Effect of novel *N*-arylurea- substituted 3-morpholino arecoline derivatives as muscarinic receptor 1 agonists in Alzheimer's dementia models

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Abstract

The cholinergic hypothesis of Alzheimer's disease has spurred the development of numerous compounds aimed at increasing central cholinergic neurotransmission. Symptomatic treatment can be given by cholinomimetics with the pharmacological profile of muscarinic receptor 1 (M1 receptor) agonist and/or acetylcholineesterase (AChE) inhibitors. Novel bioactive six-membered *N*-arylurea- substituted 3-morpholino arecoline derivatives were synthesized by *N*-benzyl amino-ethanol coupling with α-bromoacetylpyridine followed by reduction and cyclization. Five of the derivatives showed high M1 receptor binding affinity *in vitro* and elicited beneficial effects in *in vivo* memory and learning models in rats.

Keywords: Alzheimer's diseases, M1 agonist, morpholino arecolines, displacement assay, rat brain

Introduction

Neurochemical examination of the brain material from patients having Alzheimer's disease (AD) has demonstrated the loss of the presynaptic marker enzyme, choline acetyltransferase, and the muscarinic receptors of the M2 subtype which are mainly responsible for causing deficits in central cholinergic transmission in Alzheimer's patients.[1-3] The postsynaptic muscarinic receptors, which are primarily of the M1 subtype, seem to a large extent to survive the loss of cholinergic nerve endings.[4] These findings have led to attempts at restoring cholinergic function by means of cholinomimetic drugs such as acetylcholinesterase (AChE) inhibitors and muscarinic agonists, the hypothesis being that enhancement of cholinergic neurotransmission

would alleviate the symptoms of the diseases, and particularly the deficits in cognition and memory.[4] Pharmacological investigation of muscarinic receptor subtypes using both functional and binding studies has identified three distinct muscarinic receptor subtypes,[5] M1, M2 and M3. Identifying M1 selective muscarinic agonists which are capable of crossing the blood–brain barrier is the subject of active research for pharmacological application.[6]

Arecoline, an alkaloid obtained from the betel nut (*Areca catechu*), the fruit of a palm tree, has been used previously as a leading centrally active muscarinic agent.[7] The lack of M1 selectivity and efficacy due to dose limiting side effects associated with M2 and M3 muscarinic receptor subtype stimulation have produced disappointing results.[7] Replacement of the ester functionality of arecoline with either the 3-alkoxy-1,2,5 thiadiazole[8] or the 3-alkyl-1,2,4-oxadiazole[9] has produced very potent muscarinic agonists. However, the systematic removal of a heteroatom in the 3-methyl-1,2,4-oxadiazole, giving oxazoles or furans, caused a decrease in affinity for the agonist binding site. The two isomers, 2-methyl-1,2,4-oxadiazole and 5-methyl-1,2,4-oxadiazole also had lower affinities for muscarinic receptors.[9] No muscarinic M1 subtype selectivity has been reported for 2-(1-methyl-1,2,5,6-tetrahydropyridin-3-yl)morpholine. C-Functionalized morpholines are found in various naturally occurring products as well as in drugs.[10] Since the compound 2-(1-methyl-1,2,5,6-tetrahydropyridin-3-yl)morpholine is a conformationally restricted arecoline analog, we were encouraged to pursue this compound.

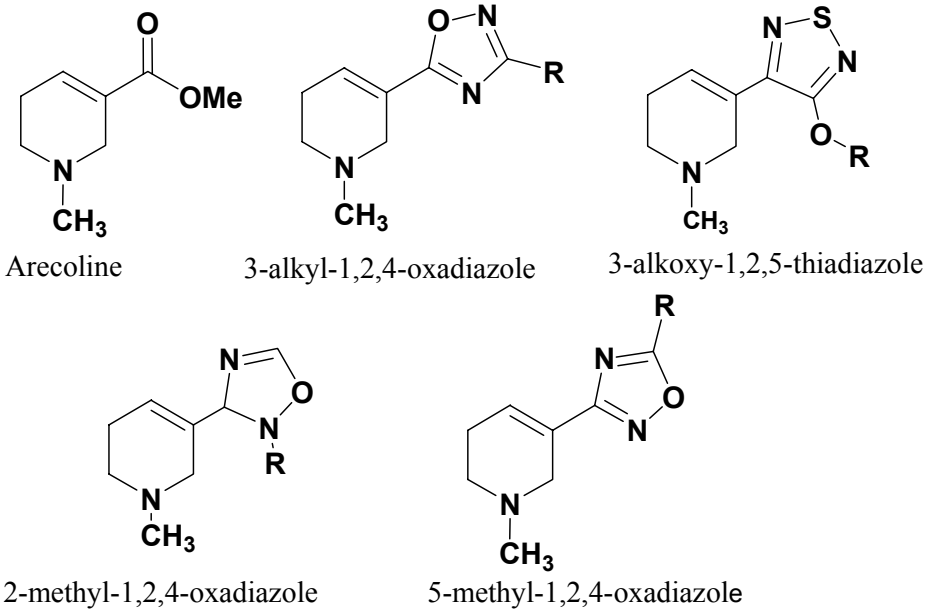


Figure 1. Arecoline and arecoline derivatives.

