Bioorganic & Medicinal Chemistry Letters, Vol.3, No.12, pp. 2619-2622, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

m-Alkyl α,α,α-Trifluoroacetophenones: A New Class of Potent Transition State Analog Inhibitors of Acetylcholinesterase

H. K. Nair and D. M. Quinn

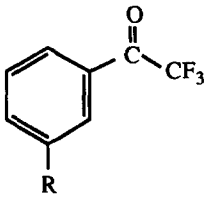
Department of Chemistry, University of Iowa
Iowa City, IA 52242, U. S. A.

(*Received in USA* 12 *August* 1993; *accepted* 26 *August* 1993)

Abstract. A series of *m*-alkyl α,α,α-trifluoroacetophenones (**1-5**) was synthesized and evaluated as inhibitors of acetylcholinesterase from *Torpedo californica*. All ketones (**1-5**) were found to be potent inhibitors of the enzyme; *m*-t-butyl α,α,α-trifluoroacetophenone (**4**) was the most potent inhibitor with a K_i value of 3.7 pM.

Acetylcholinesterase (acetylcholine hydrolase, EC 3.1.1.7) plays a vital role in the central and peripheral nervous systems, where it catalyzes the hydrolysis of the neurotransmitter acetylcholine.[1] In Alzheimer's disease, a neurological disorder, cholinergic deficiency in the brain has been reported.[2] One approach to treat Alzheimer's disease may be via inhibition of the hydrolysis of acetylcholine by reversible inhibitors.[3] Thus, synthesis and study of anticholinesterase compounds may aid the development of therapeutically useful compounds to treat such neurological disorders. As part of our program to develop potent transition state analog inhibitors, we sought to study the *m*-alkyl aryltrifluoroketones (Scheme I) as anticholinesterase compounds. In this communication, we report the synthesis of *m*-alkyl-α,α,α-trifluoroacetophenones, and provide a preliminary account of their inhibition of acetylcholinesterase from *Torpedo californica* (TC-AChE).

Scheme I



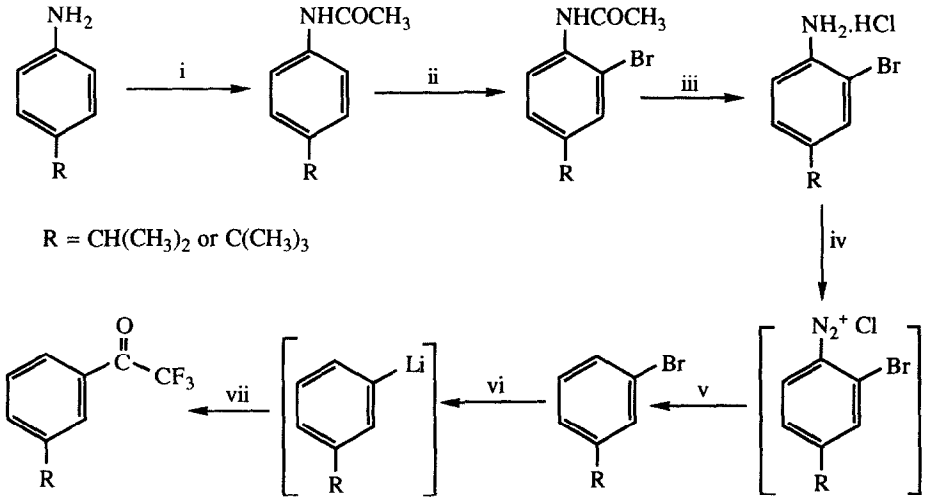
R = CH_3 (**1**), CH_2CH_3 (**2**), $CH(CH_3)_2$ (**3**), $C(CH_3)_3$ (**4**), CF_3 (**5**)

The inhibition of electric eel acetylcholinesterase by trifluoromethyl ketones has been reported.[4] We selected *m*-alkyl α,α,α-trifluoroacetophenones for the following reasons: a) the distance between the carbonyl carbon and the *m*-alkyl group (R) in **1-5** is close to that of the distance between carbonyl carbon and the quartenary nitrogen of acetylcholine (4.9Å),[5] b) the presence of electron withdrawing fluorines in **1-5** will enhance the nucleophilic addition of the active site serine to the carbonyl carbon of

the inhibitors (**1-5**), and c) we wanted to study the effect of hydrophobicity of the *m*-alkyl group (R) on the inhibition of TC-AChE, since the recent crystal structure of the enzyme reveals that the active site is surrounded by hydrophobic residues.[5]

The synthesis of the ketones (**1-5**) was accomplished via *in situ* generation of the lithium reagent from the corresponding *m*-alkyl/trifluoromethyl bromo or iodobenzene and t-BuLi at low temperature; subsequent reaction of the lithium reagent with CF_3CO_2Et afforded the desired ketones.

