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

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Abstract—A series of novel, potent, and selective muscarinic receptor 1 agonists (M1 receptor agonists) that employ a key N-substituted morpholine Arecoline moiety has been synthesized as part of research effort for the therapy of Alzheimer's diseases. The ester group of arecoline (which is reported as muscarinic agonist) has been replaced by N-substituted morpholine ring. The structure–activity relationship reveals that the electron donating 4-substituted sulfonyl derivatives (**9a**, **9b**, **9c**, and **9e**) on the nitrogen atom of the morpholine ring increases the affinity of M1 receptor binding 50- to 80-fold greater than the corresponding arecoline. Other derivatives also showed considerable M1 receptor binding affinity.

1. Introduction

Senile dementia of the Alzheimer type (SDAT) is an age-related neurodegenerative disorder, characterized by a progressive deterioration of cognitive function with impairments in faculty to use language, visuospatial ability, and memory. In recent years, the total number of individuals suffering from Alzheimer's Diseases (AD) is rapidly increasing as the percentage of elderly population is increasing due to the improved health care and quality of life. Although abnormalities in many neurotransmitter systems have been documented in AD, the most consistent deficit involves the cholinergic system.[1] The cholinergic hypothesis of aging and of dementia suggests that the loss of central forebrain cholinergic neurons contributes to the decline in cognitive abilities associated with AD.[2] The presynaptic cholinergic deficits in AD indicate that a cholinergic replacement therapy might be beneficial in alleviating some of the cognitive dysfunctions in this disorder. The clinical trials with some muscarinic agonists arecoline,[3] oxotremorine, RS 86,[4] pilocarpine,[5] and bathenechol showed results that ranked from modest improvement to lack of beneficial effects.[6] It is thus, important to understand the drawbacks of the tested muscarinic agonists in order to design better drugs.

Most of the potent muscarinic agonists evaluated in AD patients show adverse central and peripheral side effects and are neither non-selective nor M2 > M1 selective. They activate the inhibitory M2 autoreceptors resulting in decreased acetylcholine (ACh) release.[6] M1-type mAChRs are predominant in cerebral cortex and hippocampus and exhibited important roles in cognitive processes relevant to AD.[6] Hence the following probes for mAChRs were suggested as a rational treatment strategy in AD; (a) M1 agonists[6,7] (b) M2 antagonists[6]; (c) mixed M1 agonist and M2 antagonist in the same compound.[8]

N-methyl tetrahydropyridine embodying the muscarinic pharmacophore in a framework of semirigid template is one such selective M1 agonist and at the same time is flexible enough to bring about conformational change in M1 receptor during its activation. The arecoline based

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muscarinic agonists have been found to overcome the limited oral activity and short duration of action of classical agonists and they are relatively specific in their action between central and peripheral effects. Arecoline oximes and/or oxadiazoles,[9] arecoline thiadiazoles, arecoline oxazoles, arecoline amides[10] are few arecolines with the above advantages. Many arecoline bioisosters classes of M1 receptor agonists are still in clinical trials and Alvameline, Milameline, sabcomeline, xanomeline, Ly 593093, and YM 796 have been discontinued during different phases of clinical trials. Most of the derivatives were disappointing in clinical trials due to their low efficacy and high cholinergic side effects (salivation, gastric secretion, nausea and vomiting).

The effect of muscarinic agonist arecoline was investigated first on human volunteers and found to improve the performance in serial learning.[11] In patients with dementia of the Alzheimer's type[12] reported a significant enhancement of performance in a picture recognition test after the infusion of 4 mg arecoline administration compared to placebo treatment in same subjects but the effects were not statistically significant.[13] This low efficacy of arecoline can be explained by its poor tolerability paired with a short biological half-life. Many structurally modified arecoline based muscarinic agonists have been tested mainly to overcome these limitations. Both affinity and efficacy were significantly enhanced by tetrahydropyridine analogues which provide semirigid template and have good affinity for the M1 receptor.[14] Several five and six membered heterocyclic ring attached arecoline basic nuclei have been explored as M1 receptor agonists in AD research. 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.[12] Replacement of the ester functionality of arecoline with either the 3-alkyl-1,2,4-oxadiazole or the 3-alkyl-1,2,4-thiadiazole has produced very potent muscarinic agonists.[15] However, the systematic removal of a heteroatom in the 3-methyl-1,2,4-oxadiazole giving oxazoles and 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.[16]

In our previous study we have reported arecoline thiazolidinones as muscarinic receptor 1 agonists.[17] In continued efforts to discover less toxic arecoline class of muscarinic agonists and to further improve their selectivity and potency here we are reporting our finding of *N*-Aryl sulfonamide substituted 3-morpholino arecoline derivatives as another potent M1 receptor agonists for the symptomatic treatment of Alzheimer's dementia. The C-functionalized morpholines are found in a variety of natural and biologically active compounds.[18] Herein, we have described the synthesis of *N*-aryl sulfonyl morpholino arecolines **9(a–h)** along with their in vitro muscarinic binding assay by using [^{3}H] Qunclidinyl Benzillate (QNB), with male Wistar rat brain synaptosomal membrane and in vivo evaluation of memory and learning in male Wistar rats.