In an earlier paper, we reported arecoline-thiazolidinones as a muscarinic receptor 1 agonist.[11] Herein, we describe the synthesis of *N*-arylurea- substituted morpholino- arecolines **9(a-j),** along with their *in vitro* muscarinic binding assay by using [^{3}H]-QNB with male wistar rat

brain synaptosomal membrane and *in vivo* evaluation of memory and learning in male wistar rats, for the symptomatic treatment of Alzheimer's dementia.

Chemistry

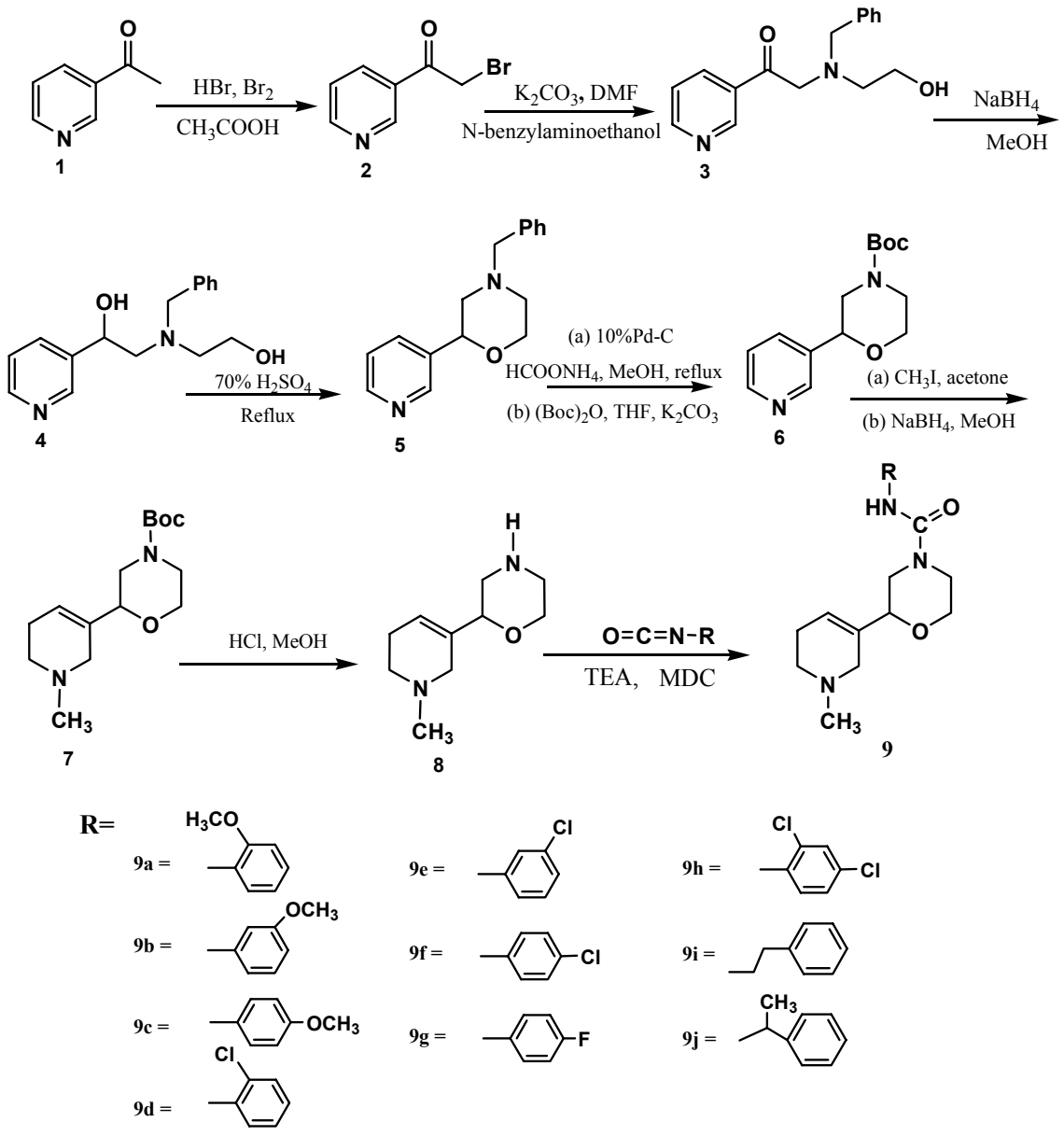
The morpholino arecoline compounds **9(a-j)** were synthesized in nine steps as shown in Scheme 1. Bromination of 3-acetylpyridine **1** with Br_2/HBr in glacial acetic acid gave the HBr salt of bromoacetylpyridine, **2**. This was converted into the amino-alcohol **3** by reaction with *N*-benzylaminoethanol in DMF in the presence of K_2CO_3. The keto group of compound **3** was reduced using $NaBH_4$ in methanol to obtain the dihydroxy compound **4**. Treatment of **4** with 70% H_2SO_4 under reflux conditions caused dehydration, to yield the cyclized product **5**. The *N*-benzyl group of **5** was removed by refluxing in methanol in the presence of 10% Pd-C and ammonium formate, and the resulting free amine was treated with Boc-anhydride in THF in the presence of K_2CO_3 to yield the Boc-protected compound **6**. This was converted by reaction with methyl iodide in acetone into the corresponding methylamine hydro-iodide salt. This on treatment with sodium borohydride in methanol gave the reduced product **7**. Finally, the Boc group was removed using methanolic HCl to yield the free amine **8** as its HCl salt. The detailed procedure for the synthesis of compound **8** has been reported in our previous paper.[12] This on reacting with the respective isocyanates gave the *N*-arylurea- substituted 3-morpholino-arecoline derivatives **9**(**a-j**). ^{1}H NMR spectra of all compounds **9(a-j)** showed a multiplet at δ 9-7 due to aromatic protons and 5.7-5.8 due to the double bond of tetrahydropyridine. All the synthesized compounds were characterized by IR, ^{1}H NMR, mass spectroscopy, and CHN analysis.

