNutt, R. W. *J. Pharm. Exp. Ther.* **1993**, *267*, 852.
2. Calderon, S. N.; Rothman, R. B.; Porreca, F.; Flippen-Anderson, J. L.; McNutt, R. W.; Xu, H.; Smith, L. E.; Bilsky, E. J.; Davis, P.; Rice, K. C. *J. Med. Chem.* **1994**, *37*, 2125.
3. Dondio, G.; Ronzoni, S.; Petrillo, P. *Exp. Opin. Ther. Pat.* **1999**, *9*, 353.
4. Zhang, X.; Rice, K. C.; Calderon, S. N.; Kayakiri, H.; Smith, L.; Coop, A.; Jacobson, A. E.; Rothman, R. B.; Davis, P.; Dersch, C. M.; Porreca, F. *J. Med. Chem.* **1999**, *42*, 5455.
5. Dondio, G.; Ronzoni, S.; Eggleston, D. S.; Artico, M.; Petrillo, P.; Petrone, G.; Visentin, L.; Farina, C.; Vecchietti, V.; Clarke, G. D. *J. Med. Chem.* **1997**, *40*, 3192.
6. Thomas, J. B.; Herault, X. M.; Rothman, R. B.; Burgess, J. P.; Mascarella, S. W.; Xu, H.; Horel, R. B.; Dersch, C. M.; Carroll, F. I. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3053.
7. Thomas, J. B.; Atkinson, R. N.; Herault, X. M.; Rothman, R. B.; Mascarella, S. W.; Dersch, C. M.; Xu, H.; Horel, R. B.; Carroll, F. I. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3347.
8. Su, Y.-F.; McNutt, R. W.; Chang, K.-J. *J. Pharmacol. Exp. Ther.* **1998**, *287*, 1.
9. Mattson, R. J.; Pham, K. M.; Leuck, D. J.; Cowen, K. A. *J. Org. Chem.* **1990**, *55*, 2552.
10. Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem.* **1996**, *61*, 3849.
11. Hattori, T.; Satoh, T.; Miyano, S. *Synthesis* **1996**, 514
12. Aldrich, J. V. In *Burger's Medicinal Chemistry and Drug Discovery*; Wolff, M. E., Ed.; John Wiley & Sons: New York, 1996; Vol. 3.