Scheme II



i) $(CH_3CO)_2O$ ii) glacial CH_3CO_2H, Br_2 iii) conc. HCl, C_2H_5OH, reflux iv) CH_3CO_2H, H_2O, conc. HCl, $NaNO_2$, 0 °C v) H_3PO_2, 0 °C, 72 h vi) t-BuLi, -78 °C vii) CF_3CO_2Et, -78 °C to rt

For **1** and **5**, the respective starting materials, *m*-methyl and *m*-trifluoromethylbromobenzenes, were commercially available; however, for compounds **2**, **3** and **4**, the corresponding bromo/iodo derivatives were not commercially available. *m*-Ethyl iodobenzene was obtained from *m*-ethylaniline via diazotization followed by treatment with KI,[6] while *m*-t-butyl and *m*-isopropyl bromobenzene were obtained from *p*-t-butyl and *p*-isopropyl anilines via a reported procedure[7] (Scheme II). Metal-halogen exchange between *m*-alkyl bromo/iodobenzene and t-BuLi in Et_2O at -78 °C under Ar resulted in the requisite lithium reagent, which on treatment with CF_3CO_2Et afforded the desired ketones in 35-61% yield (based on *m*-alkyl bromo/iodobenzene) (Scheme II). Compounds **2**, **3** and **4** are new; the preparation of **1** and **5** via Grignard reagents has been reported.[8] Pure ketones were obtained, as colorless liquids, on distillation. All ketones were fully characterized by spectral (^{19}F and ^{1}H NMR, FT-IR and GC/MS) analyses; purity was ascertained by elemental analysis.[9] A typical experimental procedure for the preparation of the ketone is given below.

To a solution of *m*-bromo t-butylbenzene (4.7 mmol) in dry Et_2O (15 mL) at -78 °C (dry ice/isopropanol bath), under argon, was added dropwise via syringe 2.8 mL of 1.7 M t-BuLi in pentane. After being stirred for 45 min at -78 °C, the reaction mixture was quickly transferred via syringe to another flask containing a cooled solution (-78 °C) of CF_3CO_2Et (6 mmol) in 10 mL of dry ether, under argon. The mixture was stirred for 10 minutes at -78 °C, then for one hour at rt. The resultant reaction mixture was extracted with Et_2O (50 mL), the Et_2O extract was washed with water (2x20 mL), dried over $MgSO_4$, concentrated and distilled by using a short path distillation apparatus to afford 0.51 g (yield 47%) of *m*-t-butyl trifluoroacetophenone (**4**) as a colorless liquid (bp 62-64 °C/4 mm Hg). Spectral data and elemental analysis follow: ^{19}F NMR ($CDCl_3$) δ -71.7 (s); ^1H NMR ($CDCl_3$) δ 1.37 (s, 9H), 7.48 (t, 1H, J = 8 Hz), 7.75 (m, 1H), 7.89 (m, 1H), 8.12 (s, 1H); GC/MS, m/z (% relative intensity): 230 (M^+, 12), 215 (100), 187 (51), 175 (5), 161 (19), 145 (9), 118 (14), 117 (12), 115 (13), 91 (11), 77 (7), 69 (4). FT-IR (CCl_4): 2971(m), 1716(s), 1197(s), 1170(s), 1148(s) cm^{-1}. Anal. Calcd. for $C_{12}H_{13}OF_3$: C, 62.58; H, 5.69; Found: C, 62.66, H, 5.74.

Compounds **1-5** were assayed by the Ellman method[10] in 0.05 M phosphate buffer, pH 7.26, that contained 0.125 M NaCl, and acetylthiocholine chloride as the substrate. All ketones tested are reversible inhibitors of TC-AChE; K_i values for **1- 5** are given in **Table I**. Compounds **1, 2** and **5** are competitive inhibitors while **3** and **4** are time-dependent inhibitors. Moving the t-butyl group in **4** from the meta to the para position decreases the inhibition potency by ~100 fold.[11] A detailed characterization of inhibition of AChE by trifluoroketones **1- 5** will be reported elsewhere.

Table I. Inhibition of TC-AChE by *m*-R-C_6H_4-C(O)CF_3

R =	K_i in M
CH_3	$2.4x10^{-9}$
C_2H_5	$3.9x10^{-10}$
$CH(CH_3)_2$	$8.5x10^{-12}$
$C(CH_3)_3$	$3.7x10^{-12}$
CF_3	$3.9x10^{-10}$

Acknowledgement. We thank Dr. I. Silman of Weizmann Institute, Rehovot, Israel, for the gift of purified *Torpedo californica* acetylcholinesterase and NIH for financial support (grant NS21334).

References and Notes

1. a) Rosenberry, T. L. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1975**, *43*, 103. b) Quinn, D. M. *Chem. Rev.* **1987**, *87*, 955.
2. Marchbanks, R. M. *J. Neurochem.* **1982**, *39*, 9.

