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Binding of β-carbolines at imidazoline I_2 receptors: a structure–affinity investigation

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Abstract—A series of ring-substituted (i.e., methoxy and bromo) 3,4-dihydro- and 1,2,3,4-tetrahydro-β-carbolines was examined at I_2 imidazoline receptors, as was the effect of ring-opening, ring-expansion, and translocation of the piperidinyl nitrogen atom. Several analogues were identified that bind with K_i <20 nM at I_2 sites and with reduced affinity at α_2-adrenergic receptors, and 1,2,3,4-tetrahydro-γ-carbolines were identified as a novel class of I_2 imidazoline receptor ligand.

1. Introduction

Imidazoline 'binding sites' (so-called imidazoline receptors) were proposed to explain certain actions of imidazoline-containing (or guanidine-containing) agents that could not be accounted for by their interaction with adrenergic receptors.[1] These binding sites exist at least as two populations (I_1 and I_2 receptors), and a third population (I_3 sites) has been identified in the pancreas.[2] Due to the possibility that I_2 sites might be involved in depression and other CNS disorders,[2,3] development of I_2 ligands is an attractive therapeutic target. Prior to the availability of more selective agents (e.g., see Fig. 1), pharmacological studies were conducted with agents that retained significant adrenergic character. Evidence now suggests that I_2 receptors might represent allosteric binding sites on monoamine oxidase; however, this issue is still somewhat controversial.[3]

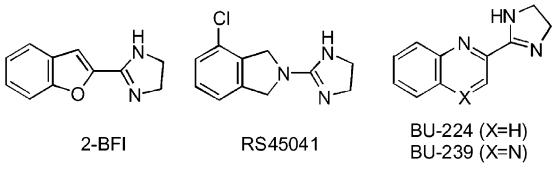
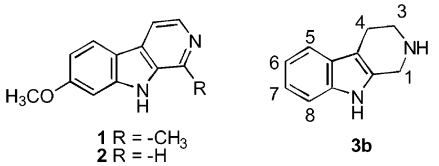


Figure 1. Structures of some commonly employed ligands with selectivity for I_2 binding sites.[3]

It recently has been shown that β-carbolines represent a novel class of I_2 ligands lacking an imidazoline moiety.[4] For example, harmane (**1**), norharmane (**2**) and 1,2,3,4-tetrahydro-β-carboline (THBC; **3b**) bind at I_2 receptors with high affinity[4] (K_i = 49 nM, 87 nM, and 9.4 nM, respectively).[5] A preliminary structure-affinity study has been conducted;[5] the fully aromatic compounds **1** and **2** displayed <10-fold) selectivity for I_2 versus I_1 binding sites, but **3b** displayed >1000-fold I_2 selectivity.



Although important structure–affinity findings were revealed, the investigation was limited to available agents and certain questions were left unanswered. For example, it was concluded that substituents might be tolerated at the aryl 6- and 7-positions, but not at the 8-position. However, only a few methoxy and hydroxy substituents were investigated, and some of the results might have been confounded by the presence of a methyl group at the 1-position. That is, a 1-methyl group might be tolerated if it is in the plane of the aryl ring (e.g., as in 3,4-dihydro-β-carbolines); but (in the tetrahydro series), an out-of-plane methyl group results in decreased affinity.[5] Hence, the role of methoxy substitution is not altogether clear. Furthermore, only one

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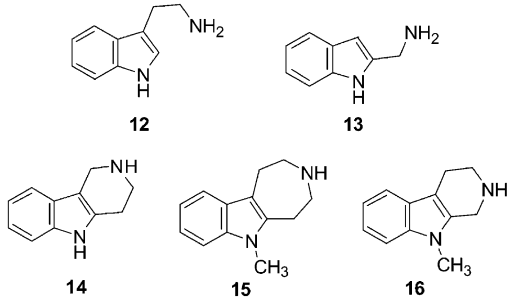
6-substituted and no 5-substituted analogues were examined. The purpose of the present investigation was to systematically examine the structure-affinity relationships of a series of 3,4-dihydro-β-carbolines and 1,2,3,4-tetrahydro-β-carbolines. To achieve this goal, the effect on I_2 binding of either an electron donating (i.e., methoxy) or an electron withdrawing (i.e., bromo) group at each of the four aryl positions was examined. In addition, the targeted compounds lacked a 1-methyl group in order to avoid the confounding influence of its affinity-reducing effects in the 1,2,3,4-tetrahydro series. Furthermore, the effect of ring opening, ring expansion, and translocation of the piperidinyl nitrogen atom was also examined. In this manner, it was hoped that a more comprehensive structure–affinity assessment could be obtained. Because many I_2 ligands also bind at α_2-adrenergic receptors, α_2-adrenergic binding data were also obtained for each compound.

