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Design and synthesis of methyl 2-methyl-7,7-dihalo-5-phenyl-2-azabicyclo[4.1.0]hept-3-ene-4-carboxylates with calcium channel antagonist activity

Javid S. Mojarrad,[a,b] Ramin Miri[a,c] and Edward E. Knaus[a,*]

[a]*Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2N8*
[b]*School of Pharmacy, Tabriz University of Medical Sciences and Health Sciences, Tabriz, PO Box 51664, Iran*
[c]*Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, PO Box 71345-1149, Iran*

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Abstract—A group of methyl 2-methyl-7,7-dihalo-5-(substituted-phenyl)-2-azabicyclo[4.1.0]hept-3-ene-4-carboxylates were prepared by reaction of dihalocarbenes (:CX_2, X = Br, Cl) with methyl 1-methyl-4-(substituted-phenyl)-1,4-dihydropyridine-3-carboxylates. In vitro calcium channel antagonist activities were determined using a guinea pig ileum longitudinal smooth muscle assay. The title compounds exhibited weaker CC antagonist activity (10^{-5}–10^{-6} M range) than the reference drug nifedipine (1.4×10^{-8} M). Structure–activity relationship studies showed that the position of a nitro substituent on the C-5 phenyl ring where the relative potency order was *ortho* > *meta* > *para*, and the size and/or electronegativity of the C-7 geminal-dihalo substituents (Br > Cl), were determinants of calcium channel antagonist activity. This class of compounds did not exhibit any inotropic effect on guinea pig left atria. A dihalocyclopropyl moiety is a potential bioisostere for the 2-methyl-3-methoxycarbonylvinyl moiety present in the calcium channel antagonist nifedipine.

1. Introduction

The design of 1,4-dihydropyridine (1,4-DHP) calcium channel modulators related to the first-generation calcium channel antagonist nifedipine (**1**), as therapeutic agents to treat cardiovascular disorders,[1] has prompted studies to investigate the geometrical requirements at the DHP binding site.[2–5] Structure–activity relationships indicate that the combination of the substituents at the C-3, C-4, and C-5 positions of nifedipine modulated activity,[2] tissue selectivity,[6–12] and the conformation[13–15] of the 1,4-DHP ring system. The nature and position of C-4 aryl ring substituents were determinants of calcium channel antagonist activity where the potency order was generally *ortho* ⩾ *meta* ≫ *para*.[3] Hantzsch 1,4-DHPs, in the solid state, exist in a boat conformation where the C-4 substituted-phenyl ring is perpendicular (pseudo-axial) to the 1,4-DHP ring. Strain due to nonbonded interactions between the C-3, C-4, and C-5 1,4-DHP substituents is relieved most notably by puckering of the 1,4-DHP ring and distortion of the bond angles about C-4 with the most potent compounds showing the smallest degree of ring distortion from planarity.[3,13] It has been suggested that synperiplanar (*sp*) carbonyl groups may be a common feature of DHP calcium channel antagonists, and that an antiperiplanar (*ap*) carbonyl group, such as the lactone group in the rigid CGP 28 392 (**2**) may be a requirement for calcium channel agonist activity.[14] Molecular orbital (MOPAC) conformational calculations suggested that both carbonyl groups in calcium channel antagonists are preferentially oriented in a plane that intersects the DHP ring with an angle between 30° and 60°. In contrast, the nitro group of the calcium channel agonist Bay K 8644 (**3**) is oriented in the plane of the DHP ring.[16] Differences in the molecular electrostatic potentials between calcium channel antagonist and agonist structures, with respect to binding at the C-3 and C-5 regions, may provide a mechanism by which the receptor distinguishes between agonist and antagonist ligands. Accordingly, antagonists show a positive potential in this region when a C-3 ester group is present, whereas calcium channel activators are reported to have a strong negative potential in the region adjacent to the C-3 nitro

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* Corresponding author. Tel.: +1-780-492-5993; fax: +1-780-492-1217; e-mail: eknaus@pharmacy.ualberta.ca