Results and Discussion

A structure–activity relationship (SAR) can be drawn from the *in vitro* affinity assay for the synthesized *N*-arylurea- substituted 3-morpholino arecoline derivatives **9(a-j)**. Five of them showed greater affinity towards the M1 receptor (Table 1), in the order **9i**>**9b**>**9e**>**9f**>**9j**. The most potent compound among all tested derivatives is the one with the ethylbenzene group, **9i** (K_i = 5 μM), (Scheme 1 and Fig. 2).

Substitution of an electron-donating methoxy group, at the *meta* position (**9b**) of the aryl group attached to the nitrogen of urea, also showed good affinity towards the M1 receptor *in vitro*. However, when the same is introduced at an *ortho*- (**9a**) or a *para*- (**9c**) position it reduces the affinity of the compound towards the M1 receptor. A chlorine atom on the benzene ring, which acts as an electron- donating group by resonance, and electron withdrawing by the inductive effect, increases the affinity of the compound when present at a *meta* position (**9e**). However when it is present either at an *ortho*- (**9d**) or a *para*- position, (**9f**), it decreases the affinity of the compounds towards the M1 receptor. Introduction of a fluoro group at the *para*-position (**9g**), or chloro groups *ortho* or *para* (**9h**) does not increase the affinity of the compound

for the receptor. Compound **9j** with an α-methylbenzyl group substituted on the nitrogen of urea showed moderate activity towards the receptor *in vitro*.



Scheme 1

Table 1. *In vitro* affinity and potency of *N*-arylurea- substituted 3-morpholino-arecoline derivatives **9**(**a-j**) towards M1 receptor of male wistar rat cortex synaptosomal membrane

Compounds	Ki (μM)	IC_{50} (μM)
9a	38±4.36	112±6.47
9b	12±2.08	46±4.21
9c	110±12.36	512±19.23
9d	89±4.92	234±11.69
9e	17±2.98	61±11.87
9f	26±3.22	90±9.65
9g	64±7.28	169±15.12
9h	40±5.36	126±13.56
9i	05±0.90	21±8.56
9j	31±5.36	98±8.56
Arecoline	86±8.39	469±17.54

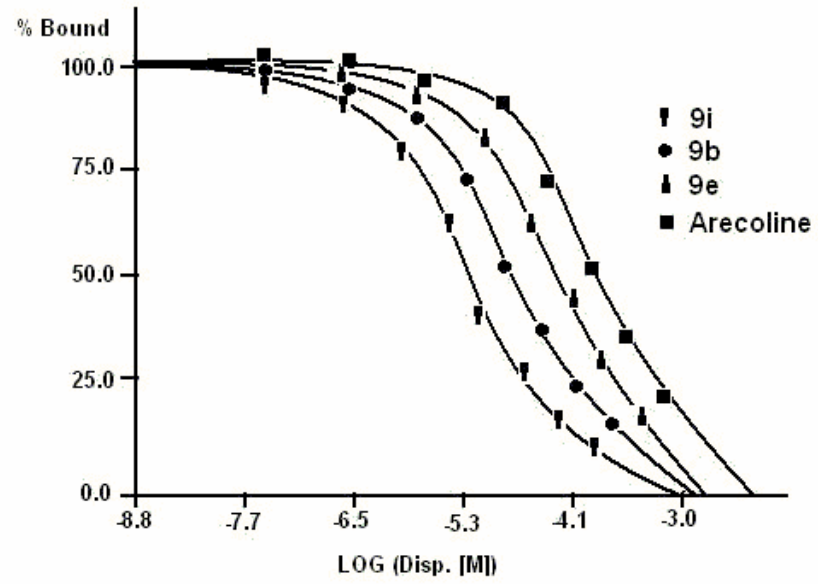


Figure 2. Displacement graph of three potent compounds **9i**, **9b** and **9e**. The displacement studies were done with 0.2 n*M* [^{3}H]QNB and different concentration of *N*-arylurea- substituted 3-morpholino-arecolines **9**(**a-j**). The mean values of % bound are plotted against the log of the displacer concentration. IC_{50} and *Ki* values are obtained from the Ligand-Drug program software.

