Synthesis of spiroisoxazolines through cycloadditions of nitrile oxides with 3-methylenequinuclidine

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This paper is dedicated to Professor James M. Coxon (Jim) on the occasion of his 65th birthday, and in recognition of his many contributions to chemistry

Abstract

Four spiroisoxazolines have been prepared, by cycloaddition of aceto-, pivalo-, benzo- and mesito-nitrile oxide with 3-methylenequinuclidine. The reactions were completely regioselective, within the limits of detection. None of the isoxazolines showed significant inhibition of acetylcholinesterase.

Keywords: Isoxazolines, nitrile oxides, cycloadditions, quinuclidine, Alzheimer’s disease, acetylcholinesterase

Introduction

Alzheimer’s disease is the most common cause of adult onset dementia. It is an incurable neuropsychiatric condition, where progressive impairment of cognitive functions, as well as affective and behavioral disturbances are observed in sufferers of the disease. Through both human and animal studies, a direct correlation has been established between the severity of memory impairment and a decline in choline acetyltransferase activity. This enzyme is responsible for production of the neurotransmitter acetylcholine (1) in the brain. Consistent with this observation, compounds that increase acetylcholine (1) concentration are beneficial in alleviating cognitive deficits associated with the disorder and, currently, the only marketed treatments for Alzheimer’s disease are inhibitors of acetylcholinesterase, the enzyme responsible for hydrolytic breakdown of acetylcholine (1) to choline (2) (Scheme 1).
Acetylcholine (1) mediates a diverse range of responses, both excitatory and inhibitory, via muscarinic receptors in the central nervous system and periphery. An alternative approach to developing pharmaceutical agents for the treatment of Alzheimer’s disease involves identifying muscarinic agonists. Clinical trials of some of these, such as arecoline (3), pilocarpine (4) and RS86 (5), have shown modest alleviations of cognitive deficits in patients.

Assays of structures bearing the quinuclidine and 1-azabicyclo[2.2.1]heptane cores tethered to oxadiazole rings and oximes, such as 6 and 7, and of compounds incorporating the quinuclidine nucleus spiro-annellated to another heterocyclic ring via a spirolide bridge, such as the dioxolane cis-AF30A (8) and its closely related analogue, the oxathiolane cis-AF102B (9), have also indicated muscarinic activity. Given the activity of these compounds, we set out to prepare the structurally related spiroisoxazolines 10a–d, as potential acetylcholinesterase inhibitors and muscarinic agonists.
Results and Discussion

The spiroisoxazolines 10a–d were prepared through cycloadditions of nitrile oxides with 3-methylenequinuclidine (12). This was obtained by base neutralization of the commercially available hydrochloride salt of the ketone 11, followed by Wittig olefination using methyltriphenylphosphonium bromide (Scheme 2). Initially, the alkene 12 was synthesized using sodium amide as the base, but better yields were obtained using n-butyllithium.

Scheme 2. Reagents and conditions: i. 11 (1.0 equiv.), MePh3PBr (2.0 equiv.), n-BuLi (1.5 equiv.), THF, −40°C for 40 min, reflux for 16 h.

The methods used to access the nitrile oxides 16a–d and their precursor hydroximinoyl chlorides 15a–d are summarized in Scheme 3. Acetaldoxime (14a) and benzaldoxime (14c) were obtained commercially. Pivaldoxime (14b) and mesitaldoxime (14d) were prepared through oximation of pivaldehyde (13b) and mesitaldehyde (13d), respectively. The hydroximinoyl chlorides 15a–d were prepared through chlorination of the oximes 14a–d using N-chlorosuccinimide. Due to the propensity of the nitrile oxides 16a–c to dimerize, they were generated in situ through dehydrohalogenation of the corresponding hydroximinoyl chlorides 15a–c with 3-methylenequinuclidine (14), which functioned as an internal base in the cycloaddition reactions. Mesitonitrile oxide (16d), which by contrast is inert to dimerization, was pregenerated and used directly in the reaction with the dipolarophile 12.

