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Original article

Synthesis and pharmacological evaluation of novel *N*-alkyl/aryl substituted thiazolidinone arecoline analogues as muscarinic receptor 1 agonist in Alzheimer's dementia models

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ABSTRACT

Earlier we have reported the effect of arecoline thiazolidinone and morpholino arecoline analogues as muscarinic receptor 1 agonist in Alzheimer's dementia models. To elucidate further our SAR study on the chemistry and muscarinic receptor binding efficacy, a series of novel *N*-alkyl/aryl substituted thiazolidinone arecoline analogues **6**(**a**–**m**) were designed and synthesized from 3-pyridine carboxaldehyde by reacting with different amines in the presence of γ-ferrite as catalyst and subjected to in vitro muscarinic receptor binding studies using male Wistar rat brain membrane homogenate and extended to in vivo pharmacological evaluation of memory and learning in male Wistar rats. Derivative **6j** having diphenylamine moiety attached to nitrogen of thiazolidinone showed significant affinity for the M1 receptor binding.

1. Introduction

Degeneration of cortically projecting cholinergic neurons is a pathological hallmark of Alzheimer's disease (AD). 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 [1]. 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. Thus, the discovery of cholinergic deficit in AD patient's brain has triggered research efforts to test cholinomimetic approaches for their efficacy in AD therapy. It is also demonstrated that the loss of presynaptic marker enzyme choline acetyltransferase and the muscarinic receptors of the M2 subtype are mainly responsible in causing deficits in central cholinergic transmission in Alzheimer's patients [2–4].

M1 muscarinic receptors play a role in an apparent linkage of three major hallmarks of AD: β-amyloid (Aβ) peptide; tau hyperphosphorylation and paired helical filaments (PHFs); and loss of cholinergic function conducive to cognitive impairments. Muscarinic acetylcholine (mACh) receptor is a growing number of G protein-coupled receptors, each specifically regulates a different physiological and biochemical function in the body. Four types of muscarinic receptors are known, named M1–M4 [5] and five subtypes of muscarinic receptors have been cloned and designated m1–m5 [6,7]. Identifying M1 selective muscarinic agonists, which are capable of crossing the blood–brain barrier is the subject of active research for pharmacological application [8]. Most of the potent muscarinic agonists, including those which were evaluated in AD patients, show adverse central and peripheral side effects, and are either non-selective or M2 > M1 selective. Thus, they may also activate inhibitory M2 autoreceptors resulting in decreased acetylcholine (ACh) release [9]. M1-type mAChRs are predominant in cerebral cortex and hippocampus and might have important roles in cognitive processes relevant to AD. To this end, the following probes for mAChRs were suggested as a rational treatment strategy in AD; (a) M1 agonists [10]; (b) M2 antagonists; (c) mixed M1 agonist and M2 antagonist in the same compound [11]. However, clinical trials with some muscarinic agonists (e.g. arecoline, oxotremorine, RS 86, pilocarpine and bathanechol) showed the results that ranked from modest improvement to lack of beneficial effects.

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N-Methyl tetrahydropyridine embodying the muscarinic pharmacophore in a framework of semirigid template is one such selective M1 agonist and at the same time, it is flexible enough to bring about conformational change in M1 receptor during its activation. In research, many structurally novel arecoline-based muscarinic agonists have been synthesized which potentially may overcome the limited oral activity, short duration of action, or lack of separation between central and peripheral effects of classical agonists. Arecoline oximes or oxadiazoles, arecoline thiadiazoles, arecoline oxazoles, arecoline amides are the new generation muscarinic agonists [12]. Arecoline bioisosteres like Alvameline, Milameline, Sabcomeline, Xanomeline, LY 593093 and YM 796 were in clinical trials and have been discontinued during different phases of clinical trials due to their low efficacy and high cholinergic side effects.

In our recent studies, we have reported arecoline thiazolidinones [13], *N*-arylsulphonamide substituted 3-morpholino arecoline analogues [14] and *N*-arylthiourea substituted 3-morpholino arecoline analogues [15] as muscarinic receptor 1 agonist. Inspired by the results of our earlier investigation, as a part of our continuing effort aimed at the development of less toxic arecoline class of muscarinic agonist and to further improve their selectivity and potency, a series of *N*-alkyl/aryl substituted thiazolidinone arecoline analogues **6**(**a**–**m**) were designed and synthesized. All the synthesized thiazolidinone arecolines were subjected to in vitro muscarinic binding studies by using [^{3}H]QNB with male Wistar rat brain membrane homogenate and in vivo evaluation of memory and learning in male Wistar rats.

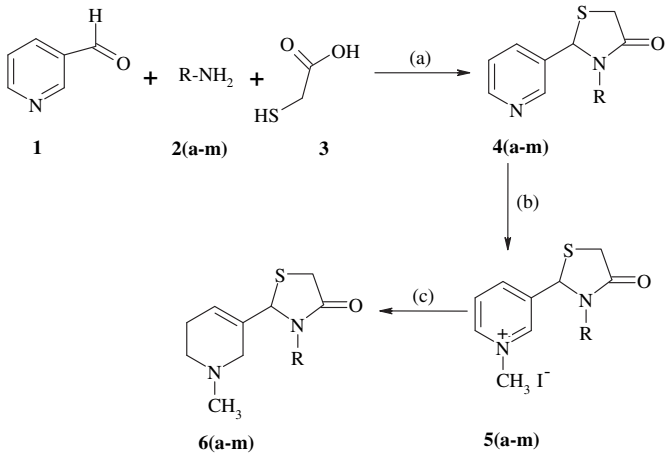
2. Results and discussion

2.1. Chemistry

The *N*-alkyl/aryl substituted thiazolidinone arecoline analogues **6**(**a**–**m**) were synthesized as depicted in Scheme 1. These analogues were synthesized in three steps, starting with 3-pyridine carboxaldehyde, allowing it to react with different amines and thioglycolic acid in the presence of γ-ferrite as catalyst and tetrahydrofuran as solvent. The successive thiazolidinone products were subjected to *N*-methylation and sodium borohydride reduction to obtain desired products. The physical characterization of the synthesized derivatives is presented in Table 1.