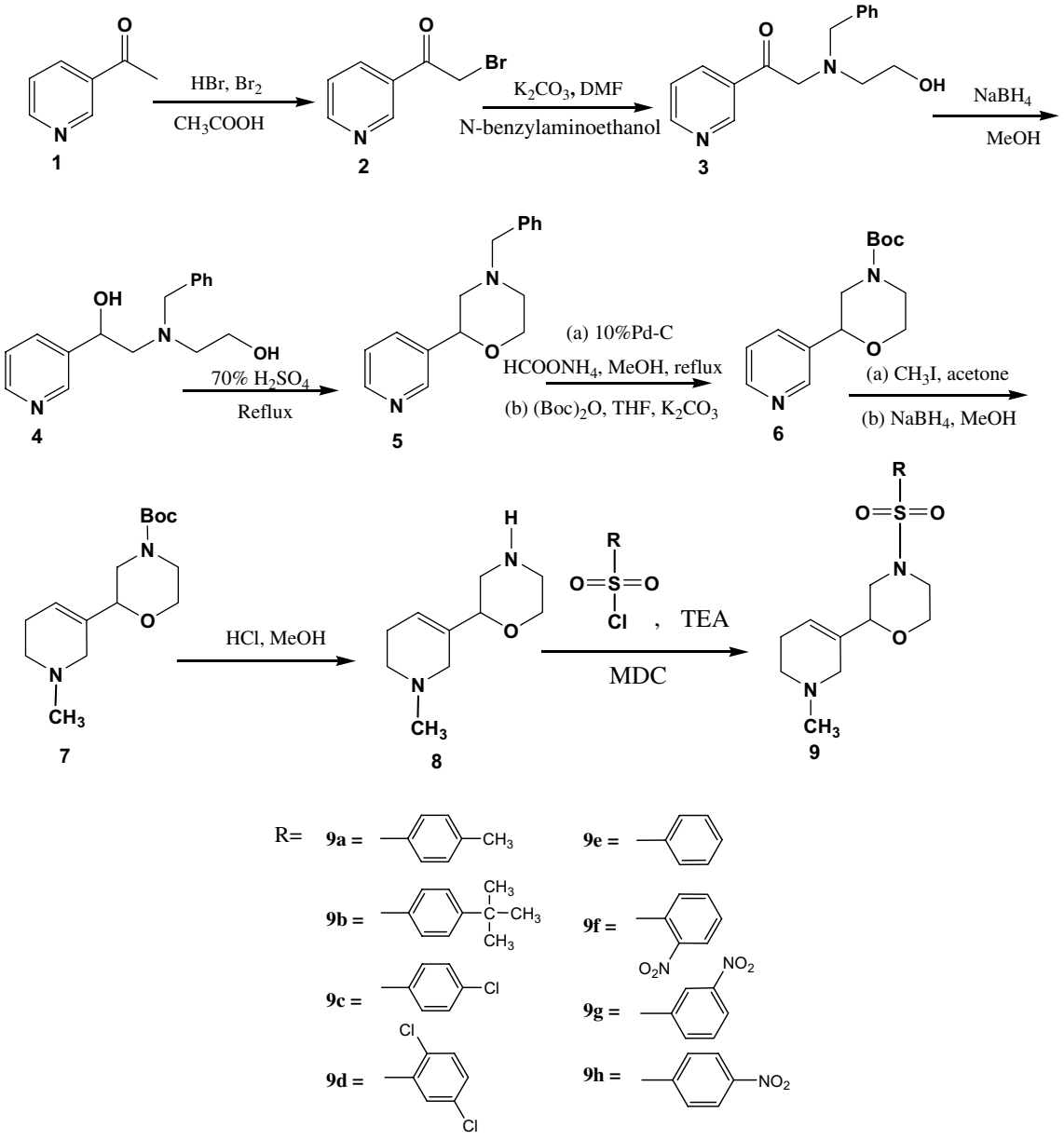
2. Chemistry

The synthetic route utilized for the preparation of the compounds is shown in Scheme 1. The morpholino arecoline compounds **9(a–h)** were synthesized in total nine steps. Bromination of 3-acetylpyridine **1** with Br_2/HBr in glacial acetic acid gave the HBr salt of bromoacetylpyridine **2**. This was converted to 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 with $NaBH_4$ in methanol to offer the dihydroxy compound **4**. Treatment of compound **4** with 70% H_2SO_4 under reflux conditions caused dehydration to yield the cyclized product **5**. The *N*-benzyl group was removed by refluxing amine **5** in methanol in the presence of 10% Pd–C and ammonium formate. 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 to the corresponding methylamine hydroiodide salt by reaction with methyl iodide in acetone. 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 as HCl salt **8**. The detail procedure for the synthesis of compound **8** has been previously reported.[14] This on reacting with respective sulfonyl chlorides gave sulfonamides **9(a–h)**. ^{1}H NMR spectra of all compounds **9(a–h)** showed a multiplet at 8–7 due to aromatic protons and 5.7–5.8 due to alkene of tetrahydro pyridine. All the synthesized compounds were characterized by IR, ^{1}H NMR, Mass spectroscopy, and CHNS analysis.

3. Result and discussion

In the present study, we have synthesized *N*-aryl sulfonyl morpholino arecoline derivatives **9(a–h)**, which were subjected to in vitro competitive muscarinic receptor 1 studies by using radiological [^{3}H] Qunclidinyl Benzillate (QNB), in male Wistar rat brain synaptosomal membrane. Also we extended the findings of in vitro studies (Table 1) to in vivo pharmacological experiment involving evaluation of learning and memory in male Wistar rats (Rodent memory evaluation, plus and Y maze studies) to ascertain their applicability in dementia model.

Structure–activity relation (SAR) was drawn from the in vitro affinity assay for *N*-arylsulfonyl-3-morpholino arecoline derivatives **9(a–h)**. It reveals that affinity and potency of all molecules **9(a–h)** were dependent on the position and the substituents of the sulfonylated aromatic ring (Scheme 1, Table 1 and Fig. 1). The most potent compound was **9c** (K_i = 0.26 μM) with electron donating chlorine group at the para position. The disubstituted