Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 16, pp. 1813-1818, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00301-0

SYNTHESIS AND BIOLOGICAL ACTIVITY OF ENANTIOMERS OF A CONFORMATIONALLY RESTRICTED MUSCARONE ANALOG

Edwin S.C. Wu,* Robert A. Mack, Alex Kover, James T. Loch III, George Mullen, Robert J. Murray, John C. Gordon,† Anthony C. Machulskis,† Sally A. McCreedy,† James C. Blosser†

Department of Chemistry and Department of Biology,† Astra Research Corporation, P.O. Box 20890, Rochester, N.Y. 14601, USA

Abstract: A short and efficient synthesis of both enantiomers of 2,8-dimethyl-1-oxa-8-azaspiro[4.5]-decan-3-one is described. The biological activity of the racemate resides predominantly in the S-enantiomer. While the S-isomer is a full M2-agonist, the R-isomer is devoid of M2 efficacy.

Interest in cholinomimetics for the treatment of cognition dysfunction associated with Alzheimer's disease (AD) has been rekindled by the recent clinical use of tacrine,[1] an acetylcholinesterase inhibitor, and advances of muscarinic agonists xanomeline[2a] and WAL 2014[2b] in clinical trials. We previously reported[3] that 1-oxa-8-azaspiro[4.5]-decan-3-one **1**,[4] a racemate, was a functionally non-selective but highly efficacious muscarinic partial agonist. In light of the well-known fact that enantiomers can exhibit quite different pharmacological profiles and pharmacokinetics from their corresponding racemate,[5,6] the possibility exists that individual enantiomers of **1** might demonstrate unique selectivity with respect to various known muscarinic receptor subtypes. Potential differences for affinity or efficacy of the optical isomers of **1** for M1, M2, and M3 receptors would provide useful information about the unique difference in agonist interactions at these receptor subtypes during the formation of the ligand-receptor complex (binding) and receptor activation (efficacy). In this communication the synthesis and biological activity of the enantiomers of **1** will be described.

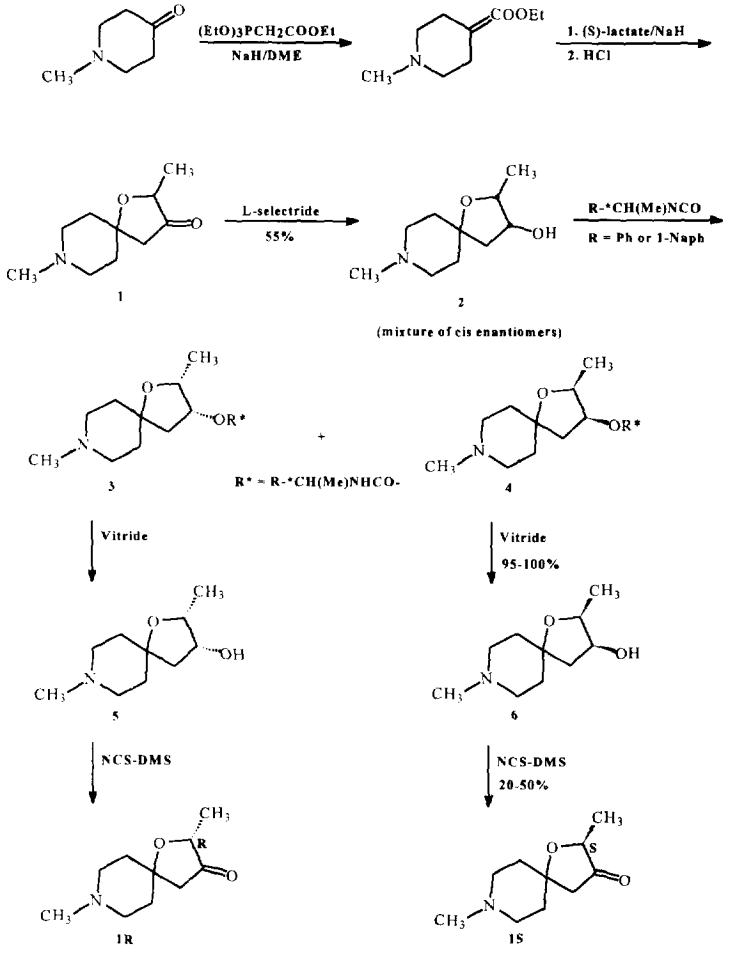
Difficulty in the classical resolution of **1** by fractional crystallization of diastereomeric salts[7a] prompted us to find a new approach to prepare the optical isomers[8] of **1**. As the proton at the chirality center, the C-2 position of the furanone, is acidic, the focal point of our synthetic strategy is to minimize racemization during the synthesis. The best way to achieve this goal is to find a latent synthon of a ketone or to form the ketone group at the end of the synthesis. Retrosynthetically, a precursor to **1** is an alcohol such as **2** with a fixed geometry preferably as the *cis* configuration (Scheme 1). Using the Tsukamoto method[4] the furanone **1** can be prepared in large quantity from 4-methylpiperidone in a two-step synthesis despite the low yield.[7b] This sets the stage for the chiral synthesis of the furanone **1** starting from the racemic