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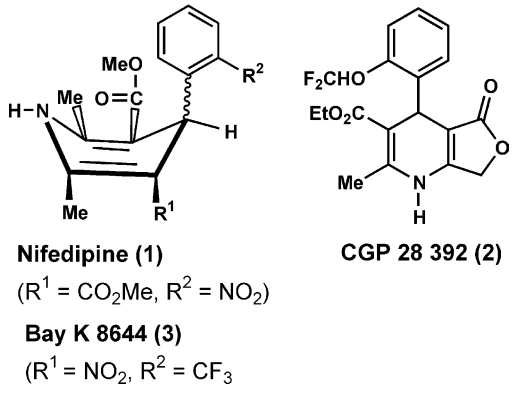


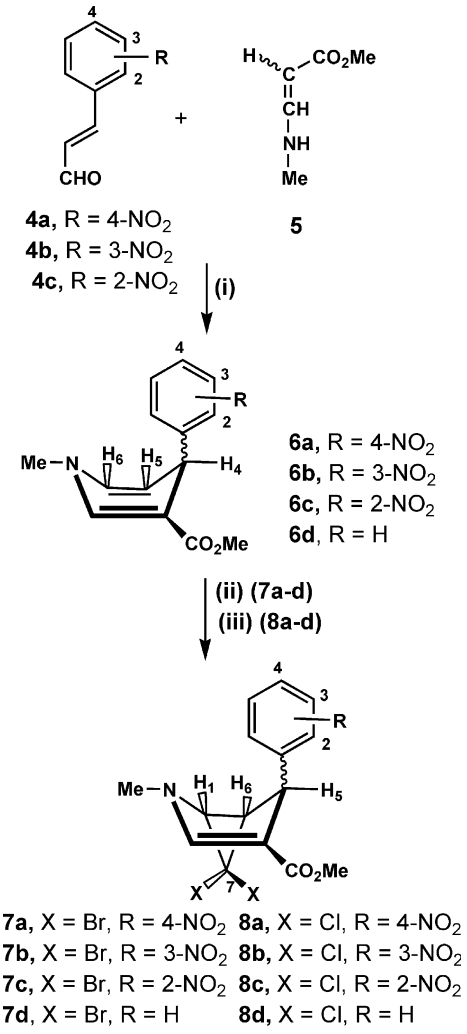
Figure 1. Structures of nifedipine (**1**), CGP 28 392 (**2**) and Bay K 8644 (**3**).

substituent.[17] It would ultimately be of interest to replace one of the 2-methyl-3-methoxycarbonyl-2,3-vinyl moieties of nifedipine (**1**) by a potentially bioisosteric dihalocyclopropyl moiety to determine the effect on calcium channel modulation. In this regard, we now report the synthesis and calcium channel antagonist activities for a group of bicyclic methyl 2-methyl-7,7-dihalo-5-phenyl-2-azabicyclo[4.1.0]hept-3-ene-4-carboxylates (**7**–**8**) that are structurally related to nifedipine (Fig. 1).

2. Chemistry

The piperidine catalyzed condendation[18] of 4-nitrocinnamaldehyde (**4a**),[19] 3-nitrocinnamaldehyde (**4b**),[20] or 2-nitrocinnamaldehyde (**4c**) with methyl 3-aminoacrylate (**5**), prepared by reaction of methylamine with methyl propiolate,[21,22] afforded the respective methyl 1-methyl-4-(4-, 3-, or 2-nitrophenyl)-1,4-dihydropyridine-3-carboxylate (**6a**, 30%; **6b**, 8%; **6c**, 27%) as illustrated in Scheme 1. Methyl 1-methyl-4-phenyl-1,4-dihydropyridine-3-carboxylate (**6d**) was prepared by the CuI catalyzed regioselective reaction of phenylmagnesium chloride with 3-methoxycarbonyl-1-methylpyridinium iodide according to literature procedures.[23,24] This latter reaction was not suitable for the preparation of **6a**–**c** since 4-, 3-, or 2-chloronitrobenzene did not react with magnesium metal to prepare the required Grignard reagent.

