Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 627–630

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Yohimbine Dimers Exhibiting Binding Selectivities for Human α_{2a}- versus α_{2b}-Adrenergic Receptors

Weiping Zheng,[a,†] Longping Lei,[b,‡] Shilpa Lalchandani,[b] Guoping Sun,[a,§] Dennis R. Feller[b] and Duane D. Miller[a,*]

[a]*Department of Pharmaceutical Sciences, University of Tennessee, Memphis, TN 38163, USA*

[b]*Department of Pharmacology and National Center for Natural Products Research, School of Pharmacy, University of Mississippi, MS 38677, USA*

Received 3 November 1999; accepted 26 January 2000

Abstract—A series of yohimbine dimers was prepared and evaluated at the human α_{2a}- and α_{2b}-adrenergic receptors (ARs) expressed in Chinese hamster ovary (CHO) cells. All dimers display higher binding selectivities for α_{2a} versus α_{2b} subtype than yohimbine, and four compounds (**3d**, **3e**, **3g** and **3i**) represent the most potent and α_{2a}- versus α_{2b}-AR selective ligands identified so far.

Following the pharmacological classification of the α-adrenergic receptor (AR) into α_1- and α_2-ARs,[1] the α_2-ARs were further subdivided into $\alpha_{2A(a)}$, $\alpha_{2B(b)}$ and $\alpha_{2C(c)}$ subtypes based on pharmacological and molecular biological studies.[2] α_2-ARs belong to the seven-transmembrane G protein-coupled receptor superfamily.[3] The existence of these α_2-AR subtypes has stimulated extensive interest in exploring the physiological relevance of each subtype. Gene targeting strategies have been applied to all three α_2-AR subtypes,[4] these studies have provided some answers to this exploration. However, the availability of α_2-AR subtype-selective agents, especially antagonists, would greatly help to advance our knowledge of functions mediated by these α_2-AR subtypes. This knowledge will also help to predict the pharmacological and therapeutic properties of α_2-AR subtype-selective agents.

Although there have been a number of α_2-AR antagonists,[5] only a small set of compounds have been reported that have a varied degree of selectivity among the three subtypes of α_2-AR. However, these latter compounds suffer from either low subtype selectivity or binding to receptor sites outside the α_2-AR subfamily.[5a,6]

In this paper, we wish to report our preliminary efforts toward employing the bivalent ligand approach to identify α_2-AR subtype-selective antagonists. The bivalent ligand approach has been successfully utilized in developing highly potent and selective ligands in a diverse set of receptor systems,[7] such as the opioid and serotonergic receptors, two members of the seven transmembrane G protein-coupled receptor superfamily, as well as the growth factor receptor system. In these studies, it was demonstrated that bivalent ligands exhibit a higher degree of potency and selectivity than their monovalent counterparts. This superior activity of bivalent ligands was shown to result from the bridging between either vicinal receptors or the pharmacophore binding site and another accessory site in the same receptor molecule.[7a–d]

The following two factors were taken into consideration in designing our bivalent yohimbines **3a**–**j**. Firstly, yohimbine (**1**) is a known potent and selective α_2-AR antagonist, and has been used extensively as a pharmacological probe for studying the α_2-AR.[8] However, yohimbine does not show selectivity among three α_2-AR subtypes.[2c,d] Secondly, the point of attaching spacers on the pharmacophore should be selected so that the ligand receptor binding interactions would not be impaired. Our choice of the C-16 carboxyl of yohimbine as the spacer attaching point was based on the report that the

*Corresponding author. Tel.: +1-901-448-7529; fax: +1-901-448-6828; e-mail: dmiller@utmem.edu

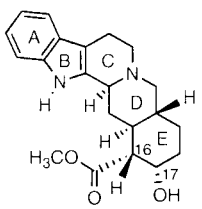
†Present address: Department of Pharmacology and Molecular Sciences, Johns Hopkins University, Baltimore, MD 21205, USA.

‡Present address: Department of Anesthesia, Brigham and Women's Hospital, Boston, MA 02115, USA.

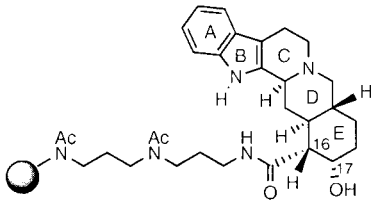
§Present address: National Institutes of Health, National Eye Institute, Laboratory of Ocular Therapeutics, Bethesda, MD 20892, USA.

