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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, characterization, and pharmacological evaluation of benzothiopyran derivatives as a novel class of calcium channel blockers

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Abstract The current research aimed to investigate the importance of the heterocyclic ring system in the structure of the cardiovascular drug Diltiazem for its calcium channel blocking activity. The manuscript describes the design, synthesis, and biological testing of Benzothiopyran derivatives (7a–7f). The new compounds maintain some Diltiazem pharmacophores. Benzothiopyran has two pharmacophore: the aromatic benzene ring fused with the heterocyclic thiopyrans ring, and the stereo-chemical centers (alkyl ether). In vitro evaluation of Benzothiopyran derivatives (7a–7f) for calcium channel blocking effects revealed moderate activities. Compounds of the current series showed optimum activity when the alkyl ether chain was substituted on the 3-chloro-3,4-dihydro-2H-1-benzothiopyran-4-ol derivatives (7a–7f).

Keywords Calcium channel blocker · Diltiazem · Benzothiopyran · Cardiovascular agents

Introduction

Calcium channel blockers (CCBs) are a group of heterogeneous drugs whose main pharmacological effect is to prevent or reduce the entry of calcium into cell via specialized calcium channel (Gennaro, 2000; Mehanna and Kim, 2003). Commercially available calcium channel blockers belong to either one of three chemical classes: Dihydropyridines (Amlodipine, Nifedipine, Felodipine, Isradipine), Phenyl alkyl amines (Verapamil, Gallopamil), and Benzothiazepine (Diltiazem) (Delgado and Remers, 1998). The intracellular concentration of calcium plays an important role in maintaining the tone of smooth muscle and in the contraction of the myocardium. Calcium enters muscle cells through special voltage-sensitive calcium channels. Calcium channel antagonists block the inward movements of calcium by binding it to L-type calcium channels in the heart and in smooth muscle of the coronary and peripheral vasculature (Harvey *et al.*, 2000). These antagonists prevent the calcium ions needed for muscle contraction from entering the cells of smooth and cardiac muscle. This decreases intracellular calcium leading to a reduction in muscle contraction. In heart, a decrease in calcium available for each beat results in decreased cardiac contractility. The current research explores the development of new and facile synthetic methods for such heterocyclic considered for activity (Rios *et al.*, 2006). Benzothiopyran is a heterocyclic compound that contains sulfur as a heteroatom, which is responsible for biological and pharmacological activity (Mehanna and Kim, 2005). Changing the heterocyclic ring size generated derivatives that not only retained the calcium channel blocking activity but also resulted in generation of several other compounds that were more active than Diltiazem (Rios *et al.*, 2006). A receptor-binding model identifies the benzene ring as a lipophilic group that facilitates transport into the channel, and the absolute stereochemistry for the selective binding (Dodda *et al.*, 2009). Benzothiopyran has two pharmacophores: aromatic benzene ring fused with heterocyclic thiopyrans ring and the stereo-chemical centers (alkyl ether). The benzene ring as a lipophilic group facilitates

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transport into channel, and provides absolute stereochemistry for the selective binding. Substitution of alkyl ethers was undertaken as it is highly lipophilic due to the presence of long chain of carbon atoms (Rios *et al.*, 2006). Substituents such as methoxy, ethoxy, propoxy, butoxy, pentoxy, and phenoxy were found to be highly active for coronary vasodilating activity, and substitution of chlorine at the 7 position in benzothiopyran ring led to slight reduction in activity (Kimball *et al.*, 1993).