Due to their extreme reactivity, carbenes often undergo contraindicated insertion reactions that decrease the yield of the olefin addition product.[25] On the other hand, dihalocarbenes ($:CX_2$) are much less reactive species that do not furnish insertion products.[26–28] Singlet state carbenes afford stereospecific *syn* addition products upon reaction with olefins[29,30] that likely proceeds via a concerted mechanism.[31] In this regard, the thermal in situ generation of dihalocarbenes ($:CX_2$, X = Br, Cl)[32,33] from the Seyferth reagents[34] $PhHgCBr_3$, or $PhHgCBrCl_2$, in the presence of the methyl 1-methyl-4-(substituted-phenyl)-1,4-dihydropyridine-3-carboxylates (**6a**–**d**) yielded the respective methyl 2-methyl-7,7-dihalo-5-(substituted-phenyl)-2-azabicyclo[4.1.0]hept-3-ene-4-carboxylates (**7a**–**d**, X = Br; **8a**–**d**, X = Cl) in



Scheme 1. Reagents and conditions: (i) piperidine, MeOH, reflux, 12 h; (ii) $PhHgCBr_3$, dry benzene, reflux, 3 h; (iii) $PhHgCBrCl_2$, dry benzene, reflux, 3 h.

7–13% chemical yield (see Scheme 1). Electronegative halogen substituents decrease the rate of reaction of carbenes, which are electrophilic species.[35,36] Accordingly, it was expected that dihalocyclopropanation ($:CX_2$) would occur exclusively at the C5–C6 olefinic bond of **6a**–**d**, rather than the deactivated C2–C3 olefinic bond to which the 3-methoxycarbonyl substituent is attached. Regiospecific dihalocyclopropanation of the C5–C6 olefinic bond, rather than the C=O bond of the 3-methoxycarbonyl substituent, is also in agreement with the observation that dihalocarbenes rarely add to the C=O bond of aldehydes or lactones.[37] 1,4-Dihydropyridines such as **6a**–**d** exist in a boat conformation with the C-4 aryl ring pseudoaxial to the 1,4-DHP ring.[3,13,24,33] The concerted addition of $:CX_2$ should therefore occur from the sterically least hindered lower face of the boat-shaped 1,4-DHP ring where the orientation of the C-4 aryl ring remains unchanged. In this context, these stereochemical and regiochemical assignments are in agreement with the X-ray structure of 2-benzoyl-5-benzyl-7,7-dibromo-4-ethoxycarbonyl-2-azabicyclo[4.1.0]hept-3-ene that was prepared by reaction of 1-benzoyl-4-benzyl-3-ethoxycarbonyl-1,4-dihydro-

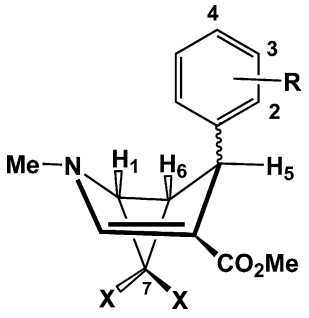
pyridine with :CBr_2, in which the C=O moiety of the C-4 CO_2Et substituent is *antiperiplanar* (*ap*) to the C3–C4 olefinic bond.[38]

3. Results and discussion

As part of an ongoing program to design novel calcium channel modulators, it was anticipated that replacement of the C5–C6 olefinic bond of 1-methyl-3-methoxycarbonyl-4-(substituted-phenyl)-1,4-dihydropyridines (**6a–d**) by a potentially bioisosteric dihalocyclopropyl moiety would provide a novel class of methyl 2-methyl-7,7-dihalo-5-(4-, 3-, or 2-nitrophenyl)-2-azabicyclo[4.1.0]hept-3-ene-4-carboxylates (**7–8**) that bear some structural relationship to the calcium channel antagonist nifedipine (**1**). This drug design concept is based on the fact that cyclopropyl C–C bonds have a number of similarities to olefinic bonds that include: (i) the hybridization of cyclopropyl C–C bonds is intermediate in character between a sigma (σ) and a pi (π) bond,[37] (ii) a cyclopropyl C–C bond, like a C=C bond, is able to conjugate with an olefinic bond to which it is attached,[37] but unlike a C=C it does not transmit electronic effects,[39] and (iii) a cyclopropyl ring interacts with adjacent π-electron systems and p-electron centers much like a vinyl group.[40,41] In this regard, a cyclopropane group has been investigated as a alkene bioisostere.[42–44] Rigid tetracyclic compounds derived from structural analogs of the calcium channel antagonist nifedipine having a fused cyclopropane ring have been synthesized but no pharmacological properties were reported.[45] It was therefore anticipated that the fused dihalocyclopropyl ring in the bicyclic compounds (**7–8**), which is oriented below the plane of the boat-shaped tetrahydropyridine ring, may serve as a bioisostere of the 2-methyl-3-methoxycarbonyl-2,3-olefinic moiety present in nifedipine (**1**). In compounds **7–8**, one C-7 halo substituent on the fused planar cyclopropyl ring system is *syn* to the H-1 and H-6 hydrogen atoms, while the other C-7 halogen substituent is *syn* to the C3–C4 vinyl bond.