2.2. Pharmacological activity

The effect of muscarinic agonist arecoline was investigated first on human volunteers and found to improve performance in serial learning [16]. It is reported that after infusion of 4 mg arecoline administration, effects were not statistically significant when compared to placebo treatment in patients with preside dementia of Alzheimer's type [17]. The low efficacy of arecoline can be explained by its poor tolerability paired with a short biological half-life. To overcome the limited oral activity and short duration of action or lack of separation between central and peripheral effects of arecoline and its bioisosteres previously tested for AD, many structurally altered novel arecoline-based muscarinic agonists have been synthesized. Both their affinity and efficacy are significantly enhanced by tetrahydropyridine analogues to M1 receptor, providing semirigid template, which has good affinity for the M1 receptor [18].



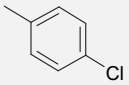
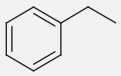
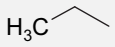
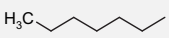
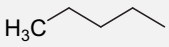
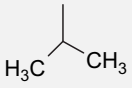
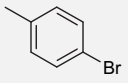
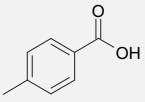
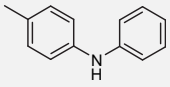
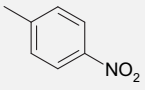
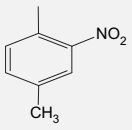
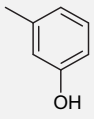
Scheme 1. (a) Benzene, γ-ferrite, 60 °C; (b) CH_3I, acetone; (c) $NaBH_4$, methanol.

Many five-membered heterocyclic ring attached to arecoline basic nuclei have been explored as M1 receptor agonist on AD research. Sauerberg et al. developed a series of very potent muscarinic agonists based on the 1,2,5-thiadiazole group, which serve as an ester isostere [19]. A unique feature of this series of compounds is that the extended amino groups can be accommodated while agonist activity is retained. In our previous report we have shown the efficacy of arecoline thiazolidinones [13], *N*-arylsulphonamide substituted 3-morpholino arecoline derivatives [14] and *N*-arylthiourea substituted 3-morpholino arecoline derivatives [15] as muscarinic receptor 1 binding. In this regard, we were interested in synthesizing similar five-membered systems, which resemble thiazole (or thiadiazole). To probe the structural requirement for the potent binding affinity, in the present study we have synthesized *N*-alkyl/aryl substituted thiazolidinone arecoline derivatives **6**(**a**–**m**) and were subjected to in vitro competitive muscarinic receptor studies and radiological (^{3}H) quinuclidinyl benzilate (QNB) using male Wistar rat brain membrane homogenate (Table 2). We have extended in vitro findings to in vivo pharmacological results involving evaluation of learning and memory in male Wistar rats (Rodent memory evaluation and plus maze studies) to ascertain their applicability in dementia (Table 3).

Structure–activity relationship (SAR) can be drawn from the in vitro assay for the synthesized derivatives **6**(**a**–**m**). Among all the synthesized compounds **6**(**a**–**m**), four derivatives **6f**, **6i**, **6j** and **6k** showed greater affinity and potency towards the M1 receptor (Table 2, Fig. 1), the potency of the molecules follows the order **6j** > **6f** > **6i** > **6k**. The remaining derivatives did not show any agonistic activity. The most potent compound **6j** ($K_i = 19 \pm 1.97$ μM and $IC_{50} = 48 \pm 6.23$ μM) is one which is having biphenyl amine attached to nitrogen of thiazolidinone arecoline. Among the compounds with alkyl chain as substituent, **6c** having ethyl group did not show any agonistic activity. But replacement of the same with isopropyl chain or *n*-butyl chain increases the activity. Interestingly, when we further increase the length of the alkyl chain by another two methylene groups (hexyl, **6e**) activity decreases drastically. This suggests that, alkyl group with four carbon atoms may be the optimum length for the binding affinity. Among the compounds with aryl group as substituents, different compounds exhibited different affinity and binding potency towards M1 receptor based on the atoms/groups attached to the phenyl ring.

It has been demonstrated that the M1 receptor subtypes predominate in the cerebral cortex and hippocampus areas in which cholinergic transmission appears to be essential for learning and memory [20,21]. The in vitro muscarinic receptor binding studies formed a basis for extending correlation further to in vivo pharmacological studies to ascertain applicability of the synthesized *N*-aryl/alkyl thiazolidinone arecolines **6**(**a**–**m**) in scopolamine-induced dementia models (male Wistar rats) using memory and learning experiments (passive avoidance, plus maze tasks). In accordance with the degree of affinity and potency of synthesized arecoline analogues **6**(**a**–**m**) in vitro binding experiments elicited

Table 1
The physical characterization of the novel *N*-alkyl/aryl substituted thiazolidinone arecoline analogues **6**(**a**–**m**).