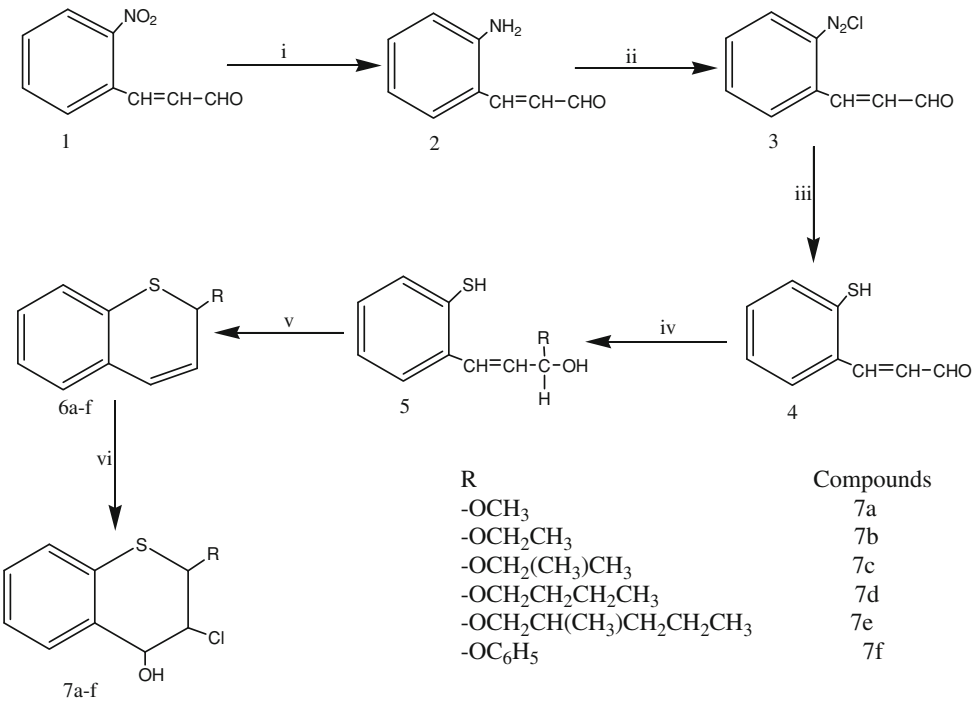
Results and discussion

Chemistry and synthesis

Scheme 1 depicts the route to synthesize the target benzothiopyran derivatives (compounds 7a–7f, Scheme 1). Substituents such as methoxy, ethoxy, propoxy, butoxy, pentoxy, and phenoxy were found to be highly active for coronary vasodilating activity, and substitution of chlorine at the 7 position in benzothiopyran ring led to slight reduction in activity (Kimball *et al.*, 1993). Compounds 7a–7f are benzothiopyran derivatives that mimic diltiazem pharmacophoric group. Compound(s) 7a–7f have an alkyl ether chain part of a benzothiopyran ring system substituted with different alcohols as shown in Scheme 1 which depicts the synthetic route to prepare the target compounds. Reaction of 3-(2-nitrophenyl)prop-2-enal(1) with $SnCl_2$ in ethanol provided the common intermediate 3-(2-aminophenyl)prop-2-enal(2). Reaction of 3-(2-aminophenyl) prop-2-enal(2) with sodium nitrate, hydrochloric acid, and potassium hydrogen sulfide provided the common intermediate 3-(2-sulfanylphenyl)prop-2-enal(4). Reaction of 3-(2-sulfanylphenyl)prop-2-enal(4) with different alcohols gave the corresponding intermediate (6a–6f). Reaction of 6a–6f with hypochlorous acid and water provided the final compounds 3-chloro-2-methoxy-3,4-dihydro-2H-1-benzothiopyran-4-ol(7a), 3-chloro-2-ethoxy-3,4-dihydro-2H-1-benzothiopyran-4-ol(7b), 3-chloro-2-(propan-2-yloxy)-3,4-dihydro-2H-1-benzothiopyran-4-ol(7c), 2-butoxy-3-chloro-3,4-dihydro-2H-1-benzothiopyran-4-ol(7d), 3-chloro-2-(2-methylbutoxy)-3,4-dihydro-2H-1-benzothiopyran-4-ol(7e), and 3-chloro-2-phenoxy-3,4-dihydro-2H-1-benzothiopyran-4-ol(7f).

In vitro biological evaluation

All six compounds (7a–7f) were tested for In vitro calcium channel blocking activity, on contractions of potassium depolarized isolate of guinea-pig ileum according to a previously reported protocol. A series of substituted 3-chloro-3,4-dihydro-2H-1-benzothiopyran-4-ol(7b–7e) has been investigated for their calcium channel blocking activity on guinea-pig ileum. The guinea-pig ileum showed spontaneous contraction when KCl solution was added to tissue bath at concentration of 80 mM, after that different derivatives were tested for their antagonistic activity with reference to nifedipine. It was observed that with increase in concentration of test compounds, significant antagonistic activity was exhibited. In all derivatives, the 100 mM concentration of test compound showed the highest antagonistic activity that was the highest muscle relaxation