The in vitro calcium channel antagonist activities exhibited by compounds **7–8** were determined using a guinea pig ileum longitudinal smooth muscle (GPILSM) assay,[6] and the results are summarized in Table 1. Compounds **7–8** exhibited weaker calcium channel antagonist activity (10^{-5}–10^{-6} M range) relative to the reference drug nifedipine ($IC_{50} = 1.40\times10^{-8}$ M). Structure–activity relationships showed that the position of a nitro substituent on the C-5 phenyl ring was a determinant of calcium channel antagonist activity where the potency profile was *ortho* > *meta* > *para* (**7c** > **7b** > **7a**; **8c** > **8b** > **8a**). It appears that the size (van der Waal's radius for Br and Cl are 1.95 and 1.80 Å, respectively), and/or electronegativity (Cl > Br) of the C-7 halogen atoms may be a determinant of CC antagonist activity. Although the differences in antagonist activity were small, compounds having C-7 geminal-bromo substituents were generally more potent that the corresponding C-7 chloro derivatives (**7a** > **8a**; **7b**≈**8b**; **7c** > **8c**; **7d** ⩾ **8d**). This class of compounds **7–8** did not exhibit an inotropic effect (antagonist or agonist) on guinea pig left atria.

Table 1. In vitro calcium channel antagonist activities for methyl 2-methyl-7,7-dihalo-5-substituted-phenyl-2-azabicyclo[4.1.0]hept-3-ene-4-carboxylates (**7–8**)



Compd	R	X	Calcium channel antagonist activity GPILSM: IC_{50} (M)[a]
7a	4-NO_2	Br	$8.82\pm0.65\times10^{-6}$
7b	3-NO_2	Br	$5.85\pm1.19\times10^{-6}$
7c	2-NO_2	Br	$2.25\pm0.11\times10^{-6}$
7d	H	Br	$7.17\pm1.26\times10^{-6}$
8a	4-NO_2	Cl	$2.01\pm0.36\times10^{-5}$
8b	3-NO_2	Cl	$5.79\pm0.33\times10^{-6}$
8c	2-NO_2	Cl	$4.55\pm0.26\times10^{-6}$
8d	H	Cl	$7.97\pm1.02\times10^{-6}$
Nifedipine			$1.40\pm0.19\times10^{-8}$

[a] The molar concentration of the test compound causing a 50% decrease in the slow component or tonic contractile response ($IC_{50}\pm$SEM, $n = 3$) in guinea pig ileum longitudinal smooth muscle (GPILSM) induced by the muscarinic agonist carbachol (1.6×10^{-7} M) was determined graphically from the dose–response curve.

4. Conclusions

A geminal-dihalocyclopropyl moiety (i) is a potential bioisostere for the 2-methyl-3-methoxycarbonylvinyl moiety of nifedipine (**1**), and (ii) the dihalocyclopropyl compounds **7–8** provides further structure–activity data that may be of value to probe the structure–function relationship of calcium channel modulation.