chlorine compound **9d** at ortho and meta positions reduces the affinity of the M1 receptor (K_i = 35 μM). Electron donating groups at para position such as tertiary butyl **9b** (K_i = 4 μM) and methyl group **9a** (K_i = 14 μM) also exhibited considerably high affinity and potency for the M1 receptor. On the other hand, substitution of electron withdrawing group such as NO_2 on the aromatic ring **9(f–h)**, reduced the affinity



Scheme 1.

Table 1. In vitro affinity and potency of morpholino arecoline derivatives **9(a–h)** towards M1 receptor of male Wistar rat cortex synaptosomal membrane

Compounds	K_i (μM)	IC_{50} (μM)
9a	14 ± 2.26	61 ± 1.23
9b	4 ± 0.82	19 ± 1.27
9c	0.26 ± .04	0.92 ± .11
9d	35 ± 4.56	115 ± 9.73
9e	27 ± 3.22	90 ± 2.95
9f	96 ± 1.98	314 ± 11.87
9g	62 ± 3.28	201 ± 7.12
9h	64 ± 5.36	208 ± 8.56
Arecoline	86 ± 1.11	469 ± 2.18

and potency of the compounds for the M1 receptor. Among the derivative having electron withdrawing groups, NO_2 at meta position **9g** (K_i = 62 μM), showed considerable high affinity when compared to ortho position **9f** (K_i= 96 μM) and para position **9h** (K_i = 64 μM). This is may be due to the strong electron withdrawing affinity of NO_2, which is in order of ortho > para > meta. Compound **9e** having no substituent showed intermediate affinity with K_i value of 27 μM.

The foresaid in vitro M1 receptor binding studies formed a basis for extending correlation further to in vivo pharmacological studies to ascertain applicability of the *N*-aryl sulfonamide morpholino arecoline derivatives **9(a–h)** in scopolamine induced dementia models (male Wistar rats) using memory and learning experiments (passive avoidance, plus and Y maze tasks). In accordance with the degree of affinity and potency of synthesized morpholino arecoline derivatives **9(a–h)** in vitro binding experiments elicited almost anticipated

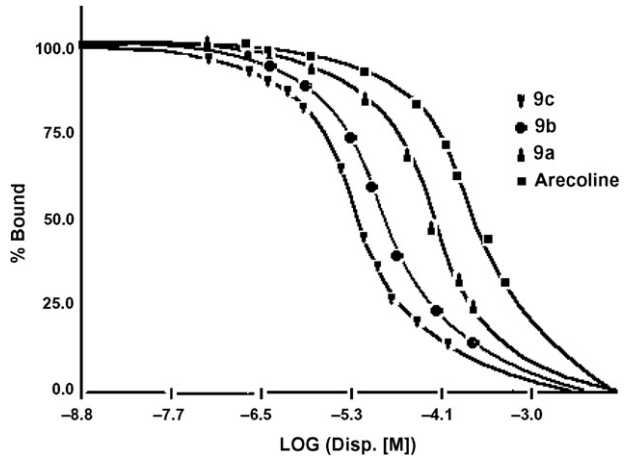


Figure 1. Displacement graphs of three most potent compounds **9c**, **9b** and **9a**. The displacement studies were done with 0.2 nM [^{3}H] QNB and different concentrations of *N*-aryl sulfonamide substituted 3-morpholino arecoline derivatives **9(a–h)**. The mean values of % bound are plotted against log of displacer concentration. IC_{50} and K_i values are obtained from Ligand–Drug programme.

level of pharmacological actions in reversing scopolamine induced dementia.