Thiazolidinone arecolines **6**(**a**–**m**)	R–NH_2	Solvent	Reaction time (h)	Eluent used in separation	Yield (%)
6a		Benzene	10	Chloroform:methanol, 9.5:0.5	60
6b		Benzene	10	Chloroform:methanol, 9.5:0.5	60
6c		Benzene	10	Chloroform:methanol, 9.5:0.5	55
6d		Benzene	8	Chloroform:methanol, 9.5:0.5	60
6e		Benzene	8	Chloroform:methanol, 9.5:0.5	60
6f		Benzene	8	Chloroform:methanol, 9.5:0.5	55
6g		Benzene	9	Chloroform:methanol, 9.5:0.5	60
6h		Benzene	9	Chloroform:methanol, 9.5:0.5	60
6i		Benzene	9	Chloroform:methanol, 9.5:0.5	60
6j		Benzene	8	Chloroform:methanol, 9.5:0.5	55
6k		Benzene	9	Chloroform:methanol, 9.5:0.5	55
6l		Benzene	10	Chloroform:methanol, 9.5:0.5	60
6m		Benzene	8	Chloroform:methanol, 9.5:0.5	60

almost anticipated level of pharmacological actions in reversing scopolamine-induced dementia (Fig. 2). Our results obtained from in vivo passive avoidance using Rodent memory evaluator in male Wistar rats suggest that **6f**, **6i** and **6j** reversed scopolamine-induced dementia (Table 4, Fig. 2) by making rats commit less number of mistakes [number of mistakes done in 12 (**6f**), 14 (**6i**) and 16 (**6j**)] as compared to scopolamine-treated group (number of mistakes done = 35). On the other hand, **6b** (30), **6d** (32) and **6g** (30) fail to reverse scopolamine-induced mistakes in rats.

The in vivo plus maze for the synthesized *N*-alkyl/aryl thiazolidinone arecoline analogues **6**(**a**–**m**) in male Wistar rats measures the transfer latency (TL) in seconds taken to reach from one extreme end of open arm to one of the closed arms in a plus maze (Fig. 3). Difference in TL in seconds between first day and second

Table 2
In vitro affinity and potency of *N*-alkyl/aryl substituted thiazolidinone arecoline analogues **6**(**a**–**m**) towards M1 receptor of male Wistar rat cortex homogenate.

Compounds	K_i (μM)	IC_{50} (μM)
6a	200 ± 14.76	653 ± 37.65
6b	149 ± 9.56	789 ± 46.98
6c	230 ± 15.95	670 ± 38.54
6d	105 ± 6.98	886 ± 42.65
6e	110 ± 7.35	632 ± 37.25
6f	**32 ± 4.27**	**68 ± 6.35**
6g	97 ± 6.98	579 ± 32.92
6h	122 ± 8.01	536 ± 30.13
6i	**37 ± 4.97**	**74 ± 7.85**
6j	**19 ± 1.97**	**48 ± 6.23**
6k	**43 ± 5.35**	**158 ± 9.75**
6l	125 ± 14.12	626 ± 36.35
6m	102 ± 6.23	563 ± 30.89
Arecoline	86 ± 4.86	469 ± 28.83

Results are expressed as mean (±S.E.M.). Bold values represent the potent molecules.

day for scopolamine-treated group and test compound along with scopolamine-treated groups was compared to evaluate learning (TL1) and memory (TL2). Derivatives **6f**, **6i**, **6j** and **6k** reversed acute memory loss and learning impairment in male Wistar rats. **6j** produced lesser TL for first day (TL1 = 16 s) compared to scopolamine-treated group (TL1 = 62 s), but on second day (TL2 = 12 s) TL was lesser than first day, indicating how **6j** is helpful in reversing learning impairment as compared to rest of the derivatives. Overall difference between TL2 and TL1 was lesser for **6j**. However other derivatives showed moderate efficiency in reversing acute memory loss and learning impairment in male Wistar rats. TL for all the synthesized molecules is given in Table 4.

3. Conclusion

In summary, in vitro competitive M1 receptor affinity assay and in vivo pharmacological experiments conducted for the synthesized compounds **6**(**a**–**m**) to test the relation between affinity of these molecules to M1 receptor, their ability to reverse scopolamine-induced memory loss and learning impairment in male Wistar rats, have ascertained their applicability in dementia. Our results emphasize that the nature of the substituent attached to the

Table 3
Acute studies of *N*-alkyl/aryl substituted thiazolidinone arecoline analogues **6**(**a**–**m**) on learning and memory against scopolamine-induced memory loss and learning impairment on elevated plus maze in male Wistar rats.

Sl. no.	Treatment group	Treatment (dose) (mg/kg ip)	Transfer latency (TL)		Average difference transfer latency (TL)
			TL1	TL2	
1	Control group	–	12	10	11
2	Scopolamine-treated group	0.4	62	56	59
3	**6a**-Treated group	0.1	46	38	42
4	**6b**	0.1	20	18	19
5	**6c**	0.1	48	42	45
6	**6d**	0.1	46	42	44
7	**6e**	0.1	34	26	30
8	**6f**	**0.1**	**19**	**15**	**17**
9	**6g**	0.1	38	32	35
10	**6h**	0.1	40	36	38
11	**6i**	**0.1**	**20**	**16**	**18**
12	**6j**	**0.1**	**16**	**12**	**14**
13	**6k**	**0.1**	**26**	**16**	**21**
14	**6l**	0.1	45	35	40
15	**6m**	0.1	28	16	22

Results are expressed as mean (± S.E.M.), $n = 6$. Bold values represent the potent molecules.

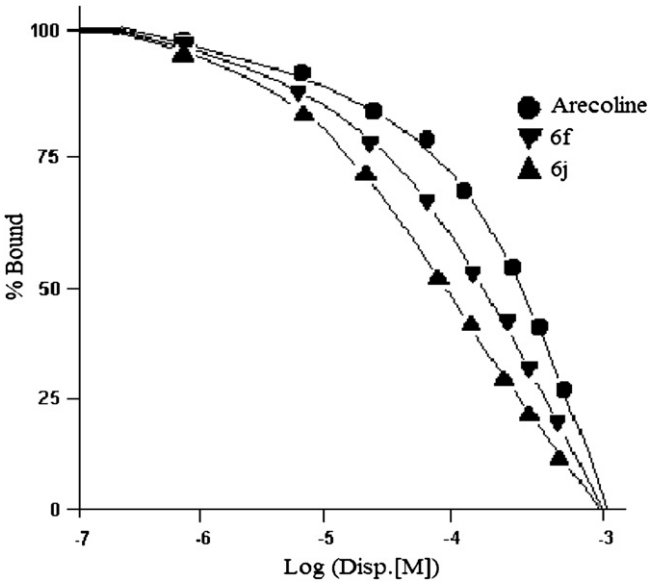


Fig. 1. Displacement studies were done with 0.2 nM [^{3}H] QNB for different derivatives (showing considerable activity) among *N*-alkyl/aryl substituted thiazolidinone arecolines **6**(**a**–**m**) and arecoline at varying concentrations. The mean values of percentage bound are plotted against log of displacer concentration IC_{50} and K_i values are obtained from Ligand-Drug programme.