Scheme 3. Reagents and conditions: for 15a: ii. 14a (1.0 equiv.), NCS (1.0 equiv.), DMF, 20–25°C, 3.5 h; for 15b: i. 13b (1.0 equiv.), NH2OH.HCl (1.1 equiv.), 50% w/v aq. NaOH (1.1 equiv.), 30% aq. EtOH, 18°C, 3 h; ii. 14b (1.0 equiv.), NCS (1.0 equiv.), DMF, 20–25°C, 3.5 h; for 15c: ii. 14c (1.0 equiv.), NCS (1.0 equiv.), DMF, 20–25°C, 3.5 h; for 16d: i. 13d (1.0 equiv.), NH2OH.HCl (1.1 equiv.), 50% w/v aq. NaOH (1.1 equiv.), 30% aq. EtOH, 18°C, 3 h; ii. 14d (1.0 equiv.), NCS (1.0 equiv.), DMF, 20–25°C, 3.5 h; iii. 15d (1.0 equiv.), NEt3 (1.2 equiv.), Et2O, 18°C, 2 h.

Preparation of the spiroisoxazoline 10a (Scheme 4) was initially hampered due to difficulties associated with the short half-life of acetonitrile oxide (16a) and the instability of the
hydroximinoyl chloride 15a. After extensive experimentation, the optimum procedure was eventually formulated. It involved addition of acetohydroximinoyl chloride (15a) to a two-fold excess of the alkene 12 over 48 hours at 20°C and stirring at 20°C for a further 24 hours. This was followed by addition of another equivalent of acetohydroximinoyl chloride (15a) over 48 hours, and stirring for a further 24 hours. Temperature control was crucial for reaction. To prevent decomposition of the hydroximinoyl chloride 15a, it was kept at a temperature of 4°C whilst the reaction mixture was maintained at 20°C to promote the cycloaddition. This was accomplished by carrying out the reaction in a 4°C cold room and the reaction vessel containing the dipolarophile 12 was then kept at 20°C using a heated oil bath. This procedure afforded the spiroisoxazoline 10a in 82% yield.

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\begin{align*}
12 & \xrightarrow{i} 10 \\
\text{a} & \quad \text{R} = \text{Me} \\
\text{b} & \quad \text{R} = \text{t-Bu} \\
\text{c} & \quad \text{R} = \text{Ph} \\
\text{d} & \quad \text{R} = 2,4,6-\text{Me}_3\text{C}_6\text{H}_2
\end{align*}
\]

**Scheme 4. Reagents and conditions:** for 10a: i. 15a (0.5 equiv.) at 4°C added over 48 h, 12 (1 equiv.), CH₂Cl₂, 20°C, 24 h; for 10b: i. 15b (0.5 equiv.) at 4°C added over 48 h, 12 (1 equiv.), CH₂Cl₂, 20°C, 24 h; for 10c: 15c (0.5 equiv.) at 4°C added over 48 h, 12 (1 equiv.), CH₂Cl₂, 20°C, 24 h; for 10d: 15d (1 equiv.), 12 (1 equiv.), THF, reflux, 3 days.

Similar methods were used to prepare the spiroisoxazolines 10b and 10c (Scheme 4), which were obtained in yields of 82 and 90%, respectively. The synthesis of the mesitylspiroisoxazoline 10d was technically more straightforward, since mesitonitrile oxide (16d) is much less prone to dimerization. Reaction of the dipole 16d with the alkene 12 in refluxing THF gave rise to the isoxazoline 10d in 85% yield (Scheme 4).

The cycloadditions of the nitrile oxides 16a–d with the alkene 12 were completely regioselective, to the extent that no other cycloadducts were detected in NMR spectra of the crude product mixtures. This may be attributed to steric effects controlling the reactions, such that the oxygen of the dipoles 16a–d becomes bonded to the most substituted olefinic carbon of the dipolarophile 12. Good yields of the isoxazolines 10a–d were obtained, which is particularly noteworthy for nitrile oxide cycloadditions to a 1,1-disubstituted alkene that is not activated by conjugating substituents.

