SYNTHESIS, CHARACTERIZATION AND EVALUATION OF SOME POTENT MEMORY ENHANCING AGENTS DERIVED FROM P-CRESOL

DR. RUCHI MALIK*  
RICHA GUPTA**  
VIRENDRA SHUKLA***  
MANISH SHARMA****  

*Dept. of Pharmacy, Central University of Rajasthan, Bandarsindri, Ajmer, India  
**Dept. of Chemistry, Bhagwant University, Ajmer, Rajasthan, India.  
***Dept. of Pharmaceutical Chemistry, Sardar Bhagwan Singh Institute of Biomedical Sciences & Research, Balawala, Dehradun, India.  
****Dept. of Medicinal and Pharmaceutical Chemistry, B.R.Nahata College of Pharmacy, Mandsaur, M.P. India

ABSTRACT

Memory or cognition dysfunction is a neurodegenerative disorder of brain, in which selective apoptosis of cholinergic neurons, in a specific region of the brain, leads to paucity of acetylcholine resulting to loss of memory and learning functions. The present study aims to develop some newer p-cresol derivatives, as drug candidate, as potential cognition enhancers. p-cresol was condensed with 1-Bromo-3-chloropropane to afford 1-(3-chloropropoxy)-4-methylbenzene which was fused with different heterocyclic/acyclic amines to obtain target compounds 7a-7f. These synthesized compounds were evaluated for their pharmacological activity by using elevated plus maze model at 1, 3 and 5mg/kg of doses. Compounds 7a and 7f were found to be potent memory enhancers at 5mg/kg of dose with percent retention of 65.43±4.17 and 63.45±5.34 when compared to reference drug piracetam.

KEYWORDS: Acetylcholine, Cognition dysfunction, Cognition enhancer, Elevated plus maze, p-cresol

INTRODUCTION

Alzheimer’s disease (AD), vascular and mixed dementia are the three commonest forms of dementia. Alzheimer’s disease is the most common cause of dementia in the elderly and increasingly significant health concern in our aging population. In the past 10 years, our understanding of this disease has increased dramatically. Due to the rapid pace of recent advances, it has not been easy for the health care professionals, researchers and the general public to keep abreast of these developments (Swartz et.al. 1996). The pathophysiology of Alzheimer’s disease is complex and involves several different biochemical pathways. AD is classified as a neurodegenerative disorder. The cause and progression of the disease are not well understood; it is associated with plaques and tangles in the brain (Tiraboschi et.al. 2004).
These include defective beta-amyloid protein metabolism, abnormalities of glutamatergic, adrenergic, serotonergic and dopaminergic neurotransmission, and the potential involvement of inflammatory, oxidative and hormonal pathways. Consequently, these pathways are all potential targets for Alzheimer’s disease treatment and prevention strategies (Doraiswamy et.al. 2002). The literature survey conducted on the various classes of antiamnesic agents showed that antiamnesic activity resides in a wide variety of chemical entities. This survey enlisted the 2-pyrrolidinones, dialkylaminoethylethanols and ethers, acetamide derivatives etc. Drugs like alkyl aminoethyl ethers, 4-alkoxy quinazolines (John Humprey et.al. 2006) and 2-naphthyloxyderivatives (Piplani et.al. 2004), which are most stable analogues of choline-O-acetate are quite active in amnesia reversal. Based on the structural data some common features can be deduced in all the pharmacological divergent classes i.e. the basic nitrogen which may be part of heteroaromatic ring or cyclic/acyclic system intended to interact electrostatically with the appropriate target, a hydrogen bond acceptor function and an appropriate linker group giving optimal spacing to the hydrogen bond acceptor function from the basic nitrogen.

The nootropics bifemelane (1), indeloxazine (2) and piracetam (3) also demonstrate certain structural resemblance to various cholinergic compounds in containing one hydrogen bond acceptor moiety and a basic nitrogen (Weiser 2004). An introspection of the active compounds of different types reveals the correlation of the compounds with the structure of endogenous neurotransmitter acetylcholine and is considered in postulating the design strategy for the compounds included in the current research project.