1. The Sandoz report[8] that oxidition of the chiral alcohol **2** to the corresponding chiral ketone **1** without racemization led us to investigate the possibility of preparing the enantiomers of **1** via diastereomeric derivatives of the alcohol **2**.[3]

CHEMISTRY

The preparation of the (S)-(-) and (R)-(+) enantiomers was accomplished as shown in Scheme 1. The Wadsworth-Emmons reaction of triethyl phosphonoacetate (1.25 eq.; 1.1 eq. NaH in ethylene glycol dimethyl ether) with 4-methylpiperidone gave 1-methyl-4-carbethoxymethylidinylpiperidine (85-90% yield)

SCHEME 1



which was cyclized with ethyl (S)-(-)-lactate (4 eq. in THF; NaH 2.38 eq.) followed by decarboxylation in concentrated HCl to **1** (~25% yield).[3,4] As reported by Shapiro, *et al*,[8] selective reduction of **1** with L-Selectride provided the alcohol **2** as a mixture of *cis* enantiomers.[3] The stereochemical outcome of the reduction was also confirmed by comparison with products obtained from the LAH or Vitride reduction.[3] The latter reductions usually gave a mixture of the *trans* and *cis* isomers in a 3:1 ratio. Treatment of **1** with R-(+)-α-methylbenzyl isocyanate in the presence of dibutyltin dilaurate[9a] in refluxing toluene produced a mixture of carbamate diastereomers **3** and **4** from which the 2S,3S,1'R isomer **4** was readily separated as its hydrochloride salt[10] (40% yield of the theoretical maximum 100% of the two diastereomers) with a 99.5-100% diastereomeric excess (de).[11] The rate of carbamation was drastically accelerated when dibutyltin dilaurate was used as a catalyst. The reaction was completed in 3-11 h as compared to 3 days in the presence of *N,N*-dimethylaminoethanol.[9a] Other attempts using the Mosher (MPTA) ester or (+)-camphanic acid ester did not give any separable diastereomers. The Vitride reduction of **4** followed by the Corey-Kim oxidation[12] provided the desired S-(-) enantiomer **1S** with a 98-100% ee (20-50% yield). [13]The configuration at the C-2 position was assigned based on the comparison of the optical rotation for the maleate salt of **1S**: $[\alpha]_D$ = -46.75 (C= 0.2588 in EtOH) with that of the HCl salt of the (S)-isomer of **1**: $[\alpha]_D$ = -58.5 (C= 0.2588 in EtOH) in the literature.[8] Similarly, the use of S-(-)-α-methylbenzyl isocyanate afforded the corresponding R-(+) enantiomer **1R**.[13c] Furthermore, R- or S-1-(1-naphthyl)ethyl isocyanate could be substituted for the α-methylbenzyl analogs (under similar conditions) but were less efficient and more expensive. Thus, synthesis of the chiral isomers of **1** using R- or S-1-(1-naphthyl)ethyl isocyanate was proven to be an effective process. In this process the chiral isocyanate could be recycled when the carbamate **3** or **4** was treated with trichlorosilane or with sodium ethoxide followed by trichlorosilane.[9a,9b] This 2-step recycling procedure gave the cis-alcohol **5** or **6** in 83-93% yield with retention of chirality.