Table 2. Study of anti-amnesic effect of *N*-arylurea- substituted 3-morpholino-arecoline derivatives **9(a-j)** against scopolamine- induced memory loss

Sl. No.	Experimental groups	Treatment (dose) mg/kg i.p.	Basal latency (sec.) of rat to reach shock- free zone (SFZ)			Memory parameters		t-test
			I	**II**	**III**	Latency (Seconds)	Mistakes	
1.	Control group*	Saline (0.9%)	18	9	5	2	8±2.3	t_{10}= 10.54
2.	Scopolamine treated group	0.4	38	12	10	7	33±7.9	
3.	**9a*** + Scop.	0.1+0.4	26	14	7	5	19±5.6	t_{10}= 4.69
4.	**9b*** + Scop.	0.1+0.4	20	10	7	3	10±3.0	t_{10}= 9.32
5.	**9c** + Scop.	0.1+0.4	38	14	10	7	32±8.4	t_{10}= 0.26
6.	**9d** + Scop.	0.1+0.4	34	14	9	6	30±6.6	t_{10}= 0.93
7.	**9e*** + Scop.	0.1+0.4	21	11	6	4	11±2.5	t_{10}= 9.18
8.	**9f*** + Scop.	0.1+0.4	24	12	7	4	15±3.9	t_{10}= 6.87
9.	**9g**** + Scop.	0.1+0.4	30	13	8	5	22±4.8	t_{10}= 3.92
10.	**9h*** + Scop.	0.1+0.4	28	13	8	5	20±4.5	t_{10}= 4.74
11.	**9i*** + Scop.	0.1+0.4	20	10	6	3	10±2.6	t_{10}= 9.54
12.	**9j*** + Scop.	0.1+0.4	25	13	7	4	18±6.3	t_{10}= 4.76

Results expressed as mean (± SEM), n = 8 (Scop = Scopolamine 0.4 mg/kg, i.p). *P=<0.001, **P= <0.002, Degrees of freedom for each parameter is 14.

The aforesaid *in vitro* M1 receptor binding studies formed a basis for extending the correlation further to *in vivo* pharmacological studies, to ascertain the applicability of the synthesized *N*-arylurea- substituted morpholino arecoline derivatives **9(a-j)** in scopolamine-induced dementia models (male wistar rats) using memory- and learning- experiments (passive avoidance tasks). In accordance with the degree of affinity and potency of the compounds **9(a-j)**, *in vitro* binding experiments elicited almost the anticipated level of pharmacological actions in reversing scopolamine-induced dementia *in vitro* (Table 2).

Three of the synthesized morpholino- arecoline derivatives, **9i**, **9b** and **9e**, reversed scopolamine-induced dementia by making rats make fewer mistakes (number of mistakes made, 10, 10 and 11, respectively) when compared with the number of mistakes made by the control rats (8 mistakes) and the scopolamine-treated group (33 mistakes). Compounds **9f**, **9j** and **9a** also significantly reversed the scopolamine- induced memory loss. Compounds **9c** and **9d** were among the least potent compound among the all tested derivatives (Figure 3).

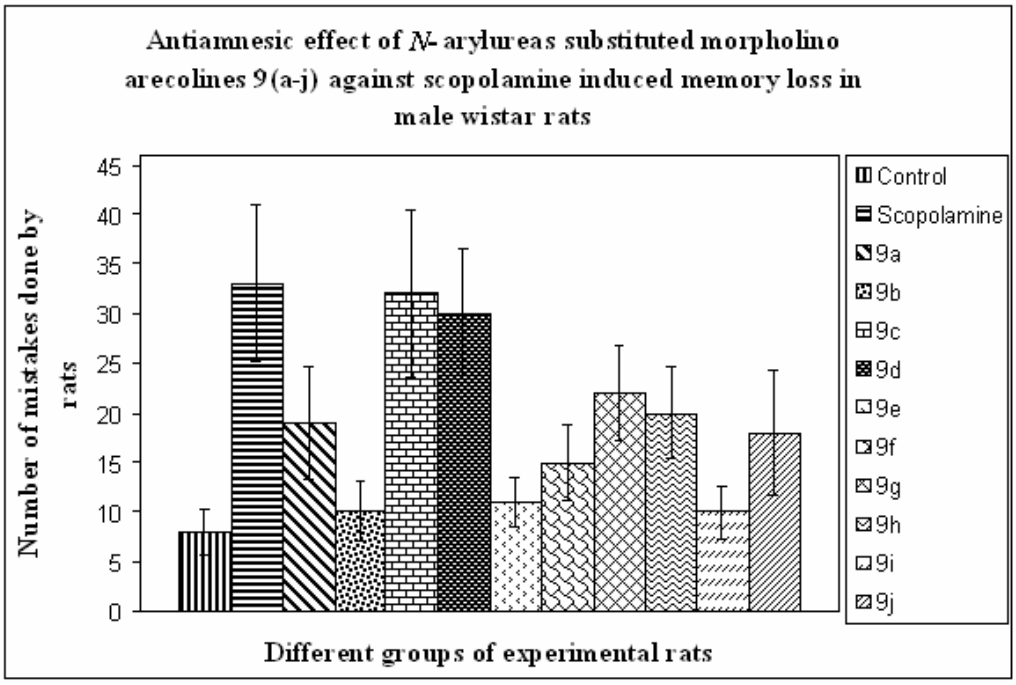


Figure 3. Anti-dementia activity of *N*-arylurea- substituted 3-morpholino-arecolines **9(a–j)**. Mean (± SEM).