nitrogen of thiazolidinone moiety determines the potency of the compound for the M1 receptor affinity. Derivatives **6f**, **6i**, **6j** and **6k** displayed significant activity in in vitro and in vivo experiments tested. Among these four derivatives **6j**, having diphenylamine is found to be the most potent molecule for M1 receptor binding. However, structural modifications of this molecule and more in-depth studies both at in vitro and in vivo levels are required for strengthening our findings. *N*-Alkyl substituted derivatives showed weak, moderate and strong activity. All the synthesized *N*-alkyl/aryl substituted thiazolidinone arecolines **6**(**a**–**m**) showed no visible cholinergic toxicity at the dose tested.

4. Experimental protocols

The melting points were determined on a SELCO-650 hot stage apparatus and are uncorrected. All the synthesized compounds were characterized by IR, ^{1}H NMR and CHNS analysis. Infrared (IR) spectra were recorded using Nujol on JASCO-FTIR, 4100 series.

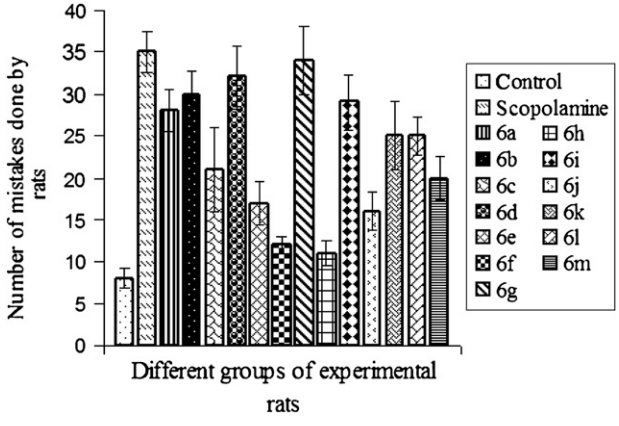


Fig. 2. Antiamnesic effect of *N*-alkyl/aryl substituted thiazolidinone arecoline analogues **6**(**a**–**m**) against scopolamine-induced memory loss.

Table 4
Antiamnesic effect of *N*-alkyl/aryl substituted thiazolidinone arecoline analogues **6**(**a**–**m**) against scopolamine-induced memory loss.

Sl. no.	Experimental groups	Treatment (dose) (mg/kg ip)	Basal latency (s) of rat to reach SFS			Memory parameters	
			I	II	III	Latency (s)	No. of mistakes
1	Control groups	–	18	3	0.8	1	8 ± 1.3
2	Scopolamine-treated groups	0.4	36	10	8	4	35 ± 2.6
3	**6a**-Treated groups	0.1	30	6	3	3	28 ± 1.8
4	**6b**	0.1	33	9	7	4	30 ± 2.1
5	**6c**	0.1	20	8	4	2	21 ± 1.3
6	**6d**	0.1	34	9	8	4	32 ± 2.4
7	**6e**	0.1	28	8	7	4	21 ± 1.2
8	**6f**	**0.1**	**20**	**7**	**5**	**4**	**12 ± 1.1**
9	**6g**	0.1	34	9	8	3	30 ± 1.9
10	**6h**	0.1	25	8	7	3	29 ± 1.6
11	**6i**	**0.1**	**27**	**8**	**6**	**4**	**14 ± 1.4**
12	**6j**	**0.1**	**22**	**6**	**5**	**3**	**16 ± 1.2**
13	**6k**	0.1	28	9	7	4	25 ± 1.9
14	**6l**	0.1	24	7	5	3	25 ± 1.5
15	**6m**	0.1	22	8	6	3	23 ± 1.8

Bold values represent the potent molecules.

Nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Shimadzu AMX 400-Bruker, 400 MHz spectrometer using DMSO as a solvent and TMS as internal standard (chemical shift in δ ppm). Spin multiplets are given as s (singlet), d (doublet), t (triplet) and m (multiplet). Elemental (CHNS) 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) and Rankem Pvt. Ltd. (India), and were used without further purification.

*4.1. General procedure for the synthesis of N-alkyl/aryl substituted thiazolidinone arecoline derivatives **6**(**a**–**m**)*

To an equimolar mixture of 3-pyridine carboxaldehyde (**1**) and amines **2**(**a**–**m**), anhydrous γ-ferrite (Fe_2O_3; 2 equiv.) was added and the reaction mixture was refluxed with constant stirring in dry benzene (20 ml) for 1/2 h, followed by the addition of thioglycolic acid (**3**). The refluxing and stirring were continued for another 6 h. The reaction was monitored by TLC. After the completion of the reaction, a reddish brown amorphous solid $Fe_2O_3 \cdot 2H_2O$/FeO(OH) was removed by filtration, filtrate was concentrated to dryness under reduced pressure to achieve compounds **4**(**a**–**m**). Compounds **4**(**a**–**m**) on stirring for 3 h with methyl iodide (2 equiv.) in dry acetone at 0 °C afforded 3-(thiazolidin-4-one)-*N*-methyl pyridinium iodides **5**(**a**–**m**). Finally, title compounds **6**(**a**–**m**) were achieved by reduction of compounds **5**(**a**–**m**) with sodium borohydride (1.2 equiv.) in methanol at −15 °C using acetone and dry ice as freezing mixture (Table 1 and Scheme 1).