RESULTS AND DISCUSSION

As shown in Table 1, the racemate **1** is a highly efficacious muscarinic agonist. The affinities of the S-enantiomer are similar (M1, Ki = 4.1 μM; M2, 2.5 μM) to or higher (M3, K_A = 0.28 μM) than those of the racemate at muscarinic receptor subtypes, however, the R-isomer demonstrates an approximate order of magnitude lower affinity. Both the S- and R-isomers display higher affinity at the M3 receptor with selectivity of approximately 10- and 6-fold, respectively, over M1 and M2 receptors. The binding activity of the racemate appears to reside predominantly in the S-enantiomer, with R/S affinity ratios at the M1, M2 and M3 receptors of 6.3, 14.8 and 16 respectively. Examination of the intrinsic activities of the optical isomers reveals that differences in efficacy between the enantiomers are qualitatively distinct from the affinity profiles. Thus, the S- and R-enantiomers exhibit similar intrinsic activity at M1 and M3 subtypes,

while the S-isomer is nearly a full agonist and the R-isomer is essentially *inactive* at the M2 receptor. That the affinity can not be used to predict the efficacy of muscarinic agonists is well documented and , in fact, the G-protein coupling site (efficacy site) has been postulated to be distinct from the ligand recognition site (binding site).[14] On the basis of this model it would appear that the R-isomer has lower affinity for a recognition or binding site at all three receptor subtypes but it can still be accommodated in the efficacy site for M1 and M3, but not M2, receptors. Therefore, the R-isomer is a low affinity agonist which is selective for M1 and M3 receptors by virtue of intrinsic activity. The S-isomer, on the other hand, is a relatively high affinity M3 agonist with 10-fold or greater binding selectivity for M3 than M2 or M1 receptor subtypes. In summary, this study indicates that while the biological activity of the racemate resides predominantly in the S-enantiomer, differences in functional and binding selectivity of individual enantiomers for different receptor subtypes exist which can provide insight for drug design.

TABLE 1. Affinities and Intrinsic Activities of 1S and 1R at the Three Muscarinic Receptor Subtypes[15]

	AFFINITY (μM)			INTRINSIC ACTIVITY (%)[a]		
Compound	^{3}H-Pirenz[b] Ki (M1)	^{3}H-NMS[c] Ki (M2)	K_A (M3)[d]	Hip[e] (M1)	Heart[f] (M2)	Tr[g] (M3)
1	3.8	5.3	0.70	64	100	83
S-isomer (**1S**)	4.1	2.5	0.28	61	84	84
R-isomer (**1R**)	26	37	4.5	49	7	76
Pilocarpine	3.1	9.8	1.8	37	48	63

[a]The maximum response in reference to that of carbachol. [b][^{3}H]pirenzepine; a radioligand displacement assay in rat hippocampal membranes. [c][^{3}H] N-methyl-scopolamine; a radioligand displacement assay in rat heart membranes. [d]Ka is a dissociation constant derived from potency in an isolated tissue assay (guinea pig trachea). [e]Stimulation of phosphatidylinositol (PI) hydrolysis in rat hippocampal slices. [f]Inhibition of adenylate cyclase in rat heart membranes. [g]Isometric contractionns of the isolated guinea pig trachea.

Acknowledgements: We are indebted to Mr. Bruce Shepardson for his excellent assistance in the separation of enantiomers or diastereomers by chiral HPLC and Mr. Patrick R. Verhoest and Matthew J. Snyder for their technical assistances in the synthetic work.

References and Notes

1. Catabelos, R.; Norberg, A.; Caamano, J.; Franco-Maside, A.; Fernadez-Novoa, L.; Gomez, M.

J.; Alvarez, X. A.; Takeda, M.; Prous, J. Jr.; Nishimura, T., Winblad, B. *Drugs Today*, **1994**, *30*, 295.