0960-894X/00/$ - see front matter
PII: S0960-894X(00)00068-8

yohimbine-agarose conjugate (**2**) is an excellent affinity chromatography matrix for large scale and micro-purification of multiple α_2-AR subtypes.[9]



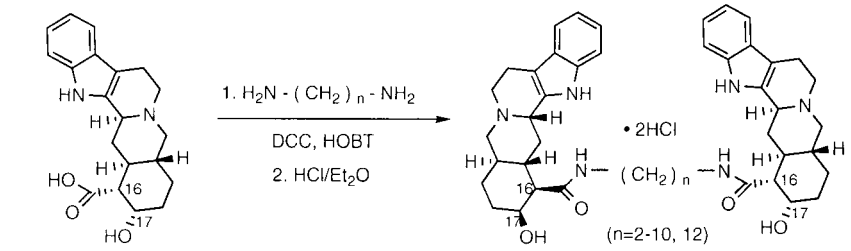
1



2

The designed yohimbine dimers were prepared by coupling the commercially available yohimbinic acid with aliphatic α,ω-diamines under standard peptide coupling condition (Scheme 1),[10] and were evaluated on human α_{2a}- and α_{2b}-AR expressed stably in Chinese hamster ovary (CHO) cells. The binding affinities of yohimbine and these dimers are given in Table 1. [^{3}H]Rauwolscine was used as the radioligand in equilibrium competition binding experiments. Yohimbine was included as the monovalent compound against which bivalent yohimbines were compared.

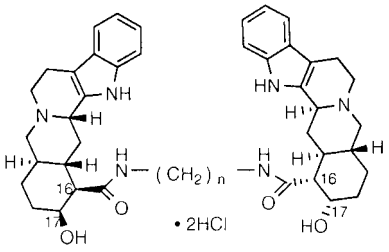
Several features are evident from the data in Table 1. Firstly, all of the yohimbine dimers displayed equal or lower affinity than the parent antagonist for the α_{2a} subtype; affinity for the α_{2b} adrenoceptor was reduced to a much greater extent. The lack of enhanced affinity of a dimer for either subtype may imply that the spacers of these bivalent ligands are not long enough to achieve the bridging of vicinal receptors. This consideration has been the basis for our preparing further bivalent yohimbines with longer spacers which are being evaluated



Yohimbinic Acid **3a-j**

Scheme 1.

Table 1. Binding affinities (K_i) of bivalent yohimbines on human α_{2a}- and α_{2b}-AR expressed in CHO cells[a]



Compound	*n*	Formula[b]	$K_i \pm$ SEM (nM)[c]		α_{2a}/α_{2b}
			α_{2a}-AR	α_{2b}-AR	Selectivity[d]
Yohimbine			0.42 ± 0.04	2.01 ± 0.29	4.8
3a	2	$C_{42}H_{52}N_6O_4 \cdot 2HCl \cdot 2.5H_2O$	26.4 ± 7.3	1510 ± 262	57.2
3b	3	$C_{43}H_{54}N_6O_4 \cdot 2HCl \cdot 2H_2O$	52 ± 3.2	990 ± 85.2	19.0
3c	4	$C_{44}H_{56}N_6O_4 \cdot 2HCl \cdot 2H_2O$	15.3 ± 6.2	188.6 ± 38.1	12.3
3d	5	$C_{45}H_{58}N_6O_4 \cdot 2HCl \cdot 1.8H_2O$	1.73 ± 0.26	134.3 ± 38.1	77.6
3e	6	$C_{46}H_{60}N_6O_4 \cdot 2HCl \cdot 3H_2O$	1.35 ± 0.27	166.2 ± 56.7	123.1
3f	7	$C_{47}H_{62}N_6O_4 \cdot 2HCl \cdot 3H_2O$	0.87 ± 0.18	27.6 ± 4	31.7
3g	8	$C_{48}H_{64}N_6O_4 \cdot 2HCl \cdot 2.2H_2O$	0.76 ± 0.12	46 ± 7.6	60.5
3h	9	$C_{49}H_{66}N_6O_4 \cdot 2HCl \cdot 2.5H_2O$	1.25 ± 0.29	44.7 ± 1.9	35.8
3i	10	$C_{50}H_{68}N_6O_4 \cdot 2HCl \cdot 2.5H_2O$	0.39 ± 0.08	18.6 ± 1.6	47.7
3j	12	$C_{52}H_{72}N_6O_4 \cdot 2HCl \cdot 2.5H_2O$	1.18 ± 0.38	18.5 ± 0.57	15.7