The in vivo passive avoidance results (Fig. 2) for compounds **9(a–h)** using rodent memory evaluator in male Wistar rats show reversed scopolamine-induced dementia by making rats to commit less number of mistakes. Compounds **9c**, **9b**, and **9a** (No. of mistakes done 9, 9 and 10, respectively) were highly potent when compared with the number of mistakes done by control rats (8 mistakes) and scopolamine treated group (33 mistakes). Compounds **9e**, **9d**, and **9g** were also significantly reversed the scopolamine induced memory loss. Compound **9f** was the least potent.

The in vivo plus maze experiment for synthesized morpholino arecoline derivatives **9(a–h)** in male Wistar rats, measures the transfer latency (TL) in seconds to reach from one extreme ends of open arm to one of the closed arms in plus maze. Difference in TL in

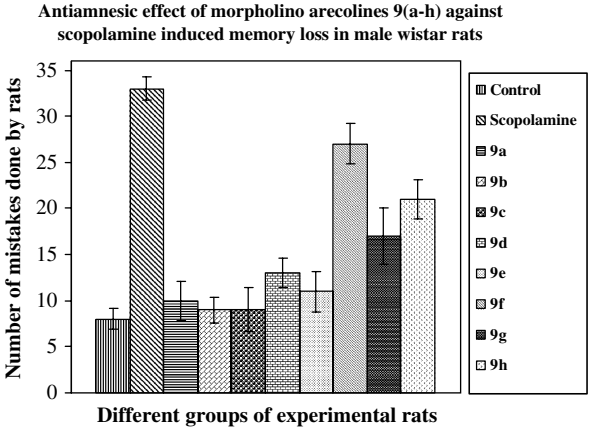


Figure 2. Antidementia activity of morpholino arecoline derivatives **9(a–h)**.

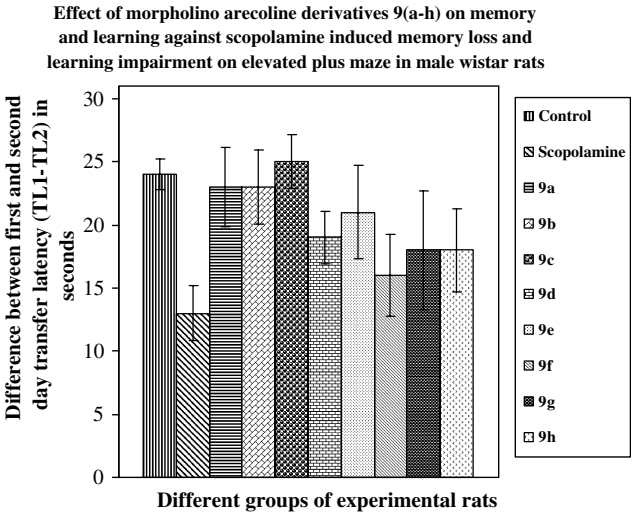


Figure 3. Effect of morpholino arecoline derivatives **9(a–h)** on memory and learning.

seconds for 2nd day and 1st day for scopolamine treated group and test compound along with scopolamine treated groups are compared to evaluate learning (TL1) and memory (TL2). Derivatives **9c**, **9b**, **9a**, and **9e** reversed acute memory loss and learning impairment in a better way than other synthesized derivatives. Compound **9c** produced lesser TL for 1st day (TL1 = 32 s) compared to scopolamine treated group (TL1 = 67 s.), but on 2nd day (TL2 = 08 s) TL was even lesser than 1st day indicating helpful in reversing learning impairment as compared to rest of the derivatives. Overall difference between TL2 and TL1 was lesser for **9c** among the other synthesized compounds. In contrary to this, compound **9f** produced longer 1st day TL (TL1 = 35 s.) and 2nd day TL (TL2 = 12 s) overall difference differs (TL2–TL2 = 16 s), implying that **9f** is less significant than other derivatives, to reverse acute memory and learning impairing in male Wistar rats. TL for remaining of the compounds is given in Figure 3.