2. a) Hannon, H. E.; Bymaster, F. P.; Calligaro, D. O; Greenwood, B; Mitch, C. H; Sawyer, B. D.; Ward, J. S.; Wong, D. T; Olesen, P. H; Sheardown, M. J.; Swedberg, D. B.; Suzdak, P. D.; Sauerberg, P. *J. Pharmacol. Exp. Ther.* **1994**, *269*, 271. b) Prous, J.; Mealy, N.; Castaner, J. *Drugs Future*, **1994**, *19*, 35.
3. Wu, E. S. C.; Griffith, R. C.; Loch, J. T. III; Kover, A.; Mullen, G. B.; Murray, R. J.; Blosser, J. C.; Machulski, A. C.; McCreedy S. A. *J. Med. Chem.* **1995**, *38*, 1558.
4. Tsukamoto, S.-I.; Nagaoka, H.; Usuda, S.; Harada, M.; Tamura, T. U.S. Patent 4 940 795, **1990**; *Chem. Abstr.* **1989**, *111*, 194613f.
5. Ariens, E. J. *TIPS*, **1986**, *7*, 200.
6. Ariens, E. J. *Eur. J. Clin. Pharmacol.* **1984**, *26*, 663.
7. a) Attempted resolution of the enantiomers employing salts of **1** with tartaric acid and its derivatives was not very successful. This failure might, in part, be due to racemization of the ketone during fractionalization. b) The yield was improved (~25%) when a modified condition (THF replacing ether as a solvent) was used.
8. Shapiro, G.; Buechler, D.; Hennet, S. *Tetrahedron Lett.* **1990**, *31*, 5733.
9. a) Pirkle, W. H.; Hoekstra, M. S. *J. Org. Chem.* **1974**, *39*, 3904. b) Pirkle, W. H.; Hauske, J. R. *J. Org. Chem.* **1977**, *42*, 2781.
10. 2S,3S,1'R-2,8-Dimethyl-1-oxa-8-azaspiro[4.5]decan-3-yl N-(1-phenylethyl)carbamate (**4**, R = Ph): Under a nitrogen atmosphere, a solution of **2** (6.28 g, 33.9 mmol), R-(+)-1-phenylethyl isocyanate (5.4 mL, 37.7 mmol), and dibutyltin dilaurate (0.11 mL, 0.65 mmol) in anhydrous toluene (170 mL) was heated at reflux for 3.5 h, cooled to ambient temperature, and concentrated. The residue was dissolved in CH_2Cl_2 (300 mL), washed successively with saturated aqueous $NaHCO_3$ (50 mL) and brine (50 mL), dried ($MgSO_4$), and evaporated. The residue was treated with anhydrous HCl in 2-propanol to obtain 4.91 g (39%) of the S,S,R-diasteriomer **4** (R = Ph) as its HCl salt: 99.97% dp; CIMS (M+H)$^+$ *m/z* 333.
11. The purity of all carbamates, alcohols and enantiomers of **1** were determined by chiral HPLC, using a 25 cm CHIRALCEL OD column (Chiral Technologies, Inc.) at λ = 210 nm. (Abbreviation: dp = diasteriomeric purity). Typically, the carbamate diastereomers and *cis* alcohol enantiomers were produced in 99.5-100% de and ee respectively, whereas the S- and R-enantiomers of **1** were obtained in 98-100% ee.
12. Corey, E. J.; Kim, C. U. *J. Am. Chem. Soc.* **1972**, *94*, 7586.
13. a) 2S,3S-2,8-Dimethyl-1-oxa-8-azaspiro[4.5]decan-3-ol (**6**): 2S,3S,1'R-(1-phenylethyl)carbamate **4** (1.67 g, 5 mmol) was added portionwise under nitrogen over 30 min to a solution of 3.4 M Vitride (6.6 mL, 22.44 mmol) in anhydrous tetrahydrofuran (50 mL). The reaction was heated at reflux for 2 h, cooled (ice bath), and treated with water dropwise until the precipitated salts formed. The solvent layer was decanted, the salts were extracted with tetrahydrofuran and the combined organics were concentrated. Purification by flash chromatography (78:20:2 ammoniated $CHCl_3$/MeOH/H_2O) gave 908 mg (98%) of S,S-**6**, hydrochloride salt, m.p. >250 °C (2-propanol).

b) S-(-)-2,8-Dimethyl-1-oxa-8-azaspiro[4.5]decan-3-one (**1S**): A solution of dimethylsulfide (8.7 mL, 120 mmol) in dry CH_2Cl_2 (23 mL) was added dropwise to a solution of N-chlorosuccinimide (12.4 g, 92 mmol) in ice-cold dry CH_2Cl_2 (380 mL) under nitrogen, producing a white precipitate. The suspension was stirred at 0 °C for 90 min, then cooled to -28 °C. A solution of the S,S-alcohol **6** (11.5 g, 62 mmol) in CH_2Cl_2 (78 mL; adjusted to pH 6 using trifluoroacetic acid) was added dropwise to the reaction. The resulting mixture was stirred at -28 °C (90 min) and treated a solution of triethylamine (12.9 mL, 92 mmol) in CH_2Cl_2 (28 mL). The reaction was stirred for an additional 5 min, allowed to warm to ambient temperature, treated with saturated aqueous $NaHCO_3$ (320 mL), and stirred vigorously for 15 min. The layers were separated, extracted with chloroform (3 x 500 mL), the organic layers were dried ($MgSO_4$), filtered, and

acidified with 2.2 equiv of maleic acid in 2-propanol. The salt was collected; mp 153-155 °C (ethyl acetate), CIMS $(M+H)^+$ *m/z* 184; optical rotation for the maleate salt: [α] = -46.75 C=0.2588 in EtOH ([α] = -58.5 C=0.2588 in EtOH for the HCl salt of (S)-**1** isomer in the literature[8]).