R	Compounds
$-OCH_3$	7a
$-OCH_2CH_3$	7b
$-OCH_2(CH_3)CH_3$	7c
$-OCH_2CH_2CH_2CH_3$	7d
$-OCH_2CH(CH_3)CH_2CH_2CH_3$	7e
$-OC_6H_5$	7f

Scheme 1 Synthesis of benzothiopyran derivatives. Reagents and conditions: (1) $SnCl_2$, Ethanol, HCl; (2) $NaNO_2$, HCl; (3) KSH, HCl; (4) Different Alcohol, HCl; (5) Cyclization; (6) HOCl, H_2O

as compared to KCl-induced muscle contraction. Table 1 and Figs. 1, 2, 3, 4 list the calcium channel blocking activities for the new compounds, expressed, with Nifedipine as the reference drug. Table 1 and Figs. 1, 2, 3, 4 data indicate that in spite of lacking heterocyclic ring system, the new compounds were moderately active as calcium channel blockers.

Chemistry

General information: Melting points were determined using digital melting point apparatus model VMP-DS, Veego apparatus and are uncorrected. FT-IR spectra were obtained with KBr on FT-IR 8400 S (Shimadzu, Japan) spectrophotometer model using Nujol and potassium bromide and on Perkin Elmer RX1 using potassium bromide cell for liquid sample and potassium pellets for solid sample(υ_{max} in cm^{-1}). ^{1}HNMR spectra were recorded on a Brucker Avance II 400 NMR spectrometer with tetramethylsilane (TMS) as an internal standard. The values of chemical shift (d) are given in parts per million (ppm) and coupling constants (J) in Hertz (Hz). The reaction progress was monitored using TLC on silica gel plate (Merck Pvt. Ltd). Reported yields are for the purified products and are

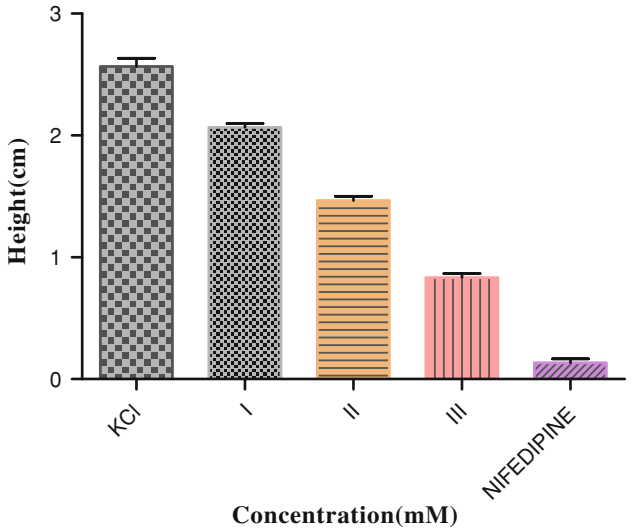


Fig. 1 *Bar diagram* showing the effect of compound (7b) on various concentration (60, 80, 100 mM) and reference drug (Nifedipine 10 mM)

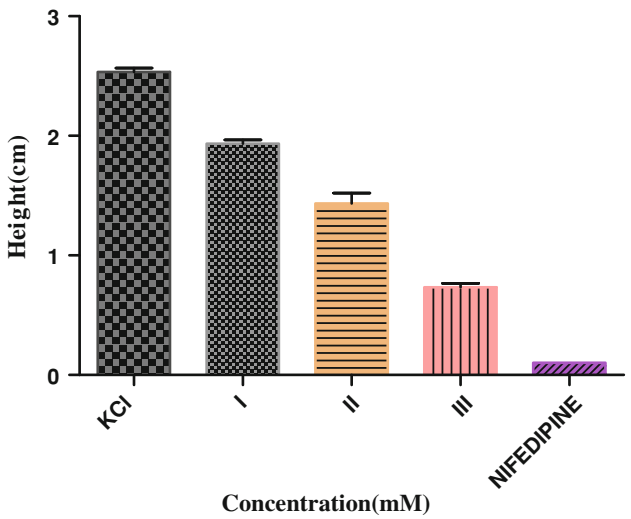


Fig. 2 *Bar diagram* showing the effect of compound (7c) on various concentration (60, 80, 100 mM) and reference drug (Nifedipine 10 mM)