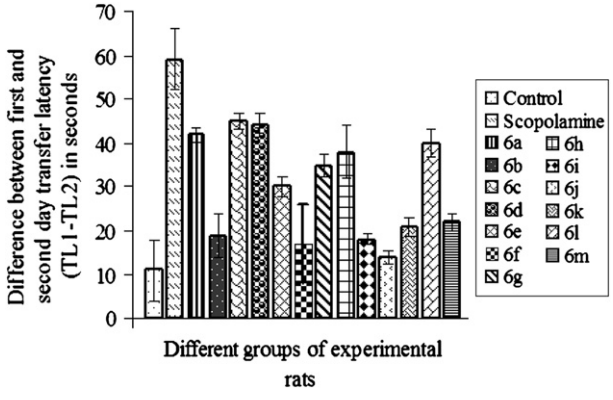


Fig. 3. Effect of *N*-alkyl/aryl substituted thiazolidinone arecoline derivatives **6**(**a**–**m**) on memory and learning against scopolamine-induced memory loss and learning impairment on elevated plus maze in male Wistar rats.

*4.1.1. Synthesis of 3-(4-chlorophenyl)-2-(1,2,5,6-tetrahydro-1-methylpyridin-3-yl)-thiazolidin-4-one (**6a**)*

The product **6a** was obtained by the reaction of 3-pyridine carboxaldehyde (**1**) (0.100 g, 0.00093 mol), *p*-chloroaniline (**2a**) (0.118 g, 0.00093 mol), anhydrous γ-ferrite (1 g), thioglycolic acid (**3**) (0.129 g, 0.0014 mol), methyl iodide (0.265 g, 0.00187 mol) and sodium borohydride (0.042 g, 0.0011 mol) as a semisolid: mp 186–188 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.0 (m, 2H, tetrahydropyridine C_5), 2.25 (s, 3H, N–CH_3), 2.45 (t, 2H, tetrahydropyridine C_6), 2.83 (s, 2H, tetrahydropyridine C_2), 3.43 (s, 2H, thiazolidin-4-one ring CH_2), 5.32 (s, 1H, thiazolidin-4-one ring CH), 5.62 (t, 1H, tetrahydropyridine C_4), 7.1 (dd, 2H, $J = 1.9$ Hz, Ar–H), 7.3 (dd, 2H, $J = 1.9$ Hz). IR ν_{max} (KBr): 1692 (C=O), 1160 (C–N) cm^{-1}. Anal. calcd for $C_{15}H_{17}ClN_2OS$ (in %): C, 58.34; H, 5.55; Cl, 11.48; N, 9.07; S, 10.38. Found: C, 58.24; H, 5.45; Cl, 11.40; N, 9.25; S, 10.42.

*4.1.2. Synthesis of 3-benzyl-2-(1-methyl-1,2,5,6-tetrahydropyridin-3-yl)-thiazolidin-4-one (**6b**)*

The product **6b** was obtained by the reaction of 3-pyridine carboxaldehyde (**1**) (0.100 g, 0.00093 mol), benzylamine (**2b**) (0.1 g, 0.00093 mol), anhydrous γ-ferrite (1 g), thioglycolic acid (**3**) (0.129 g, 0.0014 mol), methyl iodide (0.265 g, 0.00187 mol) and sodium borohydride (0.042 g, 0.0011 mol) as a semisolid: mp 195–198 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.1 (m, 2H, tetrahydropyridine C_5), 2.3 (s, 3H, N–CH_3), 2.5 (t, 2H, tetrahydropyridine C_6), 2.9 (s, 2H, tetrahydropyridine C_2), 3.1 (t, 2H, N–CH_2), 3.6 (s, 2H, thiazolidin-4-one ring CH_2), 4.46 (s, 2H, CH_2), 5.4 (s, 1H, thiazolidin-4-one ring CH), 5.7 (t, 1H, tetrahydropyridine C_4), 7.0–7.5 (m, 5H, Ar–H). IR ν_{max} (KBr): 1695 (C=O), 1190 (C–N) cm^{-1}. Anal. calcd for $C_{16}H_{20}N_2OS$ (in %): C, 66.63; H, 6.99; N, 9.71; S, 11.12. Found: C, 66.55; H, 6.81; N, 9.64; S, 11.0.

*4.1.3. Synthesis of 3-ethyl-2-(1-methyl-1,2,5,6-tetrahydropyridin-3-yl)-thiazolidin-4-one (**6c**)*

The product **6c** was obtained by the reaction of 3-pyridine carboxaldehyde (**1**) (0.100 g, 0.00093 mol), ethylamine (**2c**) (0.042 g, 0.00093 mol), anhydrous γ-ferrite (1 g), thioglycolic acid (**3**) (0.129 g, 0.0014 mol), methyl iodide (0.265 g, 0.00187 mol) and sodium borohydride (0.042 g, 0.0011 mol) as a semisolid: mp 188–190 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.20 (t, 3H, CH_3), 2.15 (m, 2H, tetrahydropyridine C_5), 2.25 (s, 3H, N–CH_3), 2.49 (t, 2H, tetrahydropyridine C_6), 2.8 (s, 2H, tetrahydropyridine C_2), 3.2 (t, 2H, N–CH_2), 3.3 (q, 2H, CH_2), 3.58 (s, 2H, thiazolidin-4-one ring CH_2), 5.42 (s, 1H, thiazolidin-4-one ring CH), 5.66 (t, 1H, tetrahydropyridine C_4). IR ν_{max} (KBr): 1698 (C=O), 1130 (C–N) cm^{-1}. Anal. calcd for $C_{11}H_{18}N_2OS$ (in %): C, 58.37; H, 8.02; N, 12.38; S, 14.17. Found: C, 58.22; H, 8.19; N, 12.15; S, 14.22.