5. Experimental

Melting points were determined using a Thomas–Hoover capillary apparatus and are uncorrected. Infrared (IR) spectra were recorded using a Nicolet 550 Series II Magna FT-IR spectrometer. ^{1}H NMR nuclear magnetic resonance spectra were recorded on a Bruker AM-300 spectrophotometer. Silica gel column chromatography was performed using Silicycle® (silica gel 70–230 mesh). Elemental analyses were determined for C, H, and N (microanalytical service laboratory, Department of Chemistry, University of Alberta). 4-Nitrocinnamaldehyde (**4a**),[19] 3-nitrocinnamaldehyde (**4b**),[20] methyl 3-aminoacrylate (**5**),[21,22] methyl 1-methyl-4-phenyl-1,4-dihydropyridine-3-carboxylate (**6d**),[23,24] phenyl(tribromomethyl)mercury ($PhHgCBr_3$),[34] and phenyl(bromodichloromethyl)mercury ($PhHgCBrCl_2$),[34] were prepared according to the literature procedures.

All other reagents, including 2-nitrocinnamaldehyde (**4c**), were purchased from Aldrich Chemical (Milwaukee, WI) and used without further purification. In vitro calcium channel antagonist activities were determined using protocols approved by the Health Sciences Animal Welfare Committee at the University of Alberta.

5.1. General method for the synthesis of methyl 1-methyl-4-(substituted-phenyl)-1,4-dihydropyridine-3-carboxylates (6a–c)

A mixture of the nitrocinnamaldehyde (**4a**, **4b**, or **4c**; 4.43 g, 25 mmol), methyl 3-methylaminoacrylate (**5**, 2.88 g, 25 mmol), and piperidine (120 μL, 1.02 mmol) in methanol (12 mL) was refluxed for 2 h. The solvent was removed in vacuo, and the respective product (**6a**, **6b**, or **6c**) was purified by elution from a silica gel column using ethyl acetate–hexanes (25:75, v/v) as eluent. Some physical and spectroscopic data for **6a**–**c** are listed below.

5.1.1. Methyl 1-methyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3-carboxylate (6a). Oil; yield 30.5%; IR (NaCl) ν 1688 (C=O), 1589 (C=C, aromatic), 1523, 1341 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.24 (d, $J_{ortho} = 8.7$ Hz, 2H, phenyl H-3, H-5), 7.54 (d, $J_{ortho} = 8.7$ Hz, 2H, phenyl H-2, H-6), 7.40 (s, 1H, DHP H-2), 6.49 (d, 1H, $J_{5,6} = 9.9$ Hz, DHP H-6), 5.14 (d, $J_{4,5} = 4.2$ Hz, 1H, DHP H-4), 5.04 (dd, $J_{5,6} = 9.9$, $J_{4,5} = 4.2$ Hz, 1H, DHP H-5), 3.73 (s, 3H, OCH_3), 2.85 (s, 3H, NCH_3). Compound **6a** is sensitive to light and it should be used as soon as possible after purification. When **6a** is stored, it should be placed in a dark container in a cool place prior to use.

5.1.2. Methyl 1-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3-carboxylate (6b). Oil; yield 8%; IR (NaCl) ν 1685 (C=O), 1637 (C=C, aromatic), 1527, 1348 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.19 (d, $J_{4,5} = 8.1$ Hz, 1H, phenyl H-4), 8.16 (s, 1H, phenyl H-2), 7.77 (d, $J_{5,6} = 7.5$ Hz, 1H, phenyl H-6), 7.58 (dd, $J_{4,5} = 8.1$, $J_{5,6} = 7.5$ Hz, 1H, phenyl H-5), 7.39 (s, 1H, DHP H-2), 6.51 (d, $J_{5,6} = 10.2$ Hz, 1H, DHP H-6), 5.25 (d, $J_{4,5} = 3.9$ Hz, 1H, DHP H-4), 5.06 (dd, $J_{5,6} = 10.2$, $J_{4,5} = 3.9$ Hz, 1H, DHP H-5), 3.73 (s, 3H, OCH_3), 2.86 (s, 3H, NCH_3). Compound **6b** is sensitive to light and it should be used as soon as possible after purification. When **6b** is stored, it should be placed in a dark container in a cool place prior to use.