2. Binding studies[6]

Affinities for the 18 compounds[7] shown in Table 1 varied over a nearly 800-fold range. In the 3,4-dihydro series, the highest affinity member is the unsubstituted compound **3a** ($K_i = 7.3$ nM). The 7-methoxy (i.e., **6a**; $K_i = 18$ nM) and 8-bromo (i.e., **11a**; $K_i = 17$ nM) derivatives also bind with high affinity. Similar results were obtained in the 1,2,3,4-tetrahydro series. That is, the unsubstituted THBC (**3b**; $K_i = 9.4$ nM) binds with high affinity, as does its 7-methoxy derivative **6b** ($K_i = 12$ nM). But here, 5-bromo and 8-bromo analogues bind with similar and enhanced affinity ($K_i = 5.4$ and 3.6 nM for **8b** and **11b**, respectively). Nevertheless, there is a significant correlation ($r > 0.9$; $n = 18$) when K_i values for binding are compared suggesting that the two series are likely binding in a similar manner.

Next investigated was the effect of ring-opening of the piperidine ring of THBC (**3b**) on I_2 affinity. Deletion of the 1-position methylene group to afford tryptamine (**12**; $K_i = 5{,}400 \pm 650$ nM) reduced affinity by >500-fold whereas deletion of the 3,4-ethylene bridge, to afford **13**[8] ($K_i = 3440 \pm 220$ nM) decreased affinity by >350-fold. Evidently the intact tricyclic system is optimal for binding.

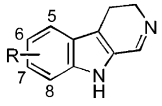


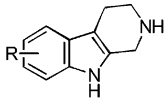
Interestingly, however, the tricyclic ring need not be a β-carboline. That is, 1,2,3,4-tetrahydro-γ-carboline **14**[9] ($K_i = 4.7 \pm 1.7$ nM) binds with about twice the affinity of THBC (**3b**). Because a two-methylene bridge was accommodated both in **3b** and **14**, it was thought that azepinoindole **15**[10] might also bind at I_2 receptors; however, **15** ($K_i = 1030 \pm 130$ nM) displayed reduced affinity. It might be reasoned that the *N*-methyl group could be responsible for the reduction in affinity, but we have previously found that methylation of the indolic nitrogen atom of THBC (**3b**) did not adversely impact affinity. That is, compound **16** ($K_i = 5.4$ nM) binds at I_2 receptors with an affinity comparable to that of **3b**.[5]

3. Selectivity

Because many I_2 ligands display affinity for α_2-adrenergic receptors, all compounds in Table 1 were examined for adrenergic binding.[6] All of the β-carbolines displayed selectivity for I_2 over α_2-adrenergic receptors. In the 3,4-dihydro series, the unsubstituted parent compound **3a** displayed 100-fold selectivity for I_2 receptors, whereas the 7-methoxy derivative **6a** displayed >500-fold selectivity. Likewise, in the 1,2,3,4-tetrahydro series, the two most selective compounds are the unsubstituted analogue **3b** (168-fold) and its 7-methoxy derivative **7b** (700-fold).