3. a) Kan, J. P. *Eur. J. Med. Chem.* **1992**, *27*, 565. b) Summers, W. K.; Majovski, L. V.; Marsh, G. M.; Tachiki, K.; Kling, A. *N. Engl. J. Med.* **1986**, *315*, 1241. c) Thai, L. J.; Fuld, P. A.; Masur, D. M.; Sharpless, N. S. *Ann. Neurol.* **1983**, *13*, 491. d) Taylor, P. In *Pharmacological Basis of Therapeutics*; Gilman, A. G., Goodman, L. S., Murad, F., Eds.; MacMillan: New York, 1985; pp 110-129.
4. a) Brodbeck, U.; Schweikert, K.; Gentinetta, R.; Rottenberg, M. *Biochim. Biophys. Acta.* **1979**, *567*, 357. b) Gelb, M. H.; Svaren, J. P.; Abeles, R. H. *Biochemistry* **1987**, *24*, 1813. c) Allen, K. N.; Abeles, R. H. *Biochemistry* **1989**, *28*, 8466.
5. Sussman, J. L.; Harel, M.; Frolow, F.; Oefner, G.; Goldman, A.; Toker, L.; Silman, I. *Science*, *253*, **1991**, 872.
6. Vogel, I. *A Text Book of Practical Organic Chemistry*, 4th edition 1978, pp 695-696.
7. Larner, B. W.; Peters, T. A. *J. Chem. Soc.* **1952**, 680.
8. Wagner, P. J.; Lam, H. M. *J. Am. Chem. Soc.* **1980**, *102*, 4167.
9. All ^{19}F and ^{1}H NMR chemical shifts (in ppm, positive shifts downfield) reported are referenced against internal $CFCl_3$ and TMS, respectively. **Compound 1** (yield 42%) bp 25°C/0.05 mm Hg(lit.[8] 60-63 °C/13 mm Hg). ^{19}F NMR ($CDCl_3$) δ -71.8(s); ^{1}H NMR ($CDCl_3$) δ 1.55(s, 3H), 7.43-7.51(m, 2H), 7.88(m, 2H); GC/MS, m/z(%relative intensity): 188(M^+, 29), 119(100), 91(85), 89(13), 69(4). FT-IR(CCl_4) 1723 cm^{-1} (>C=O). Anal. calcd. for $C_9H_7OF_3$: C, 57.43; H, 3.75; Found: C, 57.31, H, 3.72. **Compound 2** (yield 54%), bp 36° C/1 mm Hg. ^{19}F NMR ($CDCl_3$) δ -71.8(s); ^{1}H NMR ($CDCl_3$) δ 1.28(t, 3H, J = 7.6 Hz), 2.74(q, 2H, J = 7.6 Hz), 7.45(t, 1H, J = 7.7 Hz), 7.55(m, 1H), 7.87-7.90(m, 2H). GC/MS, m/z(%relative intensity): 202(M^+, 10), 187(2), 133(100), 105(36), 77(26), 69(4). FT-IR(CCl_4) 2971(w), 1718(s), 1225(s), 1204(s), 1152(vs) cm^{-1}. Anal. calcd. for $C_{10}H_9OF_3$: C, 59.39; H, 4.49; Found: C, 59.49, H, 4.51. **Compound 3** (yield 61%). bp 39 °C/0.8 mm Hg. ^{19}F NMR ($CDCl_3$) δ -71.7(s); ^{1}H NMR ($CDCl_3$) δ 1.29(d, 6H, J = 7 Hz), 3.00(heptet, 1H, J = 7 Hz), 7.46(t, 1H, J = 8 Hz), 7.58(m, 1H), 7.87-7.94(m, 2H); GC/MS, m/z(%relative intensity): 216(M^+, 14), 201(20), 147(100), 131(24), 119(17), 103(17), 77(17), 69(8). FT-IR(CCl_4) 2966(m), 1723(s), 1205(s), 1151(vs) cm^{-1}. Anal. calcd. for $C_{11}H_{11}OF_3$: C, 61.09; H, 5.13; Found: C, 61.18, H, 5.15. **Compound 5** (yield 35%) bp 36 °C/4.8 mm Hg(lit.[8] 68-71 °C/22 mm Hg). ^{19}F NMR ($CDCl_3$) δ -63.7(s,3F), -72.2(s, 3F); ^{1}H NMR ($CDCl_3$) δ 7.71(t, 1H, J = 8.8 Hz), 7.95(d, 1H, J = 7.7 Hz), 8.23(d, 1H, J = 7.9 Hz), 8.30(brs, 1H); GC/MS, m/z(%relative intensity): 242(M^+, 0.4), 223(8), 173(100), 145(93), 119(17), 95(13), 75(13), 69(8). FT-IR (CCl_4) 1731 cm^{-1} (>C=O). Anal. calcd. for $C_9H_4OF_6$: C, 44.62; H, 1.67, Found: C, 44.47, H, 1.67.
10. Ellman, G. L.; Courtney, K. D.; Andres, V. Jr.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88
11. Unpublished work of H. K. Nair.