*4.1.4. Synthesis of 2-(1-methyl-1,2,5,6-tetrahydropyridin-3-yl)-3-phenyl-thiazolidin-4-one (**6d**)*

The product **6d** was obtained by the reaction of 3-pyridine carboxaldehyde (**1**) (0.100 g, 0.00093 mol), aniline (**2d**) (0.0869 g, 0.00093 mol), anhydrous γ-ferrite (1 g), thioglycolic acid (**3**) (0.129 g, 0.0014 mol), methyl iodide (0.265 g, 0.00187 mol) and sodium borohydride (0.042 g, 0.0011 mol) as a semisolid: mp 190–193 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.1 (m, 2H, tetrahydropyridine C_5), 2.21 (s, 3H, N–CH_3), 2.42 (t, 2H, tetrahydropyridine C_6), 2.79 (s, 2H, tetrahydropyridine C_2), 3.21 (t, 2H, N–CH_2), 3.56 (s, 2H, thiazolidin-4-one ring CH_2), 5.41 (s, 1H, thiazolidin-4-one ring CH), 5.65 (t, 1H,

tetrahydropyridine C_4), 6.96–7.4 (m, 5H, Ar–H). IR ν_{max} (KBr): 1696 (C═O), 1105 (C–N) cm^{-1}. Anal. calcd for $C_{15}H_{18}N_2OS$ (in %): C, 65.66; H, 6.61; N, 10.21; S, 11.69. Found: C, 65.55; H, 6.5; N, 10.1; S, 11.51.

*4.1.5. Synthesis of 3-hexyl-2-(1-methyl-1,2,5,6-tetrahydropyridin-3-yl)-thiazolidin-4-one (**6e**)*

The product **6e** was obtained by the reaction of 3-pyridine carboxaldehyde (**1**) (0.100 g, 0.00093 mol), hexylamine (**2e**) (0.0945 g, 0.00093 mol), anhydrous γ-ferrite (1 g), thioglycolic acid (**3**) (0.129 g, 0.0014 mol), methyl iodide (0.265 g, 0.00187 mol) and sodium borohydride (0.042 g, 0.0011 mol) as a semisolid: mp 191–194 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.96 (t, 3H, CH_3), 1.29–1.55 (m, 8H, hexyl CH_2), 2.1 (m, 2H, tetrahydropyridine C_5), 2.3 (s, 3H, N–CH_3), 2.5 (t, 2H, tetrahydropyridine C_6), 2.9 (s, 2H, tetrahydropyridine C_2), 3.1 (t, 2H, N–CH_2), 3.6 (s, 2H, thiazolidin-4-one ring CH_2), 5.4 (s, 1H, thiazolidin-4-one ring CH), 5.7 (t, 1H, tetrahydropyridine C_4). IR ν_{max} (KBr): 1706 (C═O), 1180 (C–N) cm^{-1}. Anal. calcd for $C_{15}H_{26}N_2OS$ (in %): C, 63.61; H, 9.15; N, 9.8; S, 11.2. Found: C, 63.6; H, 9.3; N, 9.6; S, 11.4.

*4.1.6. Synthesis of 3-(4-amino-butyl)-2-(1-methyl-1,2,5,6-tetrahydropyridin-3-yl)-thiazolidin-4-one (**6f**)*

The product **6f** was obtained by the reaction of 3-pyridine carboxaldehyde (**1**) (0.100 g, 0.00093 mol), butylamine (**2f**) (0.0682 g, 0.00093 mol), anhydrous γ-ferrite (1 g), thioglycolic acid (**3**) (0.129 g, 0.0014 mol), methyl iodide (0.265 g, 0.00187 mol) and sodium borohydride (0.042 g, 0.0011 mol) as a semisolid: mp 181–183 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.96 (t, 3H, CH_3), 1.25–1.5 (m, 4H, $2CH_2$), 2.22 (m, 2H, tetrahydropyridine C_5), 2.39 (s, 3H, N–CH_3), 2.46 (t, 2H, tetrahydropyridine C_6), 2.84 (s, 2H, tetrahydropyridine C_2), 3.20 (t, 2H, CH_2), 3.28 (t, 2H, N–CH_2), 3.59 (s, 2H, thiazolidin-4-one ring CH_2), 5.46 (s, 1H, thiazolidin-4-one ring CH), 5.71 (t, 1H, tetrahydropyridine C_4). IR ν_{max} (KBr): 1690 (C═O), 1115 (C–N) cm^{-1}. Anal. calcd for $C_{13}H_{22}N_2OS$ (in %): C, 61.38; H, 8.72; N, 11.01; S, 12.60. Found: C, 61.5; H, 8.8; N, 11.2; S, 12.69.

*4.1.7. Synthesis of 3-isopropyl-2-(1-methyl-1,2,5,6-tetrahydropyridin-3-yl)-thiazolidin-4-one (**6g**)*

The product **6g** was obtained by the reaction of 3-pyridine carboxaldehyde (**1**) (0.100 g, 0.00093 mol), isopropylamine (**2g**) (0.0542 g, 0.00093 mol), anhydrous γ-ferrite (1 g), thioglycolic acid (**3**) (0.129 g, 0.0014 mol), methyl iodide (0.265 g, 0.00187 mol) and sodium borohydride (0.042 g, 0.0011 mol) as a semisolid: mp 192–195 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.25 (d, 6H, $2CH_3$), 2.1 (m, 2H, tetrahydropyridine C_5), 2.21 (s, 3H, N–CH_3), 2.42 (t, 2H, tetrahydropyridine C_6), 2.79 (s, 2H, tetrahydropyridine C_2), 3.21 (t, 2H, N–CH_2), 3.56 (s, 2H, thiazolidin-4-one ring CH_2), 3.94 (m, 1H, CH), 5.41 (s, 1H, thiazolidin-4-one ring CH), 5.65 (t, 1H, tetrahydropyridine C_4), 6.96–7.4 (m, 5H, Ar–H). IR ν_{max} (KBr): 1695 (C═O), 1115 (C–N) cm^{-1}. Anal. calcd for $C_{12}H_{20}N_2OS$ (in %): C, 59.96; H, 8.39; N, 11.65; S, 13.34. Found: C, 59.8; H, 8.23; N, 11.77; S, 13.45.