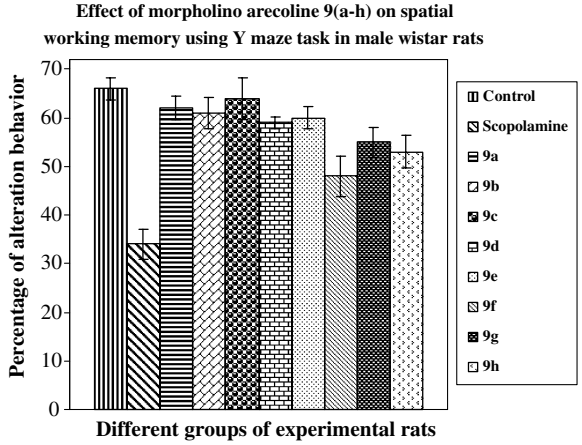


Figure 4. Effect of morpholino arecoline derivatives **9(a–h)** on acute spatial memory.

The *N*-aryl sulfonamide morpholino arecoline compounds **9(a–h)** were also subjected to in vivo Y maze study using male Wistar rats. Rats were allowed to explore three arm Y maze in which rat move in sequence, noting the percentage animal does by not entering into already entered arm before completing the sequence. The alteration behavior in terms of percentage of alteration behavior was noted for test compounds in the presence of scopolamine and was compared with scopolamine administered animal group. The percentage of alteration behavior for **9c**, **9a**, and **9b** (64%, 62%, and 61%, respectively) indicating that these derivatives significantly reverse scopolamine induced spatial working memory loss (in terms of alteration behavior). Compound **9e** and **9d** (60% and 59%, respectively) also moderately reverse scopolamine induced spatial working memory loss. The percentage of alteration behavior for the rest of the compounds is shown in Figure 4.

4. Conclusion

In the current study, in vitro competitive M1 receptor displacement assay was done using male Wistar rat brain synaptosomal membrane and in vivo pharmacological experiments for compounds **9(a–h)** to testify the reversal of scopolamine induced memory loss and learning impairment to ascertain their applicability in dementia. The derivatives with electron donating group at para position of the sulfanamide (Scheme 1) such as Cl (**9c**), $C(CH_3)_3$ (**9b**), CH_3(**9a**) showed considerable high affinity and potency for the M1 receptor in vitro and useful antidementia activity in vivo model tested. This finding can be attributed due to the delocalization of electrons from electron donating group towards the oxygen of sulfonyl group through benzene ring, which influences the affinity of the compound for the receptor. On the other hand, Cl at ortho and meta positions (**9d**) showed less affinity and potency compared to other electron donating group at para positions. Because Cl at meta position act as electron withdrawing rather donating group. This is further evidenced by the decrease in the affinity and potency of the derivatives having electron withdrawing group (NO_2) at ortho or para positions of the benzene ring. However, NO_2 at meta position showed good affinity and potency compared to NO_2 at ortho and para position, this is due to the less electron withdrawing nature of NO_2 at the meta position. All derivatives **9(a–h)** showed no visible cholinergic toxicity (salivation, defecation, etc.) at the dose tested.

5. Experimental procedure

5.1. Chemistry

5.1.1. General. All chemicals and reagents were obtained from Aldrich (USA), Spectrochem Pvt. Ltd. (India) and Rankem Pvt. Ltd. (India) and were used without further purification. The I.R. spectra were recorded using Nujol on JASCO-FTTR, 41007 series. The ^1H spectra were recorded on 400 MHz Bruker FT-NMR Spectrometer. The chemical shifts were reported as parts per million (δ ppm) tetramethyl silane (TMS) as an internal standard. Mass spectra were obtained on LCMSD-Trap-XCT instrument. Elemental analysis was performed on a variol EL, III Elementar C, H, N analyzer and values were within the acceptable limits of the calculated values. The progress of the reaction was monitored on pre coated silica gel plates (Merck) using chloroform/methanol (9:1) as a solvent system. Spectral data (IR, NMR, and mass spectra) confirmed the structures of the synthesized compounds. Elemental (C, H, and N) analysis indicated that the calculated and observed values were within the acceptable limits (±0.4%).

5.1.2. General procedure for the synthesis of compounds 9(a–h). The intermediate compound 2-(1-methyl-1,2,5,6-tetrahydropyridin-3-yl)morpholine **8** was synthesized as summarized in Scheme 1 as per reported procedure.[19] To a stirred solution of compound **8** in dichloromethane, triethylamine (5.0 equiv) was added and cooled to 0–5 °C, then sulfonyl chloride (1.0 equiv) was added dropwise. The reaction mixture was stirred for 4–5 h at room temperature (completion of reaction was confirmed by TLC) then the reaction mixture was washed with water, followed by saturated NaCl solution and dried over sodium sulfate. Dichloromethane was evaporated under reduced pressure and the crude residue obtained was purified by column chromatography using chloroform: methanol (9:1) as an eluent.

