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A CONVENIENT SYNTHESIS OF 2-AMINO-2-OXAZOLINES AND THEIR PHARMACOLOGICAL EVALUATION AT CLONED HUMAN α ADRENERGIC RECEPTORS

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Abstract: A number of 2-amino-2-oxazolines including rilmenidine (**7**) were synthesized in good yield by thermal cyclization of 2-chloroethylurea **2** in the presence of potassium fluoride on alumina. These compounds were assayed for their binding affinity and efficacy at cloned human α adrenergic receptors.

INTRODUCTION

Rilmenidine is a 2-amino-2-oxazoline derivative which shows α_2 adrenergic[1] and imidazoline receptor agonist activity,[2] and is used as an antihypertensive agent. Radioligand binding studies strongly indicate the existence of three α_2 adrenergic receptor subtypes.[3] They are designated α_{2A} , α_{2B} and α_{2C} according to their affinities for different ligands. Although an additional α_{2D} subtype was proposed,[4] it was later demonstrated to be a species homolog of the human α_{2A} subtype.[5] Recently, the human genes encoding for three α_2 subtypes have been cloned.[6] These genes have been localized to chromosomes 10, 2 and 4, and appear to express receptors corresponding to the α_{2A} , α_{2B} and α_{2C} subtypes respectively. However, their exact physiological roles have not yet been determined despite some evidence of differential tissue distribution.[7]

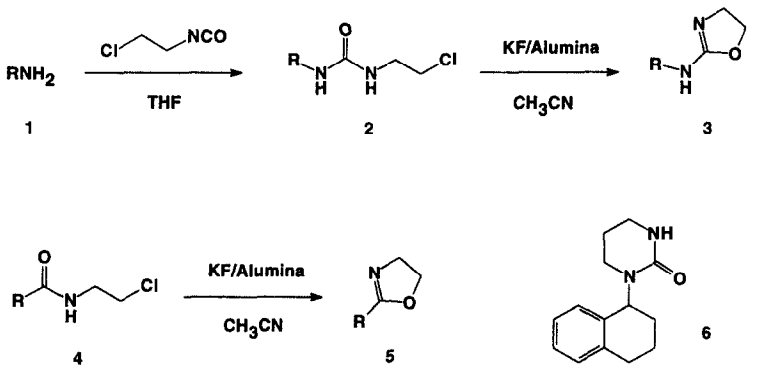
On the other hand, the imidazoline receptors are recently characterized sites which differ from the α_2 receptors in that the former may not be coupled to G-proteins and do not bind catecholamines such as norepinephrine.[8] There is strong evidence to suggest that rilmenidine exerts its antihypertensive effect through the imidazoline I_1 receptor.[9] Recently, an imidazoline I_2 site was isolated from the human liver, but no physiological role has been assigned yet and rilmenidine showed only weak affinity (pKi = 5.6) at this site.[10]

We are interested in designing novel α_2 ligands which are selective for each of the three α_2 subtypes.[11] These compounds are expected to serve as useful pharmacological tools to distinguish and localize the receptor subtypes. Moreover, an α_2 subtype selective compound may be useful for the treatment of such conditions as pain, depression, ischemia, hypertension, diarrhea, glaucoma, cognitive deficiency, asthma, obesity, diabetes and migraine[12] with reduced adverse side-effects. Since 2-amino-2-oxazolines such as rilmenidine constitute an interesting class of α_2 agonists, we have used a convenient synthesis to prepare a number of these compounds in order to study their pharmacological profile at the cloned human α adrenergic receptors (α_{1a} , α_{1b} , α_{1c} , α_{2a} , α_{2b} and α_{2c}).[13]

SYNTHESIS

2-Amino-2-oxazolines **3** (Scheme 1) have been prepared by a number of methods.[14,15] We were intrigued by the report that 2-oxazolines **5** could be obtained by thermal cyclization of *N*-(2-chloroethyl)amides **4** in the presence of potassium fluoride on alumina.[16] To extend this methodology, we investigated the reaction of several 2-chloroethylurea derivatives **2** (prepared by reaction of the corresponding amines with 2-chloroethyl isocyanate) with KF/alumina in refluxing acetonitrile. Consequently, 2-amino-2-oxazoline derivatives **3** were isolated by simple filtration of the reaction mixture through Celite. The reaction was clean and no aqueous

Scheme 1



work-up was necessary. In the absence of KF/Alumina, no reaction was observed. Table 1 shows the five 2-amino-2-oxazolines **7-11** synthesized by this method. This method can also be used to prepare 2-amino-1,3-oxazines such as structure **12**[17] by cyclization of the corresponding 3-chloropropylurea derivative. However, in this case, a significant amount of the cyclic urea byproduct **6** was isolated (41%). A typical procedure is as follows:

2-(1,2,3,4-Tetrahydro-1-naphthylamino)-2-oxazoline (9).[15] To a dry THF solution (10 mL) of 1,2,3,4-tetrahydro-1-naphthylamine (0.97 mL, d 1.026, 6.79 mmol) cooled by an ice-water bath was added dropwise 2-chloroethyl isocyanate (0.7 mL, *d* 1.237, 8.21 mmol).[18] The solution was stirred at room temperature overnight before the solvent was evaporated to give a slightly pinkish solid (1.62 g, 94%).