*4.1.8. Synthesis of 3-(4-bromophenyl)-2-(1-methyl-1,2,5,6-tetrahydropyridin-3-yl)-thiazolidin-4-one (**6h**)*

The product **6h** was obtained by the reaction of 3-pyridine carboxaldehyde (**1**) (0.100 g, 0.00093 mol), 4-bromoaniline (**2h**) (0.160 g, 0.00093 mol), anhydrous γ-ferrite (1 g), thioglycolic acid (**3**) (0.129 g, 0.0014 mol), methyl iodide (0.265 g, 0.00187 mol) and sodium borohydride (0.042 g, 0.0011 mol) as a semisolid: mp 198–200 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.2 (m, 2H, tetrahydropyridine C_5), 2.4 (s, 3H, N–CH_3), 2.45 (t, 2H, tetrahydropyridine C_6), 2.8 (s, 2H, tetrahydropyridine C_2), 3.25 (t, 2H, N–CH_2), 3.58 (s, 2H, thiazolidin-4-one ring CH_2), 5.45 (s, 1H, thiazolidin-4-one ring CH), 5.7 (t, 1H, tetrahydropyridine C_4), 6.9 (dd, $J = 1.9$ Hz, 2H, Ar–H), 7.2 (dd, $J = 1.9$ Hz, 2H, Ar–H). IR ν_{max} (KBr): 1698 (C═O), 1120 (C–N) cm^{-1}. Anal. calcd for $C_{15}H_{17}BrN_2OS$ (in %): C, 51.00; H, 4.85; Br, 22.62; N, 7.93; S, 9.08. Found: C, 51.24; H, 4.79; Br, 22.54; N, 7.8; S, 9.2.

*4.1.9. Synthesis of 4-[2-(1-methyl-1,2,5,6-tetrahydropyridin-3-yl)-4-oxo-thiazolidin-3-yl]-benzoic acid (**6i**)*

The product **6i** was obtained by the reaction of 3-pyridine carboxaldehyde (**1**) (0.100 g, 0.00093 mol), 4-amino benzoic acid (**2i**) (0.128 g, 0.00093 mol), anhydrous γ-ferrite (1 g), thioglycolic acid (**3**) (0.129 g, 0.0014 mol), methyl iodide (0.265 g, 0.00187 mol) and sodium borohydride (0.042 g, 0.0011 mol) as a solid: mp 220–222 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.15 (m, 2H, tetrahydropyridine C_5), 2.25 (s, 3H, N–CH_3), 2.49 (t, 2H, tetrahydropyridine C_6), 2.8 (s, 2H, tetrahydropyridine C_2), 3.2 (t, 2H, N–CH_2), 3.58 (s, 2H, thiazolidin-4-one ring CH_2), 5.42 (s, 1H, thiazolidin-4-one ring CH), 5.66 (t, 1H, tetrahydropyridine C_4), 7.3 (dd, 2H, $J = 1.9$ Hz, Ar–H), 8.1 (dd, 2H, $J = 1.9$ Hz), 10 (s, 1H, COOH). IR ν_{max} (KBr): 1683 (C═O), 1144 (C–N) cm^{-1}. Anal. calcd for $C_{16}H_{18}N_2O_3S$ (in %): C, 60.36; H, 5.70; N, 8.80; S, 10.07. Found: C, 60.15; H, 5.58; N, 8.71; S, 10.22.

*4.1.10. Synthesis of 2-(1-methyl-1,2,5,6-tetrahydropyridin-3-yl)-3-(4-phenylamino-phenyl)-thiazolidin-4-one (**6j**)*

The product **6j** was obtained by the reaction of 3-pyridine carboxaldehyde (**1**) (0.100 g, 0.00093 mol), 4-amino diphenylamine (**2j**) (0.1719 g, 0.00093 mol), anhydrous γ-ferrite (1 g), thioglycolic acid (**3**) (0.129 g, 0.0014 mol), methyl iodide (0.265 g, 0.00187 mol) and sodium borohydride (0.042 g, 0.0011 mol) as a solid: mp 246–248 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.01 (m, 2H, tetrahydropyridine C_5), 2.27 (s, 3H, N–CH_3), 2.47 (t, 2H, tetrahydropyridine C_6), 2.85 (s, 2H, tetrahydropyridine C_2), 3.5 (s, 2H, thiazolidin-4-one ring CH_2), 5.38 (s, 1H, thiazolidin-4-one ring CH), 5.66 (t, 1H, tetrahydropyridine C_4), 6.5–7.3 (m, 9H, Ar–H). IR ν_{max} (KBr): 1698 (C═O), 1165 (C–N) cm^{-1}. Anal. calcd for $C_{21}H_{23}N_3OS$ (in %): C, 69.01; H, 6.34; N, 11.50; S, 8.77. Found: C, 69.11; H, 6.15; N, 11.40; S, 8.6.

*4.1.11. Synthesis of 2-(1-methyl-1,2,5,6-tetrahydropyridin-3-yl)-3-(4-nitrophenyl)-thiazolidin-4-one (**6k**)*

The product **6k** was obtained by the reaction of 3-pyridine carboxaldehyde (**1**) (0.100 g, 0.00093 mol), 4-nitroaniline (**2k**) (0.13 g, 0.00093 mol), anhydrous γ-ferrite (1 g), thioglycolic acid (**3**) (0.129 g, 0.0014 mol), methyl iodide (0.265 g, 0.00187 mol) and sodium borohydride (0.042 g, 0.0011 mol) as a semisolid: mp 219–222 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.15 (m, 2H, tetrahydropyridine C_5), 2.25 (s, 3H, N–CH_3), 2.49 (t, 2H, tetrahydropyridine C_6), 2.8 (s, 2H, tetrahydropyridine C_2), 3.2 (t, 2H, N–CH_2), 3.58 (s, 2H, thiazolidin-4-one ring CH_2), 5.42 (s, 1H, thiazolidin-4-one ring CH), 5.66 (t, 1H, tetrahydropyridine C_4), 6.9–7.45 (m, 4H, Ar–H). IR ν_{max} (KBr): 1705 (C═O), 1115 (C–N) cm^{-1}. Anal. calcd for $C_{15}H_{17}N_3O_3S$ (in %): C, 56.41; H, 5.37; N, 13.16; S, 10.04. Found: C, 56.35; H, 5.3; N, 13.25; S, 10.11.