[a]Data represent the mean ± SEM of four to nine experiments. [^{3}H]Rauwolscine (2.54 nM) was used as the radioligand in the equilibrium competition binding assays, and the nonspecific binding was measured in the presence of 10 μM of yohimbine.
[b]C, H, N analyses were within 0.4% of theory.
[c]K_i (nM) was calculated according to the Cheng–Prusoff equation $K_i = IC_{50}/(1 + [L]/K_d)$[16] from determined IC_{50} values. K_d values for [^{3}H]rauwolscine at human α_{2a}- and α_{2b}-AR expressed in CHO cells were from ref. 6d.
[d]α_{2a}/α_{2b} Selectivity $= \frac{K_i(\alpha_{2b})}{K_i(\alpha_{2a})}$.

at the α_2-AR subtypes. Our decision to prepare the dimers with longer spacers was also facilitated by recent reports showing that receptor cluster formation was observed immunomicroscopically for the α_{2a}-AR,[11] and that α_{2a}- and α_{2c}-AR can exist as dimers.[12] Secondly, lower binding affinities were observed for all bivalent yohimbines on the α_{2b} than on the α_{2a} subtype. The highly divergent extracellular loops between the α_{2a} and α_{2b} subtype, in contrast to their highly homologous seven transmembrane regions, may have imparted the differential affinities of yohimbine dimers on the α_{2a} and α_{2b} subtypes.[3,13] One prominent feature of this extracellular loop diversity between α_{2a}- and α_{2b}-AR is the differential distribution of acidic and basic amino acid residues in these loops. Basic residues are in a preponderance over acidic residues at α_{2b}-AR, compared to their distributions at the α_{2a}-AR. If we assume that the extracellular loop amino acid residues have the same pK_a values when they are in the loop microenvironment and when they are free in the solution, the extracellular loops of the α_{2b}-AR are more positively charged than the extracellular loops of the α_{2a}-AR under our biological evaluation condition (pH = 7.4). Therefore, the binding of one protonated yohimbine moiety of the bivalent yohimbines at the receptor active site, which is within the seven transmembrane regions,[2c,3a] will inevitably place the second protonated yohimbine moiety in an environment where strong electronic repulsion between the protonated yohimbine moiety and the highly positively charged extracellular loops of the α_{2b}-AR is expected. This destabilizing electronic effect is expected to disturb the binding process of the yohimbine at the α_{2b}-AR active site, thus, lower binding affinities were observed. However, on the α_{2a}-AR, the weak electronic repulsion between the much less positively charged extracellular loops and the protonated yohimbine moiety is not expected to strongly disturb the binding of the yohimbine at the receptor active site. Furthermore, $\alpha_{2A(a)}$-AR from both native tissues and model cell lines are highly glycosylated at the extracellular N-terminal region,[9b,14] whereas $\alpha_{2B(b)}$-AR is not.[15] It is likely that the complex carbohydrate trees of the α_{2a} subtype shield the exposed charge, and thus further attenuate the electronic repulsion between extracellular loops and protonated yohimbine. A higher binding affinity was thus observed for a yohimbine dimer on the α_{2a} than on the α_{2b} subtype.

In conclusion, all yohimbine dimers in this study display binding selectivities for human α_{2a}- versus α_{2b}-ARs expressed in CHO cells, with peak selectivity occurring for **3e** ($n = 6$). To our knowledge, four of these dimeric compounds, i.e. **3d** ($n = 5$), **3e** ($n = 6$), **3g** ($n = 8$), **3i** ($n = 10$) represent the most potent and selective (48-fold to 123-fold) ligands identified so far for human α_{2a}- versus α_{2b}-ARs expressed in CHO cells. The functional studies for the above four compounds are in progress, and we plan to evaluate these dimers on human α_{2c}-AR expressed in CHO cells. The results from these studies will be reported in due course.