5.1.3. 4-(4-Methyl-benzenesulfonyl)-2-(1-methyl-1,2,5,6-tetrahydro-pyridin-3-yl)-morpholine (9a). Compound **9a** was obtained by reaction of compound **8** (0.2 g, 0.00078 mol) with 4-methyl benzene sulfonyl chloride (0.15 g, 0.00078 mol) and triethylamine (0.393 g, 0.0039 mol) in dichloromethane (3 ml). Yield: 80%; IR (Nujol, cm^{-1}): 1365, 1162 (O═S═O), 1675 (–RC═CH–); ^1H NMR ($CDCl_3$): δ 7.663–7.639 (d, 2H, **J** = 8.9 Hz), 7.363–7.341 (d, 2H, *J* = 8.80 Hz), 5.76 (bs, 1H, –C═C–), 3.85–3.72 (m, 3H), 3.50–3.45 (m, 1H), 2.91–2.89 (m, 3H), 2.79–2.76 (m, 2H), 2.52–2.43 (m, 2H), 2.22 (s, 3H), 2.13 (s, 3H), 2.02 (m, 2H). MS *m*/*z*: 337.45 (M^+); Anal. Calcd for $C_{17}H_{24}N_2O_3S$; C: 60.69, H: 7.19, N: 8.32, S: 9.52. Found: C: 60.79, H: 7.39, N: 8.22, S: 9.23.

5.1.4. 4-(4-*tert*-Butyl-benzenesulfonyl)-2-(1-methyl-1,2,5,6-tetrahydro-pyridin-3-yl)-morpholine (9b). Compound **9b** was obtained by reaction of compound **8** (0.2 g, 0.00078 mol) with 4-tributyl-benzene sulfonyl chloride (0.183 g, 0.00078 mol) and triethylamine (0.393 g, 0.0039 mol) in dichloromethane (3 ml). Yield: 85%; IR (Nujol, cm^{-1}): 1360, 1160 (O═S═O), 1678 (–RC═CH–); ^1H NMR ($CDCl_3$): δ 7.892–7.870 (d, 2H, *J* = 8.77 Hz), 7.243–7.221 (d, 2H, *J* = 8.73 Hz), 5.76 (bs, 1H,–C═C-), 3.83–3.76 (m, 3H), 3.49–3.44 (m, 1H), 2.89–2.85 (m, 3H), 2.77–2.75 (m, 2H), 2.54–2.48 (m, 2H), 2.24 (s, 3H), 2.08 (m, 2H), 1.02 (s, 9H). MS *m*/*z*: 379.5 (M^+); Anal. Calcd for $C_{20}H_{30}N_2O_3S$; C: 63.46, H: 7.99, N: 7.40, S: 8.47. Found: C: 64.56, H: 8.19, N: 7.48, S: 8.66.

5.1.5. 4-(2-Chloro-benzenesulfonyl)-2-(1-methyl-1,2,5,6-tetrahydro-pyridin-3-yl)-morpholine (9c). Compound **9c**

was obtained by reaction of compound **8** (0.2 g, 0.00078 mol) with 2-chloro-benzenesulfonyl chloride (0.166 g, 0.00078 mol) and triethylamine (0.393 g, 0.0039 mol) in dichloromethane (3 ml). Yield: 86%; IR (Nujol, cm^{-1}): 1368, 1162 (O═S═O), 1680 (–RC═CH–); ^1H NMR ($CDCl_3$): δ 7.89–7.87 (m, 2H), 7.52 (m, 2H), 5.76 (bs, 1H,–C═C–), 3.83–3.79 (m,3H), 3.49–3.46 (m, 1H), 2.78–2.75 (m, 3H), 2.76–2.73 (m, 2H), 2.54–2.48 (m, 2H), 2.23 (s, 3H), 2.09 (m, 2H). MS *m*/*z*: 357.5 (M^+); Anal. Calcd for $C_{16}H_{21}ClN_2O_3S$; C: 53.85, H: 5.93, N: 7.84, S: 8.99. Found: C: 54.15, H: 5.89, N: 7.92, S: 8.59.

5.1.6. 4-(2,5-Dichloro-benzenesulfonyl)-2-(1-methyl-1,2,5,6-tetrahydro-pyridin-3-yl)-morpholine (9d). Compound **9d** was obtained by reaction of compound **8** (0.2 g, 0.00078 mol) with 2,5-dichloro-benzenesulfonyl chloride (0.193 g, 0.00078 mol) and triethylamine (0.393 g, 0.0039 mol) in dichloromethane (3 ml). Yield: 90%; IR (Nujol, cm^{-1}): 1368, 1160 (O═S═O), 1680 (–RC═CH–); ^1H NMR ($CDCl_3$): δ 7.892–7.887 (d, 1H, J = 2.0 Hz), 7.720–7.699 (d, 2H, J = 8.12 Hz), 5.78 (bs, 1H, –C═C–), 3.83–3.72 (m, 3H), 3.49–3.44 (m, 1H), 2.78–2.76 (m, 3H), 2.79–2.76 (m, 2H), 2.54–2.43 (m, 2H), 2.22 (s, 3H), 2.02 (m, 2H). MS *m*/*z*: 392.1 (M^+); Anal. Calcd for $C_{16}H_{20}Cl_2N_2O_3S$; C: 49.13, H: 5.16, N: 7.15, S: 8.23. Found: C: 49.33, H: 5.36, N: 7.25, S: 8.33.