Without further purification, the above solid (332 mg, 1.31 mmol) was mixed with 40% potassium fluoride on alumina[18] (765 mg, 5.27 mmol) in CH_3CN (10 mL) and heated at reflux overnight. Filtration of the reaction mixture through Celite followed by concentration afforded a yellow solid which was flash chromatographed over silica gel (14 g) eluting with EtOAc/Hexane/Et_3N (20:40:3) to give **9** as a white solid (165 mg, 58%). An analytical sample (white crystals) was obtained by recrystallization from EtOAc/Hexane: mp 115-116°C; ^{1}H NMR

($CDCl_3$, 300 MHz) δ 7.39 (m, 1H), 7.17 (m, 2H), 7.09 (m 1H), 4.82 (t, 1H, *J*=5.6 Hz), 4.32 (t, 2H, *J*=8.4 Hz), 3.85 (t, 2H, *J*=8.5 Hz), 2.77 (m, 2H), 2.06 (m, 1H), 2.00-1.80 (m, 3H); FTIR (NaCl) 1665 cm^{-1}; EIMS m/e=216 (M^+). Anal. Calcd. for $C_{13}H_{16}N_2O$: C, 72.19; H, 7.46; N, 12.95. Found: C, 72.11; H, 7.55; N, 13.06.

PHARMACOLOGY

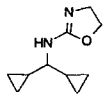
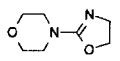
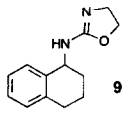
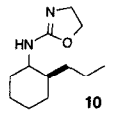
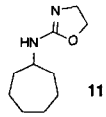
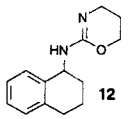
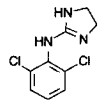
The binding and functional assays were performed using stably transfected cloned human α adrenergic receptors. The displacement of [^{3}H]prazosin from the α_1 receptors[19] and the displacement of [^{3}H]rauwolscine from the α_2 receptors[6b] were used to calculate the binding affinities (pKi). The agonistic activity (expressed as pEC_{50}) at the α_2 receptors was measured as a function of the ability to inhibit the forskolin-stimulated synthesis of cyclic adenosine monophosphate.[20]

Table 1 shows the binding and functional activities of compounds **7-12** at the cloned human α adrenergic receptors. To serve as a reference, the data for clonidine are also included. With the exception of compound **8** which is essentially inactive,[14b] all the compounds display high affinity at the α_2 receptors. As has been proposed for other biogenic amine G protein-coupled receptors, it is reasonable to assume that the basic nitrogens of these ligands, being protonated at physiological pH, probably form a salt bridge with the carboxylate anion of the aspartate residue on the third transmembrane helix of the receptors.[21] The receptor site around the oxazoline ring is apparently very limited in space such that the bridge nitrogen of the ligands cannot be fully substituted for high affinity to be maintained. This may explain why compound **8** shows weak binding affinity. Moreover, 2-amino-1,3-oxazine **12** is about 10-fold less potent than its 2-amino-2-oxazoline analog **9** at the α_2 receptors possibly because the six-membered oxazine ring is slightly too large for the receptor site. This phenomenon has also been observed in clonidine analogs whereby the binding affinity is diminished when the imidazoline ring of clonidine is expanded to a six-membered ring.[22] In contrast, the receptor site surrounding the aliphatic region of the ligands is more tolerant of ligand size such that a tetrahydronaphthyl moiety as in **9**, as well as a cycloheptyl moiety as in **11**, can be accomodated.

Although most of the compounds in this study are reasonably specific for the cloned human α_2 relative to the α_1 receptors, with **11**[23] showing the biggest difference between the pKi at α_2 and that at α_1, no ligand displays greater than 10-fold selectivity among the α_2 subtypes. At the functional level, **9** and **10**[24] are the most potent compounds, whereas clonidine shows the greatest selectivity with its potency at α_{2a} being 20-fold higher than that at α_{2b}.