Conclusions

In light of these findings, *in vitro* competitive M1 receptor displacement assay using male wistar rat-brain synaptosomal membrane and *in vivo* pharmacological experiments for the synthesized compounds **9(a-j)** testify to the reversal of scopolamine- induced memory loss and learning impairment in male wistar rats to ascertain their applicability in dementia. The derivatives with methoxy group/s at the *meta* position of the phenyl group (**9b**) showed considerably high affinity and potency for the M1 receptor *in vitro*, and useful anti-dementia activity in the *in vivo* model tested, but when the same is present at an *ortho*- or *para*- position, it decreases the affinity for the receptor. The compound **9i** which has an ethylbenzene unit is found to have the highest affinity for the M1 receptor when compared with the other tested compounds. Compound **9e**, which has a Cl group at a *meta* position showed good affinity when compared with *ortho*- Cl in **9d** or *para*- Cl in **9f**.

From the above results, we can conclude that if the Cl or OMe substitution is present at a *meta* position, the affinity of the compounds increases, whereas when it is present at an *ortho* or *para* position, the affinity of compounds decreases. This may be due to the resonance effect where electrons from OMe or Cl from *ortho*- or *para*- positions delocalize towards the nitrogen of urea *via* the benzene ring, which is not observed when they are present at a *meta* position.

These *N*-aryl morpholino arecoline derivatives **9(a-j)** showed no visible cholinergic toxicity (salivation, defecation, *etc*.) at the dose tested.

Experimental Section

General Procedures. Infrared (IR) spectra were recorded using Nujol on JASCO-FTIR, 4100 series. ^{1}H NMR spectra were recorded on Shimadzu AMX 400-Bruker, 400MHz spectrometer using $CDCl_3$ as solvent and TMS as internal standard (chemical shifts in δ ppm). Spin multiplets are given as s (singlet), d (doublet), t (triplet) and m (multiplet). Mass and purity were recorded on a LC-MSD-Trap-XCT. Elemental (CHN) analyses were obtained on Vario EL III Elementar. Silica gel column chromatography was performed using Merck 7734 silica gel (60-120 mesh) and Merck made TLC plates. All chemicals and reagents were obtained from Aldrich (USA), Spectrochem Pvt. Ltd (India), or Rankem Pvt. Ltd. (India) and were used without further purification.

General procedure for the synthesis *N*-Aryl ureas substituted 3-morpholino-Arecoline derivatives 9(a-j)

The intermediate compound 2-(1-methyl-1,2,5,6-tetrahydropyridin-3-yl)morpholine (**8**, Scheme 1) was synthesised in eight steps as described previously.[12] To a solution of compound **8** (1 eq) in dichloromethane, triethylamine (5 eq) was added and cooled to 0^0C. The respective isocyanate (1 eq) was added in the cold and stirred at room temperature for 4-5 hr (completion of reaction confirmed by TLC). Water was added and the product extracted thrice using dichloromethane. The combined organic layer was washed with brine and dried over anhydrous sodium sulphate. The dichloromethane was evaporated under reduced pressure and the crude product was purified on a silica gel (60-120 mesh) column. The compounds **9(a-j)** were eluted at 8% to 10% methanol in chloroform.

2-(1,2,5,6-Tetrahydro-1-methylpyridin-3-yl)-*N*-(2-methoxyphenyl)morpholine 4-carboxamide (9a). Obtained by reaction of compound **8** (0.2 g, 0.00078 mol) with 2-methoxy- phenyl isocyanate (0.117 g, 0.00078 mol) and triethylamine (0.394 g, 0.0039 mol) in dichloromethane (3 ml). Yield, 82%; IR: 3201 (-NH-),1623 (-CO-N-), 1675 (-RC=CH-). ^{1}H NMR: δ 8.21(s, 1H), 7.13-7.10 (m, 1H), 6.88-6.86 (m, 2H), 6.784-6.763 (d, 1H, *J* = 8.4 Hz), 5.76 (bs, 1H, -C=CH-), 3.85-3.74 (m, 3H), 3.73 (s, 3H), 3.52-3.48 (m, 1H), 3.31-3.26 (m, 3H), 2.80-2.76 (m, 2H), 2.25-2.48 (m, 2H), 2.21 (s, 3H), 2.02 (m, 2H). MS (*m/z)*: 332..51 (M^+). Anal. Calcd for $C_{18}H_{25}N_3O_3$: C, 65.23; H, 7.60; N, 12.68. Found: C, 65.48; H, 7.83; N, 12.41%.