*4.1.12. Synthesis of 3-(4-methyl-2-nitro-phenyl)-2-(1-methyl-1,2,5,6-tetrahydropyridin-3-yl)-thiazolidin-4-one (**6l**)*

The product **6l** was obtained by the reaction of 3-pyridine carboxaldehyde (**1**) (0.100 g, 0.00093 mol), 4-amino-3-nitrotoluene (**2l**) (0.142 g, 0.00093 mol), anhydrous γ-ferrite (1 g), thioglycolic acid (**3**) (0.129 g, 0.0014 mol), methyl iodide (0.265 g, 0.00187 mol) and sodium borohydride (0.042 g, 0.0011 mol) as a semisolid: mp 252–254 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.2 (m, 2H, tetrahydropyridine C_5), 2.3 (s, 3H, N–CH_3), 2.35 (s, 3H, CH_3), 2.45 (t, 2H, tetrahydropyridine C_6), 2.81 (s, 2H, tetrahydropyridine C_2), 3.21 (t, 2H, CH_2), 3.27 (t, 2H, N–CH_2), 3.58 (s, 2H, thiazolidin-4-one ring CH_2), 5.45 (s, 1H, thiazolidin-4-one ring CH), 5.72 (t, 1H, tetrahydropyridine C_4), 6.9–7.5 (m, 4H, Ar–H). IR ν_{max} (KBr): 1696 (C═O),

1115 (C–N) cm^{-1}. Anal. calcd for $C_{16}H_{19}N_3O_3S$ (in %): C, 57.64; H, 5.74; N, 12.60; S, 9.62. Found: C, 57.78; H, 5.58; N, 12.51; S, 9.72.

4.1.13. Synthesis of 3-(3-hydroxy-phenyl)-2-(1-methyl-1,2,5,6-tetrahydropyridin-3-yl)-thiazolidin-4-one (**6m**)

The product **6m** was obtained by the reaction of 3-pyridine carboxaldehyde (**1**) (0.100 g, 0.00093 mol), 3-aminophenol (**2m**) (0.1018 g, 0.00093 mol), anhydrous γ-ferrite (1 g), thioglycolic acid (**3**) (0.129 g, 0.0014 mol), methyl iodide (0.265 g, 0.00187 mol) and sodium borohydride (0.042 g, 0.0011 mol) as a semisolid: mp 210–212 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.18 (m, 2H, tetrahydropyridine C_5), 2.27 (s, 3H, N–CH_3), 2.31 (s, 3H, CH_3), 2.41 (t, 2H, tetrahydropyridine C_6), 2.81 (s, 2H, tetrahydropyridine C_2), 3.21 (t, 2H, CH_2), 3.25 (t, 2H, N–CH_2), 3.55 (s, 2H, thiazolidin-4-one ring CH_2), 5.45 (s, 1H, thiazolidin-4-one ring CH), 5.71 (t, 1H, tetrahydropyridine C_4), 6.8–7.5 (m, 4H, Ar–H), 10 (s, 1H, OH). IR ν_{max} (KBr): 1703 (C═O), 1180 (C–N) cm^{-1}. Anal. calcd for $C_{15}H_{18}N_2O_2S$ (in %): C, 62.04; H, 6.25; N, 9.65; S, 11.04. Found: C, 62.25; H, 6.35; N, 9.75; S, 11.12.

4.2. Pharmacology

4.2.1. Displacement study

The competitive inhibition study was done using all newly synthesized *N*-alkyl/aryl substituted thiazolidinone arecoline compounds **6**(**a**–**m**) to find out their affinity towards cortical M1 receptor. Male Wistar rat brain cortex was taken out and used for 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 [22]. The tissue homogenate was centrifuged at a speed of 1000 × g for 10 min at 4 °C, to remove cellular debris. The supernatant obtained was centrifuged at 32,000 × 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. [23]. 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. [24].

For the displacement studies, an aliquot of membrane proteins obtained from the cortex (50 μg) was incubated with different concentrations of compounds 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 assays was stopped by adding ice-cold assay buffer and reaction mixtures were rapidly filtered through GF/B filters under vacuum. The filtrates 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 analyzed, IC_{50} and χ values are obtained from Ligand-Drug programme. The mean values of percentage bound are plotted against log of displacer concentration [25].

4.2.2. Antiamnesic activity assay

Antiamnesic activity assay was carried out for synthesized *N*-alkyl/aryl thiazolidinone arecoline derivatives **6**(**a**–**m**) against scopolamine-induced memory loss using passive avoidance step down task paradigm in male Wistar rats (weight: 200–250 g) according to the method described by Sharma and Kulkarni [26,27].

4.2.3. Elevated plus maze

This was employed for the measurement of transfer latency (TL). The male Wistar rats (weight: 200–250 g) were placed individually at the end of one arm facing away from the central platform and time they take to move from open arm to either of the enclosed arms (TL) was measured. On the first day male Wistar rats were allowed to explore the plus maze for 90 s with scopolamine and test compounds along with scopolamine treatment to respective animal groups 30 min before the plus maze exploration. Control group animals were treated with distilled water. Second day TL was measured in the similar way on the same animals. The resultant data were subjected to statistical analysis. $p < 0.005$ was considered statistically significant [27].

4.2.4. Acute toxicity

Rats (six 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 1 week after administration under test dose.

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