This is the first report describing the binding affinity and efficacy of rilmenidine at cloned human α adrenergic receptors. As can be seen from Table 1, rilmenidine binds to the α_2 receptors with an affinity weaker than that of clonidine, and shows only slight selectivity among the α_2 subtypes. Clonidine is a well-known centrally mediated antihypertensive agent but, unlike rilmenidine, it also produces sedation at hypotensive doses. The present data, therefore, tend to support previous suggestion that rilmenidine and clonidine exert their antihypertensive effect through the imidazoline I_1 receptor.[25] In addition, the lack of sedation observed in patients

Table 1.[a] Binding and Functional Activities at Cloned Human α Adrenergic Receptors.

Structure	mp °C (% Yield)	pKi (pEC_{50})					
		α_{2a}	α_{2b}	α_{2c}	α_{1a}	α_{1b}	α_{1c}
 **Rilmenidine (7)**	140-142[b] (53)	6.74 (6.56)	7.15 (6.14)	6.54 (6.72)	5.05	4.67	5.22
 **8**	100-101 (57)	4.78	4.93	4.78	3.92	3.00	3.97
 **9**	115-116 (58)	8.63 (8.93)	8.67 (8.45)	7.92 (8.66)	6.69	5.61	6.90
 **10**	151-155[b] (88)	8.53 (8.79)	8.23 (8.03)	7.61 (8.39)	6.25	5.40	6.85
 **11**	98-99 (64)	8.27 (7.42)*	8.01 (6.67)*	7.48 (7.11)*	5.42	4.55	5.70
 **12**	165-167[b] (45)	7.59 (6.64)	7.60 (7.46)	7.07 (7.61)*	5.66	5.17	5.71
 **Clonidine**		8.10 (8.09)	8.21 (6.78)	7.25 (7.26)	6.29	5.62	6.01

*Partial Agonism

[a] $pKi = -\log(IC_{50}/(1+[Ligand]/Kd)$

$pEC_{50} = -\log([Drug]$ inducing 50% of effect)

Standard Error Mean < 0.23 (Average of 2-4 experiments)

[b] Fumarate Salt

receiving rilmenidine may be due to its low efficacy (relative to clonidine) at the α_2 receptors rather than due to α_2 subtype selectivity.

CONCLUSION

A convenient synthesis of 2-amino-2-oxazolines including rilmenidine has been described. Several of these compounds show binding specificity for the α_2 receptors but only moderate selectivity among the α_2 subtypes. Further structure-activity relationship studies on this class of compounds will be reported in due course.

REFERENCES

1. Verbeuren, T.J.; Jordaens, F.H.; Zonnekeyn, L.L.; Herman, A.G. *Arch. Int. Pharmacodyn.* **1986**, *284*, 38-52.
2. Bousquet, P.; Feldman, J.; Bricca, G.; Dontenwill, M.; Belcourt, A. *Eur. J. Pharmacol.* **1990**, *183*, 851.
3. Bylund, D.B. *Trends Pharmacol. Sci.* **1988**, *9*, 356-361.
4. Lanier, S.M.; Downing, S.; Duzic, E.; Homcy, C.J. *J. Biol. Chem.* **1991**, *266*, 10470-10478.
5. (a) Hall, J.M.; Caulfield, M.P.; Watson, S.P.; Guard, S. *Trends Pharmacol. Sci.* **1993**, *14*, 376-383.
   (b) Link, R.; Daunt, D.; Barsh, G.; Chruscinski, A.; Kobilka, B. *Mol. Pharmacol.* **1992**, *42*, 16-27.
6. (a) Kobilka, B.K.; Matsui, H.; Kobilka, T.S.; Yang-Feng, T.L.; Francke, U.; Caron, M.G.; Lefkowitz, R.J.; Regan, J.W. *Science* **1987**, *238*, 650-656.
   (b) Weinshank, R.L.; Zgombick, J.M.; Macchi, M.; Adham, N.; Lichtblau, H.; Branchek, T.A.; Hartig, P.R. *Mol. Pharmacol.* **1990**, *38*, 681-688.
   (c) Regan, J.W.; Kobilka, T.S.; Yang-Feng, T.L.; Caron, M.G.; Lefkowitz, R.J.; Kobilka, B.K. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 6301-6305.
7. (a) Ordway, G.A.; Jaconetta, S.M.; Halaris, A.E. *J. Pharmacol. Exp. Ther.* **1993**, *264*, 967-976.
   (b) De Vos, H.; Vauquelin, G.; De Keyser, J.; De Backer, J.-P.; Van Liefde, I. *J. Neurochem.* **1992**, *58*, 1555-1560.
   (c) Lawhead, R.G.; Blaxall, H.S.; Bylund, D.B. *Anesthesiology* **1992**, *77*, 983-991.
8. Reis, D.J.; Regunathan, S.; Wang, H.; Feinstein, D.L.; Meeley, M.P. *Fundam. Clin. Pharmacol.* **1992**, *6*, Suppl. 1, 23s-29s.
9. (a) Gomez, R.E.; Ernsberger, P.; Feinland, G.; Reis, D.J. *Eur. J. Pharmacol.* **1991**, *195*, 181-191.
   (b) Bousquet, P.; Feldman, J.; Tibirica, E.; Bricca, G.; Greney, H.; Dontenwill, M.; Stutzmann, J.; Belcourt, A. *Am. J. Hypertension* **1992**, *5*, 47s-50s.
10. Tesson, F.; Prip-Buus, C.; Lemoine, A.; Pegorier, J.-P.; Parini, A. *J. Biol. Chem.* **1991**, *266*, 155-160.
11. (a) Wong, W.C.; Gluchowski, C. *206th American Chemical Society National Meeting*, Chicago, IL, August 22-27, **1993**, *MEDI #51*.