4-aminophenol derived aryloxy derivatives having structural resemblance to neurotransmitter (acetylcholine) showed significant antiamnesic activity when compared to piracetam. (Malik R. et.al. 2008) In order to continue synthesis of some more cognition enhancers, similar in activity to 4-aminophenol derivatives, p-cresol (4) was exploited as starting material. The –OH group at 1- position permits to introduce various substituent at this position.
MATERIAL AND METHODS

A general synthesis of \(p\)-cresol derivatives is shown in Scheme 1. The intermediate 6 was prepared by the monomolecular nucleophilic substitution reaction mechanism using ethylmethylketone and anhydrous potassium carbonate. The intermediate so formed was fused with different amines to form the target compounds 7a-7f.

Synthesis

Melting point determined by thiele’s tube method using liquid paraffin and was uncorrected. Infrared (IR) spectra were recorded on a Shimadzu (Japan) 8400S FT-IR spectrophotometer model using nujol and potassium bromide and on Perkin Elmer RX1 using potassium bromide cell for liquid sample and potassium bromide pellets for solid samples (cm\(^{-1}\)). Proton–NMR spectra were recorded on Brucker multinuclear FT NMR spectrometer model AV-400, 400 MHz using deuterated-chloroform containing tetramethylsilane as internal standard (chemical shift in \(\delta\), ppm). The spin multiplicities are indicated by symbols, s (singlet), d (doublet), t (triplet), m (multiplet), q (quartet), quin (quintet). The purity of compounds was established by thin layer chromatography (TLC). Precoated silica gel aluminium plates 60F-254 (20 cm X 20 cm) with 250µm thickness were used for TLC (E-Merck). Iodine was used to develop the TLC plates. Elemental analyses were carried out on a Perkin-Elmer-2400 model CHN analyzer. Mass spectra on Agilent-LC-MS instrument giving \(M^+1\). All solvents were distilled prior to use according to standard procedures. Anhydrous sodium sulfate was used as a drying agent.

Synthesis of 1-(3-chloropropoxy)-4-methylbenzene (6)

\(p\)-cresol (1.00 g, 9.24 mmol) was dissolved in ethyl methyl ketone and anhydrous potassium carbonate was added to the solution. The reaction mixture was magnetically stirred refluxed for half an hour at 80°C. Then, 1-bromo-3-chloropropane (2.50 ml, 16.40 mmol) (5) was added, continued refluxing for 12 hours and reaction was monitored with the help of TLC. The slurry was filtered and the solvent was recovered under reduced pressure to obtain 6 (1.20 ml, 70.68 %). FTIR\(\nu_{\text{max}}\) (Nujol): 2977.8, 1238.1, 1086.3, 823.4cm\(^{-1}\); \(^1\)HNMR(CDCl\(_3\)): \(\delta\) 6.95(d, 2H, Ar to alkoxy group), 6.65(d, 2H, Ar to alkoxy group), 3.94(t, 2H, \(-\text{OCH}_2\text{CH}_2\text{C}_2\text{H}_5\)), 3.38(t, 2H, \(-\text{OCH}_2\text{CH}_2\text{C}_2\text{H}_5\)), 2.35(s, 3H, -\text{CH}_3) and 1.99 ppm (quin, 2H, \(-\text{OCH}_2\text{CH}_2\text{CH}_2\)). Mass: HRMS (FAB) m/e 185.07 [M+1]. Anal. For C\(_{10}\)H\(_{13}\)ClO: C, 65.04; H, 7.10; Cl, 19.20; N, 6.50; O, 8.66. Found: C, 65.24; H, 7.28; Cl, 19.59; N, 6.86; O, 8.88.
Synthesis of N-pyrrolidino-3-(p-tolyloxy)propane-1-amine (7a)

1-(3-Chloropropoxy)-4-methylbenzene (6, 1.00 ml) and pyrrolidine (1 ml, 14.08 mmol) were magnetically stirred at 80°C for 8 h. Reaction was monitored with the help of TLC. Crushed ice was added to the contents and solid obtained was crystallized from methanol to obtain 7a (1.2 g, 85.71 %), mp 166-168°C. FTIRvmax (KBr): 2960.6, 1237.9, 1045.6, 1126.1, 1028.5 cm⁻¹; ¹HNMR(CDCl₃): δ 6.85 (d, 2H, Arm to alkoxy), 6.55(d, 2H, Aro to alkoxy), 3.96(t, 2H, -OCH₂CH₂CH₂-), 3.36(t, 2H, -OCH₂CH₂CH₂-), 2.74(s, 3H, -CH₃), 2.62 [t, 4H, (-N(CH₂)₂ pyrrolidine ring], 1.67 (quin, 2H, -OCH₂CH₂CH₂) and 1.51 ppm [quin, 4H, (-CH₂CH₂)₂ pyrrolidine ring]. Mass: HRMS (FAB) m/e 220.14 [M+1]. Anal. for C₁₄H₂₁NO: C, 76.67; H, 9.65; N, 6.39; O, 7.29. Found: C, 75.99; H, 9.28; N, 6.46; O, 7.28.