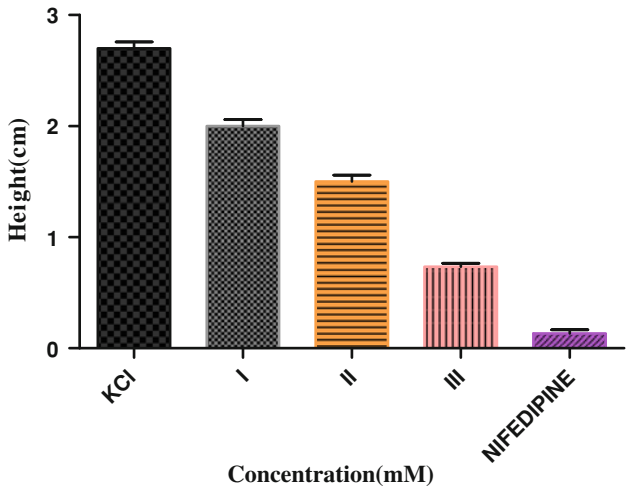


Fig. 3 *Bar diagram* showing the effect of compound (7d) on various concentration (60, 80, 100 mM) and reference drug (Nifedipine 10 mM)

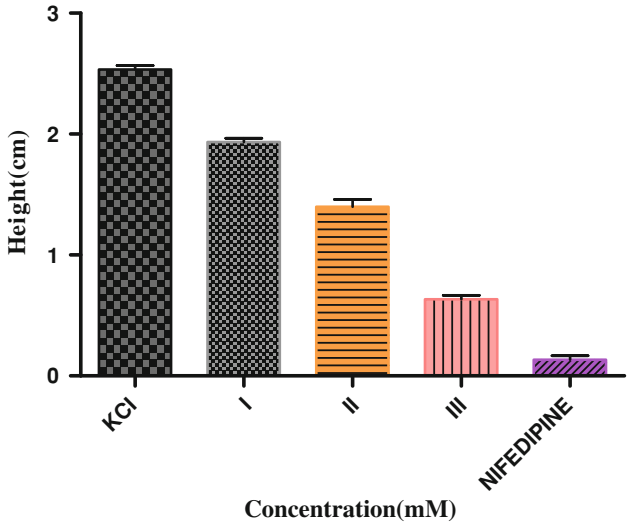


Fig. 4 *Bar diagram* showing the effect of compound (7e) on various concentration (60, 80, 100 mM) and reference drug (Nifedipine 10 mM)

Table 1 Calcium channel blocking activity of the new compounds expressed as concentration–response curve

Dose	Conc. (mM)	Mean ± S.E.M. (7b)	Mean ± S.E.M. (7c)	Mean ± S.E.M. (7d)	Mean ± S.E.M. (7e)
KCl	80	2.567 ± 0.066	2.533 ± 0.033	2.700 ± 0.057	2.533 ± 0.033
I	60	2.067 ± 0.033***	1.933 ± 0.033***	2.000 ± 0.057***	1.933 ± 0.033***
II	80	1.467 ± 0.033***	1.433 ± 0.088***	1.500 ± 0.057***	1.400 ± 0.047***
III	100	0.83 ± 0.033***	0.733 ± 0.033***	0.733 ± 0.033***	0.633 ± 0.033***
Nifedipine	10	0.13 ± 0.033***	0.100 ± 0.0***	0.133 ± 0.033***	0.133 ± 0.033***

*** Significance coefficient $P < 0.001$

not optimized. Elemental analysis were performed by Vario EL III model. All the animal experiments were carried out at Department of Pharmacology, B.R. Nahata College of Pharmacy, Mandsaur, (M.P.) and permission for conducting this experiment was obtained from Institutional Animal Ethical Committee, Mandsaur (M.P.).