5.1.7. 4-Benzenesulfonyl-2-(1-methyl-1,2,5,6-tetrahydro-pyridin-3-yl)-morpholine (9e). Compound **9e** was obtained by reaction of compound **8** (0.2 g, 0.00078 mol) with benzene sulfonyl chloride (0.138 g, 0.00078 mol) and triethylamine (0.393 g, 0.0039 mol) in dichloromethane (3 ml). Yield: 88%; IR (Nujol, cm^{-1}): 1366, 1168 (O═S═O), 1675 (–RC═CH–); ^1H NMR ($CDCl_3$): δ 7.59–7.51 (m, 5H), 5.76 (bs, 1H, –C═C–), 3.85–3.79 (m,3H), 3.49–3.47 (m, 1H), 2.78–2.75 (m, 3H), 2.77–2.75 (m, 2H), 2.54–2.48 (m, 2H), 2.23 (s, 3H), 2.09 (m, 2H). MS *m*/*z*: 323.7 (M^+); Anal. Calcd for $C_{16}H_{22}N_2O_3S$; C: 59.6, H: 6.88, N: 8.69, S: 9.99. Found: C: 59.56, H: 7.12, N: 8.99, S: 10.19.

5.1.8. 4-(2-Nitrobenzenesulfonyl)-2-(1-methyl-1,2,5,6-tetrahydro-pyridin-3-yl)-morpholine (9f). Compound **9g** was obtained by reaction of compound **8** (0.2 g, 0.00078 mol) with 2-nitrobenzenesulfonyl chloride (0.174 g, 0.00078 mol) and triethylamine (0.393 g, 0.0039 mol) in dichloromethane (3 ml). Yield: 90%; IR (Nujol, cm^{-1}): 1358, 1162 (O═S═O), 1675 (–RC═CH–); ^1H NMR ($CDCl_3$): δ 8.123–8.102 (d, 1H, J = 8.4 Hz), 8.363–8.341 (d, 1H, J = 8.8 Hz), 7.89–7.87 (m, 2H), 5.78 (bs, 1H,–C═C–), 3.83–3.72 (m, 3H), 3.49–3.44 (m, 1H), 2.79-2.76 (m, 2H), 2.78–2.76 (m, 3H), 2.54–2.43 (m, 2H), 2.19 (s, 3H), 2.02 (m, 2H). MS *m*/*z*: 368.3 (M^+); Anal. Calcd for $C_{16}H_{21}N_3O_5S$; C: 52.3, H: 5.76, N: 11.4, S: 8.73. Found: C: 51.83, H: 5.86, N: 10.94, S: 8.97.

5.1.9. 4-(3-Nitrobenzenesulfonyl)-2-(1-methyl-1,2,5,6-tetrahydro-pyridin-3-yl)-morpholine (9g). Compound **9g** was obtained by reaction of compound **8** (0.2 g, 0.00078 mol) with 3-nitrobenzenesulfonyl chloride (0.174 g, 0.00078 mol) and triethylamine (0.393 g, 0.0039 mol) in dichloromethane (3 ml). Yield: 82%; IR (Nujol, cm^{-1}): 1360, 1158 (O═S═O), 1675 (–RC═CH–); ^1H NMR ($CDCl_3$): δ 8.847–8.844 (d, 1H, J = 1.2 Hz), 8.29–8.22 (m, 2H), 7.25 (m, 1H), 5.78 (bs, 1 H, –C═C–), 3.85–3.81 (m, 3 H), 3.49–3.44 (m, 1 H), 2.79–2.76 (m, 2 H), 2.78–2.76 (m, 3 H), 2.55–2.45 (m, 2 H), 2.22 (s, 3 H), 2.02 (m, 2 H). MS *m*/*z*: 368.32 (M^+); Anal. Calcd for $C_{16}H_{21}N_3O_5S$; C: 52.3, H: 5.76, N: 11.4, S:8.73. Found: C: 52.29, H: 5.66, N: 10.95, S: 8.99.