The spiroisoxazolines 10a–d were evaluated as acetylcholinesterase inhibitors, using a combination of the methods developed by Ellman et al., and Devonshire and Moores. In this assay, the iodide of acetylthiocholine (17) is used as the enzyme substrate. In this regard it has been shown to be a satisfactory substitute for acetylcholine (1) and inhibition of the enzyme is then indicated by a reduction in the rate of production of thiocholine (18), as detected through its
reaction with the disulfide 19 to give the yellow thionitrobenzoate anion 20 (Scheme 5). Prior to screening the activity of the spiroisoxazolines 10a–d, the validity of the assay was evaluated using the known acetylcholinesterase inhibitor, Paraoxon®, which showed 89% inhibition of the enzyme when used at a concentration of 1 mg/mL. However, none of the spiroisoxazolines 10a–d displayed any significant enzyme inhibition when used at this concentration. To date it has not been practical for us to investigate the activity of these compounds as muscarinic agonists.

Scheme 5

Experimental Section

General Procedures. Acetaldoxime (14a), pivaldehyde (13b), benzaldoxime (14c), mesitaldehyde (13d), the iodide of acetylthiocholine (17), the disulfide 19, eserine sulfate and bovine erythrocyte acetylcholinesterase were purchased from Sigma–Aldrich Chemical Co. 3-Quinuclidinone (11) was prepared from the hydrochloride salt that had also been purchased from Sigma–Aldrich Chemical Co. The oximes 14b and 14d, the hydroximinoyl chlorides 15a–d and the nitrile oxide 16d were prepared using literature procedures.¹⁸

3-Methylenequinuclidine (12). To a stirred suspension of methyltriphenylphosphonium bromide (15.6 g, 43.5 mmol) in dry THF (150 mL) at −78°C (dry ice–acetone bath), n-BuLi (13.1 mL of a 2.5 M solution in hexanes, 32.7 mmol) was added dropwise. The mixture was allowed to warm to −40°C and stirred for 40 min, then it was re-cooled to −78°C (dry ice–acetone bath) and a solution of 3-quinuclidinone (11) (2.70 g, 21.6 mmol) in dry THF (50 mL) was added dropwise over 15 min. The mixture was then heated at reflux for 16 h, before it was cooled to 18°C and neutralized through the dropwise addition of aq. NH₄Cl (10 mL). After the solvent was removed under reduced pressure, Et₂O (500 mL) was added to the residue. The resultant suspension was filtered through a pad of celite and the filter cake was washed with Et₂O.
(5 x 100 mL). The combined filtrates were concentrated to ca. 100 mL and that solution was extracted with 1M HCl (3 x 50 mL). The extracts were combined and washed with CH₂Cl₂ (5 x 50 mL), then adjusted to pH 12 with 1M NaOH and extracted with Et₂O (5 x 100 mL). The combined organic extracts were washed with brine (1 x 100 mL), dried (anhydrous Na₂SO₄) and filtered. The filtrate was concentrated under reduced pressure to afford the title compound 23 (2.23 g, 84%) as a pale yellow liquid. ¹H NMR (300 MHz) δ 1.70 (m, 4H), 2.70 (m, 1H), 2.88 (m, 4H), 3.49 (s, 2H), 4.66 (m, 1H), 3.49 (s, 2H), 4.66 (m, 1H), 4.78 (m, 1H).

3-Methyl-1-oxa-2,7-diaza-7,10-ethanospiro[4.5]dec-2-ene (10a). In a 4°C cold room, a solution of ace­tohydroximinoyl chloride (15a) (302 mg, 3.25 mmol) in CH₂Cl₂ (10 mL) in a gas-tight syringe was added via a syringe pump over 48 h to a solution 3-methylenequinuclidine (12) (800 mg, 6.50 mmol) in dry CH₂Cl₂ (60 mL) heated to 20°C with an oil bath. After stirring at 20°C for 24 h, another solution of 15a (302 mg, 3.25 mmol) in CH₂Cl₂ (10 mL) at 4°C was added over 48 h to the mixture and stirring was continued for a further 24 h at 20°C. The solution was then extracted with 1M HCl (3 x 75 mL). The combined extracts were washed with Et₂O (2 x 75 mL) then adjusted to pH 12 with 1M NaOH and extracted with EtOAc (5 x 75 mL). The organic extracts were combined, washed with brine (1 x 75 mL), dried (anhydrous Na₂SO₄) and concentrated under reduced pressure. Flash column chromatography of the residue on silica, eluting with CH₂Cl₂:MeOH:10% aq. NH₄OH (63:21:1) afforded the title compound 10a (960 mg, 82%) as a colorless oil. ¹H NMR (300 MHz) δ 1.38–2.10 (m, 4H), 1.97 (s, 3H), 2.40 (m, 1H), 2.68 (d, J = 17.2 Hz, 1H), 2.68–3.00 (m, 4H), 2.92 (d, J = 14.4 Hz, 1H), 3.05 (d, J = 17.2 Hz, 1H), 3.24 (d, J = 14.4 Hz, 1H).