3-(2-Aminophenyl)prop-2-enal(2)

2 gm (0.01 mol) of 3-(2-nitrophenyl)prop-2-enal(1) was weighed and dissolved in 80 ml ethanol and 7.5 gm (0.03 mol) of tin(II) chloride dihydrate was added with 10 ml of concentrated hydrochloric acid. Reaction mixture was refluxed on oil bath for 3 h at 80 °C, the reaction mixture was cooled, and water was added to obtain the yield (Furniss *et al.*, 1989a, b, c; Desilets and Hamer, 1993). The precipitate was recrystallized using acetone to produce 0.95 gm (55.00 %) of intermediate(2) as white crystals. M.p. = 240–245 °C; IR (KBr, υ cm^{-1}): 3,482.91, 3,370.76, 3,049.25, 2,975.96, 2,736.80, 1,680.56, 1,627.81, 1,610.43, 1,442.66, 1,569.95, 1,172.64, 1,037.63, 738.69; ^{1}HNMR (400 MHz, δ ppm, CDCl3): δ 4.13 (s, 2H, amino-H), 6.59 (d, 1H, $J = 12.72$ Hz, –CH=C–), 6.62 (dd, 1H, $J = 8.0$ Hz, Ar–H), 6.84 (td, 1H, $J = 6.56$, Ar–H), 6.97 (dd, 1H, $J = 2.42$, Ar–H), 7.36 (td, 1H, $J = 6.16$, Ar–H), 7.61 (d, 1H, $J = 12.44$ Hz, C=CH–), 9.59 (s,1H, aldehyde-H).

3-(2-Sulfanylphenyl)prop-2-enal(4)

2 gm (0.01 mol) of 3-(2-Aminophenyl)prop-2-enal(2) was weighed and dissolved in 6.5 ml concentrated hydrochloric acid and 6.5 ml water. The mixture was cooled at 0 °C in an ice-salt bath. A solution of 1.6 gm (0.02 mol) sodium nitrite was added in 8 ml water at 0–5 °C for 10–15 min with addition of a little crushed ice from time to time. The cold diazonium chloride solution was poured slowly into the cold potassium hydrogen sulfide. The mixture was allowed to warm up to room temperature, on a water bath to about 60 °C (Furniss *et al.*, 1989a, b, c; Desilets and Hamer, 1993). Recrystallization was done using mixture of chloroform and ethanol to produce 1.20 gm (53.33 %) of intermediate (4) as yellow powder. M.p. = 275–280 °C; IR (KBr, υ cm^{-1}): 3,045.11, 2,925.81, 2,735.13, 1,681.81, 1,618.17, 1,519.80, 1,456.16, 671.18, 1,035.70, 964.34, 746.40; ^{1}HNMR (400 MHz, δ ppm, DMSO): 3.11 (s, 1H, mercapto-H), 6.79 (td, 1H, $J = 7.36$, Ar–H), 7.11 (d, 1H, $J = 13.36$, –C=CH–), 7.25 (dd, 1H, $J = 6.72$ Hz, Ar–H), 7.41 (td, 1H, $J = 6.80$ Hz, Ar–H), 7.57 (dd, 1H, $J = 6.96$ Hz, Ar–H), 8.11 (d, 1H, $J = 10.72$ Hz, –CH=C–), 9.69 (s, 1H, aldehyde-H).

2-Methoxy-2H-thiochromene(23a)

2 gm (0.012 mol) powder of 3-(2-sulfanylphenyl)prop-2-enal(4) was weighed and placed in 250 ml round bottom flask and 4 ml (0.13 mol) methanol was added with few microliters of concentrated hydrochloric acid. The solution was stirred over night at room temperature. The solution was concentrated by solvent evaporation of methanol with a rotary evaporator at 50 °C. The concentrated solution was heated at 60 °C in alkaline condition for 4 h (Salam and Marguerite, 1990). Recrystallization was done using mixture of chloroform and ethyl acetate to produce 1.65 gm (76.38 %) of intermediate (6a) as off white powder. M.p. = 255–260 °C; IR (KBr, υ cm^{-1}) :3,032.68, 2,867.96, 1,595.46, 1,486.10, 1,446.51, 1,370.08, 1,255.00,1,035.70, 1,080.06, 746.61, 669.25; ^{1}HNMR (400 MHz, δ ppm, $CDCl_3$): 3.43 (s, 3H, methoxy-H), 5.45 (d, 1H, $J = 6.00$, –S–CH–), 5.97 (dd, 1H, $J = 10.88$, –S–C–CH–), 6.60 (d, 1H, $J = 10.52$ Hz, –S–C–C=CH–), 7.01 (td, 1H, $J = 5.94$, Ar–H), 7.15 (dd, 1H, $J = 7.44$, Ar–H), 7.36 (td, 1H, $J = 6.98$, Ar–H), 7.43 (dd, 1H, $J = 7.54$, Ar–H).