5.1.10. 4-(4-Nitro-benzenesulfonyl)-2-(1-methyl-1,2,5,6-tetrahydro-pyridin-3-yl)-morpholine (9h). Compound **9h** was obtained by reaction of compound 8 (0.2 g, 0.00078 mol) with 4-nitrobenzenesulfonyl chloride (0.174 g, 0.00078 mol) and triethylamine (0.393 g, 0.0039 mol) in dichloromethane (3 ml). Yield: 93%; IR (Nujol, cm^{-1}): 1369, 1158 (O═S═O), 1675 (–RC═CH–); ^1H NMR ($CDCl_3$): δ 8.321–8.298 (d, 2H, **J** = 9.2 Hz), 7.931–7.908 (d, 2H, J = 9.16 Hz), 5.76 (bs, 1H, –C═C–), 3.83–3.76 (m, 3H), 3.49–3.44 (m, 1H), 2.77–2.75 (m, 3H), 2.79–2.76 (m, 2H), 2.54–2.48 (m, 2H), 2.24 (s, 3H), 2.07 (m, 2H). MS *m*/*z*: 368.2 (M^+); Anal. Calcd for $C_{16}H_{21}N_3O_5S$; C: 52.3, H: 5.76, N: 11.4, S:8.73. Found: C: 52.23, H: 5.86, N: 10.94, S: 8.97.

5.2. Biology

5.2.1. Displacement study. The competitive inhibition study was done using *N*-aryl sulfonamide morpholino arecoline derivatives **9(a–h)** to find their affinity towards cortical M1 receptor. Male Wistar rat brain cortex was used for synaptosomal membrane preparation. Crude membrane pellet was obtained from brain tissue, 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.[20] The tissue homogenate was centrifuged at a speed of 1000*g* for 10 min at 4 degrC, to remove cellular debris. The supernatant obtained was centrifuged at 15,000*g* for 20 min at 4 °C. Pellet obtained was resuspended in 50 mmol/L phosphate assay buffer (pH 7.4) containing 1 mmol $MgCl_2$. The protein concentration was estimated by method described by Lowry et al.[21]

The affinity of various compounds towards M1 receptor was estimated by using [^{3}H]QNB (0.2 nM, specific activity 48 Ci/mmol, Amersham, Little Chalfont, Bucks, UK) essentially following the procedure described by Hyttel et al.[22]; Yamamura and Snyder[23] 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 reaction volume was made up to 200 μl with assay buffer and incubated for 2 h at 37 °C. The reaction for all displacement assay 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 containing scintillation fluid, (5 ml) 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 K_i values are obtained from Ligand–Drug programme.[24] The mean values of % bound are plotted against log of displacer concentration.

5.2.2. Antiamnesic activity. It was carried out for synthesized *N*-aryl morpholino arecoline derivatives **9(a–h)** against scopolamine induced memory loss using passive avoidance step down task paradigm in male Wistar rats weighing 200–250 g ($n = 8$) according to the method described by Sharma and Kulkarni.[25,26]

5.2.3. Elevated plus maze. This was employed for the measurement of transfer latency (TL). The male Wistar rats (weighing 200–250 g, $n = 8$) were selected, grouped and were administered scopolamine and test compound along with scopolamine to respective animal group. 30 min later they were placed individually at the end of one arm facing away from the central platform and the time they take to move from open arm to either of enclosed arms (TL) was measured. On the 1st day male Wistar rats were allowed to explore the plus maze for 90 s. Control group animals were treated with 0.9% saline and on 2nd day TL was measured in the similar way on the same animals. The resultant data were subjected to statistical analysis.[26]

5.2.4. Y maze task. This task is used to measure the spatial memory through the spontaneous alteration behavior in rats. Male Wistar rats (weighing 200–250 g, $n = 8$) were administered with test compound along with scopolamine. 30 min later they were allowed to explore in Y-maze. The ability of the rats to alternate in the Y-maze requires the rat to know which arm they have already visited.[27] The series of arm entry, including possible returns into the same arm, are recorded visually. Alteration is defined as the number of successive entries into the three arms, on overlapping triplet sets. The percentage of alteration is calculated as the ratios of actual alterations to possible alterations, defined as the total number of arm entries minus two, and multiplied by 100.

5.2.5. Acute toxicity. Rats (8 per group), which had fasted 16 h, 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 test dose.

5.2.6. Dose–response curve. Different doses (0.05–0.2 mg/kg) of the derivatives were selected to find optimum dose (found to be 0.1 mg/kg) for in vivo studies.

6. Data analysis

The data from the displacement assay were analyzed using 'Ligand–Drug' software program[24] to obtain the IC50 and K_i values (both are expressed in μMol). All the data are expressed as means ± SD. The statistical analysis was done by using student's *t*-test. Differences were considered to be significant at $P < 0.05$. All analyses were performed with the 'Jandel-Scientific-Sigma stat' software, version 2.0 for windows.

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