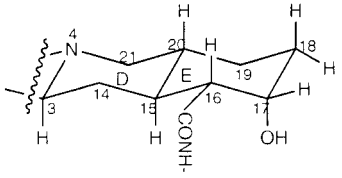
Acknowledgements

We thank the National Institute of Health (NIH R01 GM 29358) and Van Vleet foundation for their financial support for this work. We are also very grateful for Drs. Stephen White and Weixing Zhang at the Department of Structural Biology of St. Jude Children's Research Hospital (Memphis, TN 38105, USA) for their generous support and excellent NMR assistance.

References and Notes

1. Starke, K. *Rev. Physiol. Biochem. Pharmacol.* **1981**, *88*, 199.
2. For reviews, see (a) Bylund, D. B. *FASEB J.* **1992**, *6*, 832; (b) Bylund, D. B.; Eikenberg, D. C.; Hieble, J. P.; Langer, S. Z.; Lefkowitz, R. J.; Minneman, K. P.; Molinoff, P. B.; Ruffolo Jr., R. R.; Trendelenburg, U. *Pharmacol. Rev.* **1994**, *46*, 121; (c) Hieble, J. P.; Bondinell, W. E.; Ruffolo Jr., R. R. *J. Med. Chem.* **1995**, *38*, 3415; (d) Hieble, J. P.; Ruffolo Jr., R. R. *Prog. Drug Res.* **1996**, *47*, 81.
3. (a) Harrison, J. K.; Pearson, W. R.; Lynch, K. R. *TiPS* **1991**, *12*, 62; (b) Aantaa, R.; Marjamaki, A.; Scheinin, M. *Ann. Med.* **1995**, *27*, 439.
4. Link, R. E.; Stevens, M. S.; Kulatunga, M.; Scheinin, M.; Barsh, G. S.; Kobilka, B. K. *Mol. Pharmacol.* **1995**, *48*, 48; (b) Link, R. E.; Desai, K.; Hein, L.; Stevens, M. E.; Chruscinski, A.; Bernstein, D.; Barsh, G. S.; Kobilka, B. K. *Science*, **1996**, *273*, 803; (c) MacMillan, L. B.; Hein, L.; Smith, M. S.; Piascik, M. T.; Limbird, L. E. *Ibid* **1996**, *273*, 801; (d) MacMillan, L. B.; Lakhlani, P.; Lovinger, D.; Limbird, L. E. *Recent Prog. Horm. Res.* **1998**, *53*, 25.
5. For reviews, see (a) Ruffolo, R. R. Jr.; Bondinell, W.; Hieble, J. P. *J. Med. Chem.* **1995**, *38*, 3681; (b) Clark, R. D.; Michel, A. D.; Whiting, R. L. *Prog. Med. Chem.* **1986**, *23*, 1.
6. (a) Yound, P.; Berge, J.; Chapman, H.; Cawthorne, M. A. *Eur. J. Pharmacol.* **1989**, *168*, 381; (b) Devedjian, J.-C.; Esclapez, F.; Denis-Pouxviel, C.; Paris, H. *Ibid* **1994**, *252*, 43; (c) Meana, J. J.; Callado, L. F.; Pazos, A.; Grijalba, B.; Garcia-Sevilla, J. A. *Ibid* **1996**, *312*, 385; (d) Beeley, L. J.; Berge, J. M.; Chapman, H.; Hieble, P.; Kelly, J.; Naselsky, D. P.; Rockell, C. M.; Young, P. W. *Bioorg. Med. Chem.* **1995**, *3*, 1693; (e) Michel, A. D.; Loury, D. N.; Whiting, R. L. *Br. J. Pharmacol.* **1990**, *99*, 560; (f) Uhlen, S.; Porter, A. C.; Neubig, R. R. *J. Pharmacol. Exp. Ther.* **1994**, *271*, 1558; (g) Blaxall, H. S.; Murphy, T. J.; Baker, J. C.; Ray, C.; Bylund, D. B. *Ibid* **1991**, *259*, 323; (h) Bylund, D. B.; Blaxall, H. S.; Iverson, L. J.; Caron, M. G.; Lefkowitz, R. J.; Lomasney, J. W. *Mol. Pharmacol.* **1992**, *42*, 1; (i) Okumura, K.; Koike, K.; Asai, H.; Takayanagi, I. *Gen. Pharmacol.* **1988**, *19*, 463.
7. (a) Portoghese, P. S.; Larson, D. L.; Yim, C. B.; Sayre, L. M.; Ronsisvalle, G.; Lipkowski, A. W.; Takemori, A. E.; Rice, K. C.; Tam, S. W. *J. Med. Chem.* **1985**, *28*, 1140; (b) Erez, M.; Takemori, A. E.; Portoghese, P. S. *Ibid.* **1982**, *25*, 847; (c) Portoghese, P. S. *Ibid* **1992**, *35*, 1927; (d) Portoghese, P. S. *TiPS* **1989**, *10*, 230; (e) Shimohigashi, Y.; Costa, T.; Chen, H.-C.; Rodbard, D. *Nature* **1982**, *297*, 333; (f) LeBoulluec, K. L.; Mattson, R. J.; Mahle, C. D.; McGovern, R. T.; Nowak, H. P.; Gentile, A. J. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 123; (g) Cwirla, S. E.; Balasubramanian, P.; Duffin, D. J.; Wagstrom, C. R.; Gates, C. M.; Singer, S. C.; Davis, A. M.; Tansik, R. L.; Mattheakis, L. C.; Boytos, C. M.; Schatz, P. J.; Baccanari, D.; Wrighton, N. C.; Barrett, R. W.; Dower, W. J. *Science* **1997**, *276*, 1696.