5.1.3. Methyl 1-methyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3-carboxylate (6c). Red crystals; mp 112–114 °C (MeOH/H_2O); yield 27.5%; IR (NaCl) ν 1696 (C=O), 1642 (C=C, aromatic), 1528, 1307 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.91 (d, $J_{3,4} = 8.1$ Hz, 1H, phenyl H-3), 7.86 (d, $J_{5,6} = 8.1$ Hz, 1H, phenyl H-6), 7.67 (dd, $J_{4,5} = 7.2$, $J_{5,6} = 8.1$ Hz, 1H, phenyl H-5), 7.48 (s, 1H, DHP H-2), 7.45 (dd, $J_{3,4} = 8.1$, $J_{4,5} = 7.2$ Hz, 1H, phenyl H-4), 6.45 (d, $J_{5,6} = 9.9$ Hz, 1H, DHP H-6), 5.83 (d, $J_{4,5} = 4.2$ Hz, 1H, DHP H-4), 5.27 (dd, $J_{5,6} = 9.9$, $J_{4,5} = 4.5$ Hz, 1H, DHP H-5), 3.73 (s, 3H, OCH_3), 2.86 (s, 3H, NCH_3). Anal. Calcd for $C_{14}H_{14}N_2O_4$: C, 61.31; H, 5.15; N, 10.21. Found: C, 60.99; H, 5.49; N, 10.25. Compound **6c** is sensitive to light, and it should be stored in a dark container in a cool place prior to use.

5.2. General method for the preparation of methyl 2-methyl-7,7-dihalo-5-(4-, 3-, or 2-nitrophenyl)-2-azabicyclo[4.1.0]hept-3-ene-4-carboxylates (7a–d, 8a–d)

Phenyl(tribromomethyl)mercury (1.38 g, 2.6 mmol), or phenyl(bromodichloromethyl)mercury (1.15 g, 2.6 mmol), was added to stirred solution of **6a**, **6b**, **6c**, or **6d** (2.6 mmol) in dry benzene (20 mL), the reaction flask was sealed with a rubber septum, and the mixture was refluxed for 3 h. The reaction mixture was cooled to 25 °C, and the PhHgBr, which precipitated during the reaction was removed by filtration. Removal of the solvent in vacuo gave a brownish oil, which was purified by silica gel column chromatography using ethyl acetate–hexane (25:75, v/v) as eluent to afford the respective product **7a**–**d** using phenyl(tribromomethyl)mercury, or **8a**–**d** using phenyl(bromodichloromethyl)mercury. Physical and spectral data for these products are listed below.

5.2.1. Methyl 7,7-dibromo-2-methyl-5-(4-nitrophenyl)-2-azabicyclo[4.1.0]hept-3-ene-4-carboxylate (7a). Yellow crystals (Et_2O); mp 194–195 °C (decomp.); yield 7.5%; IR (NaCl) ν 1686 (C=O), 1621 (C=C, aromatic), 1511, 1346 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.26 (d, $J_{ortho} = 9.0$ Hz, 2H, phenyl H-3, H-5), 7.50 (s, 1H, H-3), 7.46 (d, $J_{ortho} = 9.0$ Hz, 2H, phenyl H-2, H-6), 4.29 (s, 1H, H-5), 3.80 (s, 3H, OCH_3), 2.85 (d, $J_{1,6} = 11.1$ Hz, 1H, H-1), 2.76 (s, 3H, NCH_3), 2.16 (d, $J_{1,6} = 11.1$ Hz, 1H, H-6). Anal. Calcd for $C_{15}H_{14}Br_2N_2O_4$: C, 40.39; H, 3.16; N, 6.28. Found: C, 40.40; H, 2.98; N, 6.05.

5.2.2. Methyl 7,7-dibromo-2-methyl-5-(3-nitrophenyl)-2-azabicyclo[4.1.0]hept-3-ene-4-carboxylate (7b). Yellow crystals (Et_2O–hexane); mp 154–156 °C (decomp.); yield 11%; IR (NaCl) ν 1683 (C=O), 1621 (C=C, aromatic), 1530, 1346 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.20–8.24 (m, 1H, phenyl H-4), 8.14 (s, 1H, phenyl H-2), 7.57–7.64 (m, 2H, phenyl H-5, H-6), 7.51 (s, 1H, H-3), 4.30 (s, 1H, H-5), 3.80 (s, 3H, OCH_3), 2.86 (d, $J_{1,6} = 11.1$ Hz, 1H, H-1), 2.77 (s, 3H, NCH_3), 2.18 (d, $J_{1,6} = 11.1$ Hz, 1H, H-6). Anal. Calcd for $C_{15}H_{14}Br_2N_2O_4$: C, 40.39; H, 3.16; N, 6.28. Found: C, 40.76; H, 3.03; N, 6.00.