Table 1. Imidazoline I_2 and α_2-adrenergic receptor affinities of the 3,4-dihydro- and 1,2,3,4-tetrahydro-β-carbolines examined[a]





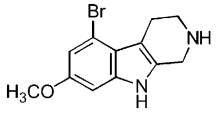
	R	K_i, nM (SEM)					K_i, nM (SEM)			
		I_2		α_2-Adrenergic			I_2		α_2-Adrenergic	
3a	H	7.3	(3.8)	700	(35)	**3b**	9.4[b]		1600[b]	
4a	5-OMe	84	(34)	330	(30)	**4b**	300	(190)	825	(330)
5a	6-OMe	480	(440)	2190	(830)	**5b**	1640	(1300)	7830	(3850)
6a	7-OMe	18[b]		>10,000		**6b**	12[b]		8840[b]	
7a	8-OMe	160	(120)	5020	(2820)	**7b**	270	(210)	640	(280)
8a	5-Br	86	(28)	3160	(1120)	**8b**	5.4	(2.1)	390	(180)
9a	6-Br	790	(140)	5290	(2360)	**9b**	2785	(1420)	11,500	(5300)
10a	7-Br	400	(210)	10,700	(4690)	**10b**	1290	(960)	15,500	(3000)
11a	8-Br	17	(5)	815	(30)	**11b**	3.6	(1.0)	460	(80)

[a] Radioligand binding studies[6] were conducted as previously reported.[5]
[b] Binding data were already reported[5] and are included only for comparison.

The other compounds in Table 1 displayed lower I_2 selectivity. Tryptamine (**12**) and 2-aminomethylindole (**13**) displayed low affinity for α_2-adrenergic receptors ($K_i = 19{,}000 \pm 4000$ nM and $14{,}700 \pm 2560$ nM, respectively), and **15** ($K_i = 415 \pm 135$ nM) showed some selectivity for adrenergic receptors over I_2 receptors. In contrast, γ-carboline **14** ($K_i = 600 \pm 30$ nM) was 125-fold selective for I_2 receptors.

High-affinity ligand **11b** (I_1 $IC_{50} = 3{,}740 \pm 650$ nM) was also found to bind with >1000-fold selectivity at I_2 versus I_1 binding sites.

We have recently demonstrated that 5-bromo- and 8-bromo-1,2,3,4-tetrahydro-β-carboline (**8b** and **11b**; $K_i = 180$ nM and 22 nM, respectively) also bind at 5-HT_{2A} serotonin receptors.[7] We have also found that introduction of a 7-methoxy group is not tolerated by 5-HT_{2A} receptors. Consequently, we prepared compound **17**[11] with the expectation that it would bind with high affinity at I_2 binding sites and with reduced affinity at 5-HT_{2A} receptors. However, compound **17** (I_2 $K_i = 1{,}740 \pm 340$ nM) displayed low affinity for I_2 receptors.



17

Of the compounds investigated in this study, two of the most promising for subsequent evaluation are **6b** and **11b**. Bromo analogue **11b** might find applicability in studies addressing I_2 versus I_1 or α_2-adrenergic receptors, but possesses limitations in certain types of studies due to its high affinity for 5-HT_{2A} serotonin receptors. 7-Methoxy-1,2,3,4-tetrahydro-β-carboline (**6b**) binds with good affinity and selectivity at I_2 ($K_i = 12$ nM) versus I_1 ($K_i > 10{,}000$ nM) and α_2-adrenergic receptors (8840 nM).[5] Most recently, we have found that **6b** lacks affinity ($K_i > 1000$ nM) for 5-HT_{2A} and 30 other populations of neurotransmitter receptors and transporters, with the exception of α_{2B}-adrenergic receptors ($K_i = 140$ nM).[7] Compound **6b** is targeted for further evaluation.

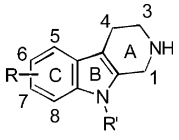


Figure 2. A structure-affinity summary for the binding of β-carbolines at I_2 receptors. Data are derived from the present study, and from that reported earlier.[5] **A**: an intact 'piperidinyl' moiety seems optimal and ring-opening reduces affinity; substitution at C_1 with a methyl group is tolerated in the 3,4-dihydro series but not in the 1,2,3,4-tetrahydro series (where it is out of plane); the N_2-nitrogen atom can be moved to the 3-position; a carboxylate group at C_3 abolishes affinity in the 1,2,3,4-tetrahydro series; ring-expansion to an azepinoindole decreases affinity; fully-unsaturated C-ring analogues seem to lack selectivity for I_2 versus I_1 sites; **B**: an N-methyl group is tolerated; **C**: optimal affinity (i.e., $K_i < 20$ nM) is associated with an unsubstituted ring system, or with either a 7-methoxy or 8-bromo substituent.