2-(1,2,5,6-Tetrahydro-1-methylpyridin-3yl)-*N*-(3-methoxyphenyl)morpholine 4-carboxamide (9b). Obtained by reaction of compound **8** (0.2 g, 0.00078 mol) with 3-methoxy- phenyl isocyanate (0.117 g, 0.00078 mol) and triethylamine (0.394 g, 0.0039 mol) in dichloromethane (3 ml). Yield: 83%; IR (Nujol, cm^{-1}): 3206 (-NH-), 1628 (-CO-N-) 1676 (RC=CH-). ^{1}H NMR ($CDCl_3$): δ 8.02 (s, 1H), 7.35-7.21 (m, 1H), 6.90-6.86 (m, 2H), 6.67-6.65 (dd, 1H, *J* = 1.6 and 1.6 Hz), 5.74 (bs, 1H, -C=CH-), 3.84-3.73 (m, 3H), 3.70 (s, 3H), 3.53-3.49 (m, 1H), 3.33-3.28

(m, 3H), 2.81-2.76 (m, 2H), 2.52-2.47 (m, 2H), 2.23 (s, 3H), 2.01 (m, 2H). MS (*m/z)*: 332.8 (M^+); Anal. Calcd for $C_{18}H_{25}N_3O_3$: C, 65.23; H, 7.60; N, 12.68. Found: C, 64.98; H, 7.73; N, 12.50%.

2-(1,2,5,6-Tetrahydro-1-methylpyridin-3yl)-*N*-(4-methoxyphenyl)morpholine-4-carboxamide (9c). Obtained by reaction of compound **8** (0.2 g, 0.00078 mol) with 4-methoxy- phenyl isocyanate (0.117 g, 0.00078 mol) and triethylamine (0.394 g, 0.0039 mol) in dichloromethane (3 ml). Yield: 86%; IR (Nujol, cm^{-1}): 3204(-NH-), 1628 (-CO-N-) 1678 (-RC=CH-). ^{1}H NMR ($CDCl_3$): δ 8.22 (s, 1H), 7.315-7.296 (d, 2H, *J* = 7.6 Hz), 6.863-6.842 (d, 2H, *J* = 8.4 Hz), 5.74 (bs, 1H, -C=CH-), 3.85-3.74 (m, 3H), 3.74 (s, 3H), 3.52-3.47 (m, 1H), 3.33-3.28 (m, 3H), 2.84-2.79 (m, 2H), 2.53-2.48 (m, 2H), 2.21 (s, 3H), 2.00 (m, 2H). MS (*m/z)*: 332.41 (M^+); Anal. Calcd for $C_{18}H_{25}N_3O_3$: C, 65.23; H,7.60; N, 12.68. Found: C, 64.89; H, 7.86; N, 12.51%.

***N*-(2-Chlorophenyl)-2-(1,2,5,6-tetrahydro-1-methylpyridin-3-yl)morpholine-4-carboxamide (9d).** Obtained by reaction of compound **8** (0.2 g, 0.00078 mol) with 2-chloro-phenyl isocyanate (0.120 g, 0.00078 mol) and triethylamine (0.394 g, 0.0039 mol) in dichloromethane (3 ml).Yield: 88 %; IR: 3221 (-NH-), 1644 (-CO-N-), 1678 (-RC=CH-). ^{1}H NMR ($CDCl_3$): δ 8.22 (s, 1H), 7.472-7.453 (d, 1H, *J* = 7.6 Hz), 7.25-7.20 (m, 3H), 5.73 (bs, 1H, -C=CH-), 3.80-3.75 (m, 3H), 3.53-3.50 (m, 1H), 3.30-3.23 (m, 3H), 2.80-2.72 (m, 2H), 2.54-2.46 (m, 2H), 2.26 (s, 3H), 2.05 (m, 2H). MS (*m/z)*: 336.90 (M^+). Anal. Calcd for $C_{17}H_{22}ClN_3O_2$: C, 60.80; H, 6.60; N, 12.51. Found: C, 60.83; H, 6.75; N, 12.53%.

***N*-(3-Chlorophenyl)-2-(1,2,5,6-tetrahydro-1-methylpyridin-3-yl)morpholine-4-carboxamide (9e).** Obtained by reaction of **8** (0.2 g, 0.00078 mol) with 3-chlorophenyl isocyanate (0.120 g, 0.00078 mol) and triethylamine (0.394 g, 0.0039 mol) in dichloromethane (3 ml). Yield: 92%; IR: 3224(-NH-),1643(-CO-N-) 1677 (-RC=CH-). ^{1}H NMR ($CDCl_3$): δ 8.03 (s, 1H), 7.395-7.390 (d, 1H, *J* = 2.06 Hz), 7.26-7.20 (m, 2H), 7.132-7.116 (d, 1H, *J* = 7.2 Hz), 5.73 (bs, 1H, -C=CH-), 3.84-3.78 (m, 3H), 3.50-3.48 (m, 1H), 3.33-3.27 (m, 3H), 2.80-2.75 (m, 2H), 2.53-2.48 (m, 2H), 2.24 (s, 3H), 2.04 (m, 2H). MS (*m/z)*: 336.90 (M^+); Anal. Calcd for $C_{17}H_{22}ClN_3O_2$: C, 60.80; H, 6.60; N, 12.51. Found: C, 60.88; H, 6.75; N, 12.83%.