Synthesis of N-morpholino-3–(3–tolyloxy)propane–1–amine (7b)

1-(3-chloropropoxy)-4-methylbenzene (6, 1.00 ml) and morpholine (1.00 ml) was stirred magnetically at 80°C for 16 h. Reaction was monitored with the help of TLC. Crushed ice was added to the contents and the solid obtained was crystallized from methanol to obtain 7b (0.85 g, 66.92 %), mp 160-162°C. FTIRvmax (KBr): 2958.7, 1237.1, 1043.5, 1287.3, 1108.0, 1035.4 cm⁻¹; ¹HNMR(CDCl₃)(ppm): δ 6.85(d, 2H, Arm to alkoxy), 6.55(d, 2H, Aro to alkoxy), 3.94 (t, 2H, -OCH₂CH₂CH₂-), 3.67(t, 4H, -CH₂-NCH₂C₂H₂O- of morpholino ring), 2.36 (t, 2H, -OCH₂CH₂CH₂), 2.33(t, 4H,-CH₂-NCH₂C₂H₂O- of morpholino ring), 2.35 (s, 3H, -CH₃) and 1.81ppm (quin, 2H , -OCH₂CH₂CH₂). Mass: HRMS (FAB) m/e 236.13 [M+1].Anal. forC₁₄H₂₁NO₂: C, 71.46; H, 8.99; N, 5.95; O, 13.60. Found: C, 71.32; H, 8.26; N, 5.64; O, 14.00.

Synthesis of N-imidazole-3–(3–tolyloxy) propane–1–amine (7c)

1-(3-Chloropropoxy)-4-methylbenzene (6, 1.00 ml) and imidazole (1.00 g) were magnetically stirred at 80°C for 14 h. Crushed ice was added to the contents and solid obtained was crystallized from acetone to obtain 7c (0.72 g, 61.53 %), mp 134-136°C. FTIRvmax (KBr): 2963.4, 1241.4, 1054.6, 1169.6, 1511.7,1096.5 cm⁻¹; ¹HNMR(CDCl₃): δ 7.94 [s, 1H, (-NCH=N-) imidazolering], 7.01[d, 1H, (-NC>HCHN-) ofimidazolering], 6.94 [d, 1H, (-NCHCHN-) of imidazole ring], 6.82(d, 2H, Arm to alkoxy), 6.57(d, 2H, alkoxy), 3.94(t, 2H, -OCH₂CH₂CH₂), 3.73(d, 2H, -OCH₂CH₂CH₂) and 2.10 ppm (quin, 2H, OCH₂CH₂CH₂). Mass : HRMS (FAB) m/e 216.00 [M+1]. Anal. for C₁₃H₁₆N₂O: C, 72.19; H, 7.46; N, 12.95; O , 7.40. Found: C, 71.99; H, 7.76; N, 12.84; O, 7.20.
Synthesis of \(N,N\)-dimethyl-3-(3-tolyloxy)propane-1-amine (7d)
1-(3-chloropropoxy)-3-methylbenzene (6, 1.00 ml) and dimethylamine (1.00 ml) were magnetically stirred at 80°C for 10 h. Reaction was monitored with the help of TLC. Crushed ice was added to the contents and the solid obtained was crystallized from acetone to 7d (0.86 g, 82.69 %), mp 146-148°C. FTIR \(\tilde{\nu}\)max(KBr): 2986.2, 1233.4, 1050.6, 1158.2, 1097.5 cm\(^{-1}\); \(^1\)HNMR(CDC\(_3\)): \(\delta\) 6.90(d, 2H, Ar\(m\) to alkoxy), 6.70 (d, 2H, Ar\(o\) to alkoxy), 3.88 (t, 2H, -OCH\(_2\)CH\(_2\)CH\(_2\)Br), 2.36[t, 2H, -OCH\(_2\)CH\(_2\)CH\(_2\)Br], 2.22[s, 3H, -CH\(_3\)] , 2.27[s, 6H, (-NCH\(_3\))\(_2\) dimethyl protons] and 1.64 ppm[quin, 2H, (-CH\(_2\)CH\(_2\)CH\(_2\))] Mass: HRMS (FAB) m/e 193.12 [M+1]. Anal. for C\(_{12}\)H\(_{19}\)NO: C, 74.57; H, 9.91; N, 7.25; O, 8.28. Found: C, 74.59; H, 8.96; N, 7.24; O, 8.22.