4. Summary

A systematic structure-affinity investigation was conducted to determine the influence on I_2 affinity of electron donating and electron withdrawing groups on the aryl portion of 3,4-dihydro- and 1,2,3,4-tetrahydro-β-carbolines. Also examined was the role of the intact β-carboline nucleus, translocation of the piperidinyl nitrogen atom, and ring expansion. This information, together with our previously published report,[5] provides a much clearer structure–affinity picture for the binding of 3,4-dihydro- and 1,2,3,4-tetrahydro-β-carbolines at I_2 receptors. A general structure–affinity summary is provided in Figure 2.

Finally, we have shown (on the basis of the low affinity of **12** and **13**) that an intact carboline ring system seems optimal for I_2 binding, but that studies need not be limited to β-carboline derivatives. That is, γ-carboline **14** was identified as a member of a novel non-imidazoline structural class of I_2 ligands. Additional studies with **14**-type derivatives are planned.

Acknowledgements

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References and notes

1. Parini, A.; Moudanos, C. G.; Pizzinat, N.; Lanier, S. M. *Trends Pharmacol. Sci.* **1996**, *17*, 13.
2. Eglen, R. M.; Hudson, A. L.; Kendall, D. A.; Nutt, D. J.; Morgan, N. G.; Wilson, V. G.; Dillon, M. P. *Trends Pharmacol. Sci.* **1998**, *19*, 381.
3. Hudson, A. L.; Nutt, D. J.; Husbands, S. M. *Pharmaceut. News* **2001**, *8*, 18.
4. Hudson, A. L.; Price, R.; Tyacke, R. J.; Lalies, M. D.; Parker, C. A.; Nutt, D. J. *Br. J. Pharmacol.* **1999**, *126*, 2 P.
5. Husbands, S. M.; Glennon, R. A.; Gorgerat, S.; Gough, R.; Tyacke, R.; Crosby, J.; Nutt, D. J.; Lewis, J. W.; Hudson, A. L. *Drug Alcohol Depend.* **2001**, *64*, 203.
6. Crude P2 membranes were prepared from rat (male, Wistar ~250g) whole brains and kidneys, I_1, I_2 and α_2-adrenoceptor competition binding was performed as previously described.[5] [^{3}H]2-BFI and [^{3}H]clonidine (in the presence of rauwolscine) was used to label I_2 and I_1 receptors, respectively, and [^{3}H]RX821002 was used to label α_2-adrenergic receptors.[5] Each assay was analyzed individually using GraphPad Prism version 3.03 for Windows, (GraphPad Software; San Diego, CA) and the IC_{50} value determined. In the case of the I_2 and α_2-adrenoceptor binding, this was then used to calculate the K_i using the method of Cheng and Prusoff.[12]
7. Synthesis of the β-carbolines shown in Table 1 has been described: Grella, B.; Teitler, M.; Smith, C.; Herrick-Davis, K.; Glennon, R. A. *Bioorg. Med. Chem. Lett.*, in press.
8. Compound **13** HCl salt from MeOH, mp 254–257 °C, analyzed within 0.4% of theory for C, H, and N.
9. Compound **14** HCl was prepared as described by Ismaiel,

A. M.; Titeler, M.; Glennon, R. A. *Mansoura J. Pharm. (Egypt)* **1989**, *6*, 1.
10. Compound **15** HCl (i.e., U-22,394A) was obtained as a gift from the Upjon Company, Kalamazoo, MI.
11. Compound **17** HCl salt from MeOH, mp 276–279 °C, was prepared from 5-bromo-7-methoxy-1,2,3,4-tetrahydro-β-carbolin-1-one[13] by reduction with BH_3, and analyzed within 0.4% of theory for C, H, and N.
12. Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
13. Narayanan, K.; Schindler, L.; Cook, J. M. *J. Org. Chem.* **1991**, *56*, 359.