5.2.3. Methyl 7,7-dibromo-2-methyl-5-(2-nitrophenyl)-2-azabicyclo[4.1.0]hept-3-ene-4-carboxylate (7c). Yellow crystals (Et_2O–hexanes); mp 122–124 °C (decomp.); yield 10%; IR (NaCl) ν 1686 (C=O), 1621 (C=C, aromatic), 1523, 1341 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.02 (d, $J_{3,4} = 8.4$ Hz, 1H, phenyl H-3), 7.64 (t, $J_{5,6} = 7.5$, $J_{4,5} = 7.5$ Hz, 1H, phenyl H-5), 7.55 (s, 1H, H-3), 7.51 (dd, $J_{4,5} = 7.5$, $J_{3,4} = 8.4$ Hz, 1H, phenyl H-4),

7.38 (dd, $J_{5,6} = 7.5$, $J_{4,6} = 0.9$ Hz, 1H, phenyl H-6), 4.83 (s, 1H, H-5), 3.80 (s, 3H, OCH_3), 2.78 (d, $J_{1,6} = 11.1$ Hz, 1H, H-1), 2.75 (s, 3H, NCH_3), 2.33 (d, $J_{1,6} = 11.1$ Hz, 1H, H-6). Anal. Calcd for $C_{15}H_{14}Br_2N_2O_4$: C, 40.39; H, 3.16; N, 6.28. Found: C, 40.31; H, 2.96; N, 6.01.

5.2.4. Methyl 7,7-dibromo-2-methyl-5-phenyl-2-azabicyclo[4.1.0]hept-3-ene-4-carboxylate (7d). White crystals (Et_2O–hexanes) after purification by elution from a silica gel column using ethyl acetate–hexanes (15:85, v/v) as eluent; mp 96–98 °C; yield 13%; IR (NaCl) ν 1690 (C=O), 1630 (C=C, aromatic) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.33 (s, 1H, H-3), 7.16-7.29 (m, 5H, phenyl hydrogens), 3.92 (s, 1H, H-5), 3.53 (s, 3H,OCH_3), 3.25 (s, 3H, NCH_3), 3.11 (d, $J_{1,6} = 11.1$ Hz, 1H, H-1), 2.24 (d, $J_{1,6} = 11.1$ Hz, 1H, H-6). Anal. Calcd for $C_{15}H_{15}Br_2NO$: C, 44.92; H, 3.77; N, 3.49. Found: C, 44.86; H, 3.67; N, 3.43.

5.2.5. Methyl 7,7-dichloro-2-methyl-5-(4-nitrophenyl)-2-azabicyclo[4.1.0]hept-3-ene-4-carboxylate (8a). Yellow crystals (Et_2O); mp 197–199 °C (decomp.); yield 9.3%; IR (NaCl) ν 1686 (C=O), 1631 (C=C, aromatic), 1520, 1346 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.20 (d, $J_{ortho} = 8.7$ Hz, 2H, phenyl H-3, H-5), 7.49 (s, 1H, H-3), 7.46 (d, $J_{ortho} = 8.7$ Hz, 2H, phenyl H-2, H-6), 4.39 (s, 1H, H-5), 3.80 (s, 3H, OCH_3), 2.82 (d, $J_{1,6} = 11.1$ Hz, 1H, H-1), 2.77 (s, 3H, NCH_3), 2.08 (d, $J_{1,6} = 11.1$ Hz, 1H, H-6). Anal. Calcd for $C_{15}H_{14}Cl_2N_2O_4$: C, 50.44; H, 3.95; N, 7.84. Found: C, 50.17; H, 3.77; N, 7.62.