Synthesis of \(N,N\)-diethyl-3-(3-tolyloxy)propane-1-amine (7e)
1-(3-Chloropropoxy)-3-methylbenzene (6, 1.00ml) and diethylamine (1.00 ml) were magnetically stirred at room temperature for 15 h. Reaction was obtained was crystallized from acetone to obtain the 7e (0.94 g, 78.99 %), mp 150 –152°C. IR (KBr): 2983.2, 1243.1, 1048.9,1161.2,1093.5cm\(^{-1}\); \(^1\)HNMR (CDC\(_3\)): \(\delta\) 6.95(d, 2H, Ar\(m\) to alkoxy), 6.74 (d, 2H, Ar\(o\) to alkoxy), 3.86(t, 2H, -OCH\(_2\)CH\(_2\)CH\(_2\)Br), 2.33[t, 2H, -OCH\(_2\)CH\(_2\)CH\(_2\)Br], 2.24[s, 3H, -CH\(_3\)] , 2.10 [ q, 4H,(-NCH\(_2\)_2 diethyl protons] , 1.81 [quin, 2H, (-CH\(_2\)CH\(_2\)CH\(_2\))] and 1.00 ppm (s, 6H, -NCH\(_2\)CH\(_3\))\(_2\). Mass: HRMS (FAB) m/e 222.15 [M+1]. Anal. for C\(_{14}\)H\(_{23}\)NO: C, 75.97; H, 10.47; N, 6.33; O, 7.23. Found: C, 75.86; H, 10.66; N, 6.23; O, 7.62.

Synthesis of \(N\)-(\(N\)-methylpiperazino)-3-(3-tolyloxy)propan-1-amine(7f)
1-(3-chloropropoxy)-3-nitrobenzene (6, 1.00 ml) and \(N\)-methyl piperazine (1.00 ml) were magnetically stirred for 16 hours and then solid obtained was crystallized from methanol to obtain 7f (1.00 g, 74.62%), mp 148-150°C. IR (KBr): 2951.5, 1236.9, 1048.4, 1146.3, 1096.2cm\(^{-1}\); \(^1\)HNMR(CDC\(_3\)): \(\delta\) 6.95(d, 2H, Ar\(m\) to alkoxy), 6.74(d, 2H, Ar\(o\) to alkoxy), 3.96(t, 2H, -OCH\(_2\)CH\(_2\)CH\(_2\)Br), 2.46 (m, 8H, piperazino protons), 2.36[t, 2H, -OCH\(_2\)CH\(_2\)CH\(_2\)Br], 2.27ppm (s, 3H, -NCH\(_3\)) and 1.94 (quin, 2H, -OCH\(_2\)CH\(_2\)CH\(_2\)). Mass: HRMS (FAB): m/e 249.19 [M+1]. Anal. For C\(_{16}\)H\(_{23}\)NO: C, 75.97; H, 10.66; N, 11.28; O, 6.44. Found: C, 72.59; H, 9.76; N, 11.24; O, 6.26.

Pharmacological Activity
All the compounds were screened for the memory enhancing activity using elevated plus
maze model (Kulkarni 2007). Transfer latency (TL) on elevated plus maze was used as an index of learning and memory processes. Mice weighing 20-25 g were divided into six groups of six animals each. All the drugs were dissolved in normal saline and administered interperitonally. The time taken by each mice to move from the end of open arm to any of closed arm of elevated plus maze was measured on 1st day and after 24 h of drug treatment. The results are expressed as percent retention in Fig 2. The memory enhancing activity of these active compounds was more pronounced as compared to standard drug piracetam at 1 mg/kg, 3 mg/ g/kg kg and 5 mg/kg of dose. Piracetam is used as a positive standard. The result was expressed as percent retention.