13C NMR (75.4 MHz) δ 13.4, 21.2, 23.6, 30.8, 46.3, 46.6, 48.4, 63.1, 85.3, 154.4. IR (thin film) νmax 2948, 2874, 1630, 1457, 1434, 1388, 1332, 1219, 1072, 1050, 1038, 982, 897, 800, 688, 630 cm⁻¹. LRMS (EI) m/z (%) 180 (M⁺•, 0.8), 167 (6), 149 (21), 139 (63), 124 (7), 111 (3), 96 (100), 82 (43), 69 (49), 55 (44). HRMS (EI) Found m/z 180.1264 (M⁺). C₁₀H₁₆N₂O requires m/z 180.1263.

3-tert-Butyl-1-oxa-2,7-diaza-7,10-ethanospiro[4.5]dec-2-ene (10b). Using the procedure described above for the synthesis of the spiroisoxazoline 10a, reaction of 3-methylenequinuclidine (12) (867 mg, 6.42 mmol) and pivalohydroximinoyl chloride (15b) (867 mg, 6.42 mmol) afforded the title compound 10b (1.17 g, 82%) as a colorless oil, after flash column chromatography on silica, eluting with CH₂Cl₂:MeOH:10% aq. NH₄OH (63:21:1) afforded the title compound 10a (960 mg, 82%) as a colorless oil. ¹H NMR (300 MHz) δ 1.19 (s, 9H), 1.10–2.05 (m, 4H), 2.10–2.23 (m, 1H), 2.72 (d, J = 16.8 Hz, 1H), 2.77–3.01 (m, 4H), 2.94 (d, J = 14.5 Hz, 1H), 3.08 (d, J = 16.8 Hz, 1H), 3.26 (d, J = 14.5 Hz, 1H). ¹³C NMR (75.4 MHz) δ 13.4, 21.2, 23.6, 30.8, 46.3, 48.4, 63.1, 85.3, 154.4. IR (thin film) νmax 2948, 2874, 1630, 1457, 1434, 1388, 1332, 1219, 1072, 1050, 1038, 982, 897, 800, 688, 630 cm⁻¹. LRMS (EI) m/z (%) 222 (M⁺, 4), 165 (100), 149 (2), 139 (57), 122 (16), 111 (6), 96 (62), 82 (37), 69 (26), 55 (20). HRMS (EI) Found m/z 222.1730 (M⁺•). C₁₃H₂₂N₂O requires m/z 222.1732.

3-Phenyl-1-oxa-2,7-diaza-7,10-ethanospiro[4.5]dec-2-ene (10c). Using the procedure described above for the synthesis of the spiroisoxazoline 10a, reaction of 3-methylenequinuclidine (12) (300 mg, 2.44 mmol) and benzohydroximinoyl chloride (15c) (379
mg, 2.44 mmol) afforded the title compound 10c (531 mg, 90%) as a viscous oil, after flash column chromatography on silica, eluting with CH₂Cl₂:MeOH:10%aq. NH₄OH (75:8:1). ¹H NMR (300 MHz) δ 1.42–1.55 (m, 1H), 1.56–1.98 (m, 2H), 1.99–2.03 (m, 1H), 2.21–2.30 (m, 1H), 2.76–2.98 (m, 4H), 3.03 (d, J = 14.5 Hz, 1H), 3.12 (d, J = 16.7 Hz, 1H), 3.36 (d, J = 14.5 Hz, 1H), 3.49 (d, J = 16.7 Hz, 1H), 7.38–7.48 (m, 2H), 7.60–7.68 (m, 3H). ¹³C NMR (75.4 MHz) δ 21.9, 24.2, 31.6, 45.3, 47.0, 47.3, 63.7, 86.3, 127.0, 129.2, 130.4, 130.5, 156.2. IR (thin film) υmax 3430, 2922, 2871, 1596, 1445, 1356, 1318, 1072, 1050, 1013, 980, 923, 801, 759, 692, 645, 545 cm⁻¹. LRMS (EI) m/z (%) 242 (M⁺•, 2), 165 (5), 149 (10), 139 (100), 124 (12), 117 (3), 110 (5), 103 (8), 96 (92), 82 (47), 69 (33), 55 (20). HRMS (EI) Found m/z 242.1418 (M⁺•). C₁₅H₁₈N₂O requires m/z 242.1419.