5.2.6. Methyl 7,7-dichloro-2-methyl-5-(3-nitrophenyl)-2-azabicyclo[4.1.0]hept-3-ene-4-carboxylate (8b). Yellow crystals (Et_2O–hexanes); mp 163–165 °C (decomp.); yield 10.4%; IR (NaCl) ν 1683 (C=O), 1623 (C=C, aromatic), 1525, 1353 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.20–8.23 (m, 1H, phenyl H-4), 8.14 (s, 1H, phenyl H-2), 7.57–7.64 (m, 2H, phenyl H-5, H-6), 7.50 (s, 1H, H-3), 4.41 (s, 1H, H-5), 3.80 (s, 3H, OCH_3), 2.82 (d, $J_{1,6} = 11.1$ Hz, 1H, H-1), 2.78 (s, 3H, NCH_3), 2.10 (d, $J_{1,6} = 11.1$ Hz, 1H, H-6). Anal. Calcd for $C_{15}H_{14}Cl_2N_2O_4$: C, 50.44; H, 3.95; N, 7.84. Found: C, 50.77; H, 4.01; N, 7.66.

5.2.7. Methyl 7,7-dichloro-2-methyl-5-(2-nitrophenyl)-2-azabicyclo[4.1.0]hept-3-ene-4-carboxylate (8c). Yellow crystals (Et_2O–hexanes); mp 146–147 °C (decomp.); yield 11%; IR (NaCl) ν 1683 (C=O), 1629 (C=C, aromatic), 1520, 1346 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.01 (dd, $J_{3,4} = 8.4$, $J_{3,5} = 1.2$ Hz, 1H, phenyl H-3), 7.64 (ddd, $J_{4,5} = 7.8$, $J_{5,6} = 7.5$, $J_{3,5} = 1.2$ Hz, 1H, phenyl H-5), 7.54 (s, 1H, H-3), 7.50 (ddd, $J_{3,4} = 8.4$, $J_{4,5} = 7.8$, $J_{4,6} = 1.2$ Hz, 1H, phenyl H-4), 7.38 (dd, $J_{5,6} = 7.5$, $J_{4,6} = 1.2$ Hz, 1H, phenyl H-6), 4.93 (s, 1H, H-5), 3.79 (s, 3H, OCH_3), 2.76 (s, 3H, NCH_3), 2.75 (d, $J_{1,6} = 11.1$ Hz, 1H, H-1), 2.25 (d, $J_{1,6} = 11.1$ Hz, 1H, H-6). Anal. Calcd for $C_{15}H_{14}Cl_2N_2O_4$: C, 50.44; H, 3.95; N, 7.84. Found: C, 50.56; H, 3.86; N, 7.61.

5.2.8. Methyl 7,7-dichloro-2-methyl-5-phenyl-2-azabicyclo[4.1.0]hept-3-ene-4-carboxylate (8d). White crystals after purification by silica gel column chromatography using ethyl acetate–hexanes (15:85, v/v) as eluent; mp 95–96 °C (Et_2O–hexanes); yield 12%; IR (NaCl) ν 1688 (C=O), 1622 (C=C, aromatic) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.33 (s, 1H, H-3), 7.16–7.31 (m, 5H, phenyl hydrogens), 3.99 (s, 1H, H-5), 3.53 (s, 3H, OCH_3), 3.22 (s, 3H, NCH_3), 3.04 (d, $J_{1,6} = 11.1$ Hz, 1H, H-1), 2.14 (d, $J_{1,6} = 11.1$ Hz, 1H, H-6). Anal. Calcd for $C_{15}H_{15}Cl_2NO_2$: C, 57.71; H, 4.84; N, 4.49. Found: C, 57.84; H, 4.72; N, 4.39.

5.3. In vitro calcium channel antagonist assay

Smooth muscle calcium channel antagonist activity was determined as the molar (M) concentration of the test compound required to produce 50% inhibition of the muscarinic receptor-mediated (carbachol, 1.6×10^{-7} M) Ca^{+2}-dependent contraction (tonic response) of guinea pig ileum longitudinal smooth muscle (GPILSM) using the procedure previously reported.[6] The IC_{50} (± SEM, $n = 3$) was determined graphically from the dose–response curve.

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