RESULTS AND DISCUSSION

Chemistry

The synthetic path to various \( p \)-cresolderivatives have been shown in Scheme 1. The synthesis of 1-(3-chloropropoxy)-4-methylbenzene 6, a key component in the synthesis of all the derivatives in Scheme 1 was performed by condensing \( p \)-cresol 5 with 1-bromo-3-chloropropane (5 ) in ethyl methyl ketone in the presence of anhydrous potassium carbonate.

Compounds 7a–7f were prepared by treating 6 with requisite cyclic (pyrrolidine7a, morpholine7b, imidazole 7c, \( N \)-methylpiperazine7f) and acyclic amines \( N,N \)-dimethylamine7d, \( N,N \)-diethylamine7e) respectively, as specified in “Experimental” section. The completion of the reaction was monitored by thin layer chromatography. The structures of the compounds were characterized using various spectral analyses. \(^1\)H NMR spectra of these substituted derivatives exhibited a two protons singlet at \( \delta \approx 3.94 \) ppm for \(-OCH_2\), while peaks for other protons were observed at their expected values. Asymmetric and symmetric ethereal stretching vibrations at \( \approx 1237 \) and \( \approx 1048 \) cm\(^{-1}\), respectively. \( N \)-Methylene protons of the heterocyclic ring resonated at \( \delta 2.62 \) ppm for pyrrolidino7a, \( \delta 2.33 \) ppm for morpholino7b, \( \delta 7.94 \) ppm for imidazolo7c, \( \delta 2.46 \) ppm for methylpiperazino7f derivatives. Characteristic \(^1\)H NMR signals for \( N \)-methylene protons of the acyclic amines resonated at \( \delta 2.27 \) for dimethyl and for diethyl amine were observed at \( \delta 2.10 \) ppm. The protons for cyclic and acyclic amines at the appropriate positions validated their structures. The \( N \)-methyl of the piperazino moiety appeared as a singlet at \( \delta 2.27 \) ppm.

The evaluation of all the synthesized compounds for cognition were evaluated for their ability to prevent the memory decline using elevated plus maze model. Retention of the learnt task was assessed 24 h after the first day trial and \% retention of the memory was calculated from...
the initial transfer latency. All the behavioral data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnet’s test using Graph Pad Prism Program 5. The results are expressed as % retention in Fig. 1.

Out of the series of p-cresol derived compounds 7a –7f, all the doses level, % retention was significantly improved in active control, 7a, 7b, and 7f group as compared to control group which revealed their memory enhancing activity. whereas compounds 7c, 7d, and 7e was observed unable to improve the % retention as compared to control group at their all dose levels and indicated devoid of memory enhancing activity of them. Thus 7a and 7f may have potential to improve learning and memory at 5 mg/kg of dose.

CONCLUSION

In our study, 7a, 7b and 7c has shown dose dependent memory enhancing activity i.e. % retention at 3mg/kg and 5mg/kg of these compounds were significantly improved as compared to 1mg/kg whereas on further increment in dose there is further increase in memory enhancing activity reflected by significant difference between % retention of 3 and 5 mg/kg except compound 7b, 7d, 7e. This may propose that 7b, 7d, 7e compounds might have shown memory enhancing activity by inhibiting acetylcholinesterase which on further increase of dose might get saturated, whereas compounds 7a and 7f may have potential to improve learning and memory at 5 mg/kg of dose.

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Scheme 1: Synthetic procedure of p-cresol derivatives 7a-7f. Reagents and conditions (i) ClCH₂CH₂CH₂Br, K₂CO₃, condense; (ii) R= amines, stirring with or without heating for 4-52 h

Fig. 1 Effect of various compounds (7a-7f) and reference drug (piracetam) (1 and 5 mg/kg, i.p) on % retention measured on elevated plus maze in mice. * P ≤ 0.001 as compared to control group, † P ≤ 0.001 as compared to the reference drug (1 mg/kg). ‡ P ≤ 0.001 as compared to the reference drug (3 mg/kg). (ANOVA followed by Dunnett’s test). N=6, S.E.M., Standard Error.