c) R-(-)-2,8-Dimethyl-1-oxa-8-azaspiro[4.5]decan-3-one (**1R**): Prepared from 0.44 g (2.4 mmol) of R,R-**5** using the method described above, giving 100 mg (18%) of R-3 as its maleate salt, m.p. 148-149 °C (ethyl acetate), CIMS $(M+H)^+$ *m/z* 184.

14. a) Freedman, S. B.; Harley, E. A.; Iversen, L. L. *Br. J. Pharmacol.* **1988**, *93*, 437. b) Saunders, J.; Freedman, S. B. *Trends Pharmacol. Sci. Suppl.* **1989**, 70. c) Saunders, J. *Drug Des. Discovery*, **1993**, *9*, 213.

15. M1 Receptors: Affinity for M1 receptors was measured in a binding assay using rat hippocampal membranes in the presence of 10 nM [^{3}H]pirenzepine.[16] The assay buffer, 50 mM sodium phosphatepH 7.4, contained 0.3 mM $MgCl_2$ and 0.1 mM GppNp. Nonspecific binding for this and the M2 assay below was measured in the presence of 1 μM atropine. Bound radioactivity was collected by vacuum filtration and measured by liquid scintillation. Binding parameters were estimated with the aid of a nonlinear iterative curve fitting program developed by Lundon.[17] Intrinsic activity at M1 receptors was estimated by measuring stimulation of phosphatidylinositol (PI) hydrolysis in rat hippocampal tissue by a modification of the method described by Crews et al.[18,19] Results are expressed as a percent of hydrolysis rates in the presence of carbachol.

M2 Receptors

M2 receptor affinity was determined in rat heart membranes in 20 mM HEPES Krebs buffer containing 1.2 mM $MgCl_2$, 0.1 mM GppNp, and 0.3 nM [^{3}H]N-methyl-scopolamine (NMS).[20] Ki values were estimated as described above.

M2 inhibition of adenylate cyclase (AC) was measured in rat heart membranes.[21,22] Intrinsic activity was determined as described above with carbachol as the reference full agonist.

M3 Receptors

Agonist parameters at M3 were determined by measuring isometric contractions of the isolated guinea pig trachea, suspended in an organ bath in a Krebs buffer.[23,24] Cumulative concentration response curves were recorded for a full agonist, carbachol and the test compound on each of four tissues. Affinity and intrinsic activity were derived using a BMDP-based non-linear statistical curve fitting program based on the operational model.[24,25] Affinity, K_A, is the dissociation constant for the agonist-receptor complex.

16. Luthin, G. R.; Wolf, B. B. *J. Pharmacol. Exp. Ther.* **1984**, *228*, 648.
17. Lundon Software, Inc., "AccuFit Competition", Copyright 1989, Beckman Instruments.
18. Gonzales, R. A.; Crews, F. T. *J. Neurosci.* **1984**, *4*, 3120.
19. Pontzer, N. J.; Crews, F. T. *J. Pharmacol. Exp. Ther.* **1990**, *253*, 921.
20. Freedman, S. B.; Beer, M. S.; Harley, E. A. *Eur. J. Pharmacol.* **1988**, *156*, 133.
21. Ehlert, F. J. *J. Pharmacol. Exp. Ther.* **1986**, *240*, 23.
22. Salomon, Y.; Loudos, C.; Rodbell, M. *Anal. Biochem.* **1974**, *58*, 541.
23. Morrison, K. J.; Vanhoutte, P. M. *Br. J. Pharmacol.* **1992**, *106*, 672.
24. Leff, P.; Dougall, I. G.; Harper, D. *Br. J. Pharmacol.* **1993**, *110*, 239.
25. Black, J. W.; Leff, P. *Proc. Royal Soc. Lond.* **1983**, *220*, 141.

(*Received in USA* 1 *June* 1995; *accepted* 3 *July* 1995)