8. Goldberg, M. R.; Robertson, D. *Pharmacol. Rev*. **1983**, *35*, 143.
9. (a) Domino, S. E.; Repaske, M. G.; Bonner, C. A.; Kennedy, M. E.; Wilson, A. L.; Brandon, S.; Limbird, L. E. *Methods Enzymol*. **1992**, *215*, 181; (b) Wilson, A. L.; Seibert, K.; Brandon, S.; Cragoe, E. J. Jr.; Limbird, L. E. *Mol. Pharmacol*. **1991**, *39*, 481.
10. (a) The structures of bivalent yohimbines were confirmed by spectroscopic analysis (MS, IR, ^{1}H NMR, ^{13}C NMR) and elemental analysis; (b) Due to the conformationally rigid nature of the yohimbine polycyclic ring system,[10c] the stereo centers that are susceptible to epimerization during the coupling reaction and the subsequent reaction work up are those at C16 and C17. However, the integrity of their stereochemistry was conserved as confirmed by decoupling NMR studies; (Only rings D and E are shown for clarity). (c) Morrison, G. A. *Fortschr. Chem. Organ. Naturstoffe* **1967**, *25*, 269.
11. Uhlen, S.; Axelrod, D.; Keefer, J. R.; Limberg, L. E.; Neubig, R. R. *Pharmacol. Commun*. **1995**, *6*, 155.
12. (a) Maggio, R.; Vogel, Z.; Wess, J. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 3103; (b) Maggio, R.; Barbier, P.; Fornai, F.; Corsini, G. U. *J. Biol. Chem*. **1996**, *271*, 31055.
13. Swiss Protein Databank. **1998**, Accession Number: p08913 and p18089.
14. Guyer, C. A.; Horstman, D. A.; Wilson, A. L.; Clark, J. D.; Cragoe Jr., E. J.; Limbird, L. E. *J. Biol. Chem*. **1990**, *265*, 17307.
15. (a) Zeng, D.; Harrison, J. K.; D'Angelo, D. D.; Barber, C. M.; Tucker, A. L.; Lu, Z.; Lynch, K. R. *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 3102; (b) Lanier, S. M.; Homcy, C. J.; Patenauda, C.; Graham, R. M. *J. Biol. Chem*. **1988**, *263*, 14491.
16. Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol*. **1973**, *22*, 3099.



H_{17}: δ = 4.07-4.22 ppm (Multiplet in 600 MHz; Singlet in 300 MHz)

H_{16}: δ = 2.11-2.22 ppm

• Before irradiating H_{17} signal: dd. $^{3}J_{15,16}$ = 12.0 Hz; $^{3}J_{16,17}$ = 2.4 Hz

• After irradiating H_{17} Signal: d. $^{3}J_{15,16}$ = 12.0 Hz