***N*-(4-Chlorophenyl)-2-(1,2,5,6-tetrahydro-1-methylpyridin-3-yl)morpholine-4-carboxamide (9f).** Obtained by reaction of **8** (0.2 g, 0.00078 mol) with 4-chlorophenyl isocyanate (0.120 g, 0.00078 mol) and triethylamine (0.394 g, 0.0039 mol) in dichloromethane (3 ml). Yield: 92%; IR: 3221 (-NH-), 1646 (-CO-N-), 1669 (-RC=CH-) cm^{-1}**;** ^{1}H NMR ($CDCl_3$): δ 8.22 (s, 1H), 7.351-7.331 (d, 2H, *J* = 8.4 Hz), 7.234-7.215 (d, 2H, *J* = 7.6 Hz), 5.75 (bs, 1H, -C=CH-), 3.84-3.79 (m, 3H), 3.53-3.48 (m, 1H), 3.33-3.28 (m, 3H), 2.81-2.75 (m, 2H), 2.55-2.48 (m, 2H), 2.28 (s, 3H), 2.05 (m, 2H). MS (*m/z)*: 336.90 (M^+); Anal. Calcd for $C_{17}H_{22}ClN_3O_2$: C, 60.80; H, 6.60; N, 12.51. Found: C, 60.85; H, 6.78; N, 12.55%.

***N*-(4-Fluorophenyl)-2-(1,2,5,6-tetrahydro-1-methylpyridin-3-yl)morpholine-4-carboxamide (9g).** Obtained by reaction of **8** (0.2 g, 0.00078 mol) with 4-fluorophenyl isocyanate (0.107 g, 0.00078 mol) and triethylamine (0.394 g, 0.0039 mol) in dichloromethane (3 ml). Yield: 90%; IR: 3232 (-NH-), 1638 (-CO-N-), 1675 cm^{-1} (-RC=CH-). ^{1}H NMR δ 8.20 (s, 1H), 7.416-7.404 (d, 2H, *J* = 8.8 Hz), 7.102-7.120 (d, 2H, *J* = 7.2 Hz), 5.73 (bs, 1H, -C=CH-), 3.85-3.75 (m, 3H),

3.53-3.48 (m, 1H), 3.33-3.27 (m, 3H), 2.81-2.76 (m, 2H), 2.54-2.49 (m, 2H), 2.24 (s, 3H), 2.04 (m, 2H). MS (*m/z)*: 320.38 (M^+). Anal. Calcd for $C_{17}H_{22}FN_3O_2$: C, 63.93; H, 6.94; N, 13.16. Found: C, 64.20; H, 6.83; N, 13.34%.

***N*-(2,4-dichlorophenyl)-2-(1,2,5,6-tetrahydro-1-methylpyridin-3-yl)morpholine-4-carboxamide (9h).** Obtained by reaction of compound **8** (0.2 g, 0.00078 mol) with 2,4-dichloro- phenyl isocyanate (0.148 g, 0.00078 mol) and triethylamine (0.394 g, 0.0039 mol) in dichloromethane (3 ml). Yield: 94%; IR: 3243 (-NH-), 1633 (-CO-N-), 1676 (-RC=CH-). ^{1}H NMR ($CDCl_3$): δ 8.20 (s, 1H), 7.596-7.591 (d, 1H, *J* = 2.0 Hz), 7.368-7.347 (d, 1H, *J* = 8.4 Hz), 7.512-7.492 (d, 1H, *J* = 8.0 Hz), 5.70 (bs, 1H, –C=CH-), 3.80-3.76 (m, 3H), 3.54-3.50 (m, 1H), 3.30-3.28 (m, 3H), 2.81-2.75 (m, 2H), 2.50-2.47 (m, 2H), 2.26 (s, 3H), 2.06-2.04 (m, 2H). MS (*m/z)*: 370.86 (M^+); Anal. Calcd for $C_{17}H_{21}Cl_2N_3O_2$: C, 55.14; H, 5.72; N, 11.35. Found: C, 55.34; H, 5.84; N, 11.30%.

2-(1,2,5,6-Tetrahydro-1-methylpyridin-3yl)-*N*-phenethylmorpholine-4- carboxamide (9i). Obtained by reaction of compound **8** (0.2 g, 0.00078 mol), 1-(2-isocyanatoethyl) benzene (0.115 g, 0.00078 mol) and triethylamine (0.394 g, 0.0039 mol) in dichloromethane (3 ml). Yield: 87%; IR (Nujol): 3211 (-NH-), 1638 (-CO-N-), 1676 (-RC=CH-). ^{1}H NMR ($CDCl_3$): δ 8.22 (s, 1H), 7.30 (s, 5H), 5.76 (b, 1H, -C=CH-), 3.85-3.75 (m, 3H), 3.50-3.45 (m, 1H), 3.31-3.20 (m, 3H), 2.79-2.76 (m, 2H), 2.52-2.43 (m, 2H), 2.23 (s, 3H), 2.10-2.09 (m, 2H), 2.02 (m, 4H). MS (*m/z)*: 330.54 (M^+). Anal. Calcd for $C_{19}H_{27}N_3O_2$: C, 69.27; H, 8.26; N, 12.76. Found: C, 68.98; H, 8.38; N, 12.69%.