3-(2,4,6-Trimethylphenyl)-1-oxa-2,7-diaza-7,10-ethanospiro[4.5]dec-2-ene (10d). A mixture of 3-methylenequinuclidine (12) (482 mg, 3.92 mmol) and mesitonitrile oxide (16d) (632 mg, 3.92 mmol) in dry THF (20 mL) was heated at reflux for 3 days, then it was concentrated under reduced pressure. The residue was taken up in CH₂Cl₂ (50 mL) and the solution was extracted with 1M HCl (5 x 50 mL). The combined extracts were washed with Et₂O (2 x 50 mL), then adjusted to pH 12 with 1M NaOH and extracted with EtOAc (3 x 50 mL). The combined organic extracts were washed with H₂O (2 x 50 mL) and brine (1 x 50 mL), then they were dried (anhydrous Na₂SO₄) and concentrated under reduced pressure. Flash column chromatography of the residue on silica, eluting with CHCl₃:MeOH:10%aq. NH₄OH (33:8:1) afforded the title compound 10d (946 mg, 85%), mp 215–217°C. ¹H NMR (300 MHz) δ 1.62–1.80 (m, 2H), 1.82–1.94 (m, 1H), 2.22 (s, 6H), 2.28 (s, 3H), 2.32–2.44 (m, 1H), 2.94–3.28 (m, 5H), 3.00 (d, J = 17.5 Hz, 1H), 3.22 (d, J = 14.5 Hz, 1H), 3.30 (d, J = 17.5 Hz, 1H), 3.54 (d, J = 14.5 Hz, 1H), 6.90 (s, 2H). ¹³C NMR (75.4 MHz) δ 18.7, 19.7, 20.3, 21.0, 30.3, 45.4, 46.0, 48.3, 59.3, 82.7, 125.1, 128.5, 136.2, 139.2, 157.6. IR (thin film) υmax 3435, 2946, 2920, 2654, 2584, 1610, 1486, 1462, 1332, 1248, 1037, 1002, 890, 855, 835, 733, 667, 583 cm⁻¹. LRMS (EI) m/z (%) 284 (M⁺•, 0.7), 214 (0.4), 181 (0.3), 172 (0.5), 149 (5), 139 (100), 124 (10), 111 (7), 96 (100), 82 (49), 69 (31). HRMS (EI) Found m/z 284.1888 (M⁺•). C₁₈H₂₄N₂O requires m/z 284.1889.

Assay for inhibition of bovine acetylcholinesterase. A solution of acetylcholinesterase in water (1 mg/mL, 1 mL) was mixed with a solution of the disulfide 19 in 0.1 M phosphate buffer at pH 7.0 (1 mg/10 mL, 5 mL). Aliquots (200 µL) of the solution, along with a 50 µL aliquot of a solution of the iodide of acetylthiocholine (17) in 0.1 M phosphate buffer at pH 7.0 (75 mg/mL) and a 25 µL aliquot of a solution containing either Paraoxon® or one of the spiroisoxazolines 10a–d in DMSO (1 mg/mL) were incubated at 37°C for 10 minutes, then 25 µL of a 0.1% aqueous eserine sulfate solution was added to terminate the reaction. The extent of reaction was then determined by measuring the absorbance due to the thiolate 20 at 405 nm.
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References and Footnotes