(b) Jeon, Y.T.; Luo, C.; Gluchowski, C.; Forray, C.; Vaysse, P.J.-J.; Branchek, T.A.; Bard, J.; Weinshank, R.L.; Hartig, P.R. *206th American Chemical Society National Meeting*, Chicago, IL, August 22-27, **1993**, *MEDI #52*.

12. (a) Timmermans, P.B.M.W.M. *Receptor Pharmacology and Function*; Williams, M.; Glennon, R.A.; Timmermans, P.B.M.W.M., Ed.; Marcel Dekker: **1989**; pp. 133-185.
(b) Karjalainen, A.J.; Virtanen, R.E.; Karjalainen, A.L.; Kurkela, K.O.A. *European Patent Application* #183492, **1986**; *Chem. Abstr.* **1986**, *105*, 115070.
(c) Lammintausta, R.A.S.; Virtanen, R.E.; Riekkinen, P.J.; Riekkinen, Jr., P.; Sirvio, J.S.I.; Miettinen, R.A.; Valjakka, A.; Airaksinen, M.M.; Nieminen, S.A.; Macdonald, E. *U.K. Patent Application* #2244431, **1991**; *Chem. Abstr.* **1992**, *116*, 143885.
(d) Bloor, B.C. *Anaesthetic Pharmacol. Review* **1993**, *1*, 221-232.
13. This work was presented at the *205th American Chemical Society National Meeting*, Denver, CO, March 28-April 2, **1993**, *MEDI #132*.
14. (a) Malen, C.; Desnos, M.; Laubie, M.; Poignant, J.-C. *German Patent* #2362754, **1974**; *Chem. Abstr.* **1974**, *81*, 105533.
(b) Peet, N.P.; Sunder, S. *U.S. Patent* #4064348, **1977**; *Chem. Abstr.* **1978**, *88*, 121154.
15. Smith, Jr., D.H. *U.S. Patent* #4256755, **1981**; *Chem. Abstr.* **1981**, *95*, 12764.
16. Mitchell, M.A.; Benicewicz, B.C. *Synthesis* **1994**, 675-677.
17. Not previously reported in the literature.
18. Available from Aldrich Chemical Co.
19. Forray, C.; Bard. J.A.; Wetzel, J.M.; Chiu, G.; Shapiro, E.; Tang, R.; Lepor, H.; Hartig, P.R.; Weinshank, R.L.; Branchek, T.A.; Gluchowski, C. *Mol. Pharmacol.* **1994**, *45*, 703-708.
20. Adham, N.; Romanienko, P.; Hartig, P.; Weinshank, R.L.; Branchek, T. *Mol. Pharmacol.* **1992**, *41*, 1-7.
21. Savarese, T.M.; Fraser, C.M. *Biochem. J.* **1992**, *283*, 1-19.
22. Ruffolo, Jr., R.R.; DeMarinis, R.; Wise, M.; Hieble, J.P. *The Alpha-2 Adrenergic Receptors*; Limbird, L.E., Ed.; Humana Press: New Jersey, **1988**; pp. 115-186.
23. Hiltmann, R.; Wollweber, H.; Stoepel, K.; Puls, W. *South African Patent* #6705645, **1968**; *Chem. Abstr.* **1969**, *71*, 101842.
24. (a) Gluchowski, C. *European Patent Application* #420606, **1991**; *Chem. Abstr.* **1991**, *115*, 174682.
(b) Munk, S.A.; Ryan, C.; Wong, H.; Gluchowski, C.; Machado, C.; Padillo, E.; Kharlamb, A.; Runde, E.; Burke, J.; Wheeler, L.; Kaplan, L.; Garst, M.; Chandraratna, R. *203rd American Chemical Society National Meeting*, San Francisco, CA, April 5-10, **1992**, *MEDI # 165*.
25. Dominiak, P. *Cardiovasc. Drugs Ther.* **1994**, *8*, 21-26.

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