2-(1,2,5,6-Tetrahydro-1-methylpyridin-3yl)-*N*-(1-phenethyl)morpholine-4- carboxamide (9j). Obtained by reaction of compound **8** (0.2 g, 0.00078 mol) with 1-(1-isocyanatoethyl) benzene (0.115g, 0.00078 mol) and triethylamine (0.394 g, 0.0039 mol) in dichloromethane (3 ml). Yield: 89%; IR (Nujol, cm^{-1}): 3212 (-NH-), 1640(-CO-N-), 1674 (-RC=CH-). ^{1}H NMR ($CDCl_3$): δ 8.21 (s, 1H), 7.31-7.06 (m, 2H), 6.95-6.90 (m, 3H), 5.76 (b, 1H, -C=CH-), 3.85-3.76 (m, 3H), 3.53-3.49 (m, 1H), 3.32-3.28 (m, 3H), 2.79-2.78 (m, 2H), 2.53-2.48 (m, 2H), 2.20 (s, 3H), 2.04 (m, 2H), 1.24 (m, 3H). MS (*m/z)*: 330.50 (M^+). Anal. Calcd for $C_{19}H_{27}N_3O_2$: C, 69.27; H, 8.26; N, 12.76. Found: C, 69.30; H, 8.38; N, 12.65%.

Biology

Displacement study

The competitive inhibition study was done using various synthesized *N*-arylurea- substituted 3-morpholino arecoline derivatives **9**(**a-j**) to find their affinity towards cortical M1 receptor. Male wistar rat brain cortex was taken out and used for synaptosomal membrane preparation. A crude membrane pellet was obtained from brain tissue, and homogenized in 20 volumes of Tris-HCl buffer (50 mmol/L, pH 7.4) containing 0.32 mol/L sucrose, following the procedure described by Creese and Snyder.[13] The tissue homogenate was centrifuged at a speed of 1,000g for 10 minutes at 4^0C, to remove cellular debris. The supernatant obtained was centrifuged at 32,000g for 20 minutes at 4^0C. The pellet obtained was re-suspended in 50 mmol/L phosphate assay buffer

(pH 7.4) containing 1 mmol $MgCl_2$. The protein concentration was estimated by the method described by Lowry *et al*.[14]

The affinity of various compounds towards M1 receptor was estimated by using [^{3}H]QNB (0.2 nM, specific activity 48Ci/mmol, Amersham, Little Chalfont, Bucks, UK) essentially following the procedure described by Hyttel *et al.*[15] and Yamamura and Snyder[16] with slight modification. In brief, an aliquot of synaptosomal membrane proteins (50 μg) was incubated with different concentrations of compounds (0.1-200 μM) as a displacer and [^{3}H]QNB (0.2 nM) and the reaction volume was made up to 200 μl with assay buffer in 96 well plates and incubated for 2 hrs at 37 ^{0}C. The reaction for all displacement assays was stopped by adding ice-cold assay buffer and the reaction mixtures were rapidly filtered through GF/B filters under vacuum. The filters were transferred to vials and 5 ml of scintillation fluid was added and allowed to equilibrate overnight. Radioactivity was measured in a liquid scintillation counter (Tris-Carb 2100TR, Packard, US) at 65% efficiency.

The data from displacement were analysed and IC_{50} and Ki values are obtained from Ligand-Drug programme.[17] The mean values of % bound are plotted against the *log* of displacer concentration.

Anti-amnesic activity

This was carried out for the synthesized *N*-aryl-morpholino- arecoline derivatives **9(a-j)** against scopolamine- induced memory loss using passive avoidance step down task paradigm in male wistar rats weighing 200-250gm (*n*=8) according to the method described by Sharma and Kulkarni.[18,19]

Acute toxicity

Rats (8 per group) which had fasted 16 hr, were treated orally with various doses of the compounds and observed for 1 week after treatment; deaths were recorded daily. None of the rats died within one week after administration under the test dose.

Dose–response curve

Different doses (0.05-0.2mg/kg) of the derivatives were selected to find the optimum dose (found to be 0.1 mg/kg) for *in vivo* studies.

Data analysis

The data from the displacement assay were analyzed using "LIGAND-DRUG" software programme[17] to obtain the IC50 and Ki values (both are expressed in μMol). All the data are expressed as mean ± SD. The statistical analysis was done by using student's t-test. Differences were considered to be significant at $P<0.05$. All analysis was performed with the "Jandel-Scientific-Sigma stat" software, version 2.0 for Windows.

Acknowledgements

The authors are grateful to the Department of Science and Technology (DST), New Delhi, for financial support under the project SR/SO/HS-58/2003. The CHN, IR and other data were obtained from the instruments granted under DST-FIST and UGC-SAP (phase I) programs and are gratefully acknowledged. One of the authors (Mansih Malviya) is grateful to the Indian Council of Medical Research (ICMR) for providing Senior Research Fellowship.

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