3-Chloro-2-methoxy-3,4-dihydro-2H-1-benzothiopyran-4-ol(7a)

In a 100 ml round bottom flask with a stirrer, 7.31 ml water and 1.70 ml (0.032 mol) hypochlorous acid were taken. 1.00 gm (0.005 mol) of 2-Methoxy-2H-thiochromene(6a) was added to the reaction mixture and the temperature of the reaction was maintained between 40 and 45 °C for 4 h. The mixture was then allowed to stand overnight at room

temperature. The solvent was removed and put in water bath, and then ice-cold solution of 1 gm of sodium hydroxide in 1.8 ml of water was added. The temperature was not allowed to rise above 45 °C. This alkaline mixture solution was warmed up to 45 °C and an equal volume of ethyl acetate was added. The ethyl acetate extract was concentrated under reduced pressure (Furniss *et al.*, 1989a, b, c). Recrystallization was done using mixture of ethyl acetate and acetone to produce 1.2 gm (58.00 %) of compound (7a). M.p. = 190–195 °C; IR (KBr, υ cm^{-1}): 3,347.66, 3,048.28, 2,881.45, 1,587.30, 1,504.37, 1,434.94, 1,369.37, 1,137.70, 1,247.07, 1,039.31, 1,099.81, 747.63, 639.61; ^{1}HNMR (400 MHz, δ ppm, $CDCl_3$): 2.12 (s, 1H, hydroxy-H), 3.31 (s, 3H, methoxy-H), 4.63 (d, 1H, 14.00 Hz, –S–C–C=CH–), 4.75 (d, 1H, J = 5.40 Hz, –S–CH–), 5.03 (dd, 1H, J = 10.88 Hz, –S–C–CH–), 6.95 (td, 1H, J = 7.10 Hz, Ar–H), 7.07 (dd, 1H, J = 6.90 Hz, Ar–H), 7.19 (td, 1H, J = 7.44 Hz, Ar–H), 7.39 (dd, 1H, J = 6.76 Hz, Ar–H); Mass: MS (ESI) m/z 230.13 (M^+), m/z 231.12 (M + 1), m/z 232.01 (M + 2). Calcd for $C_{10}H_{11}ClO_2S$: C, 52.06; H, 4.81; O, 13.87. Observed: C, 51.92; H, 4.77; O, 13.85 %.

2-Ethoxy-2H-thiochromene(6b)

2 gm (0.012 mol) of crystal powder of 3-(2-Sulfanylphenyl)prop-2-enal(4) was weighed and placed in 250 ml round bottom flask with addition of 4 ml (0.09 mol) ethanol with few milliliters of concentrated hydrochloric acid. The solution was stirred over night at room temperature. The solution was concentrated by solvent evaporation of propan-2-ol with rotary evaporator at 50 °C. The concentrated solution was heated at 60 °C in alkaline condition for 4 h. Recrystallization was done using mixture of chloroform and ethyl acetate to produce 1.50 gm (64.65 %) of intermediate (6b); M.p. = 270–274 °C; IR (KBr, υ cm^{-1}): 3,074.32, 2,877.37, 1,589.23, 1,499.36, 1,456.16, 1,374.46, 1,250.91, 1,039.7, 1,191.93, 753.62, 667.32; ^{1}HNMR (400 MHz, δ ppm, $CDCl_3$): 1.18 (t, 3H, J = 5.80 Hz, ethoxy-H), 3.88 (q, 2H, J = 5.92 Hz, methoxy-H), 4.72 (d, 1H, J = 7.44 Hz, –S–CH–), 6.13 (dd, 1H, J = 13.8 Hz, –S–C–CH–), 6.39 (d, 1H, J = 11.84 Hz, –S–C–C=CH–), 6.93 (td, 1H, J = 7.36 Hz, Ar–H), 7.16 (dd, 1H, J = 7.64 Hz, Ar–H), 7.35 (td, 1H, J = 7.34 Hz, Ar–H), 7.43 (dd, 1H, J = 7.08 Hz, Ar–H).

3-Chloro-2-ethoxy-3,4-dihydro-2H-1-benzothiopyran-4-ol(7b)

In a 100 ml round bottom flask with a stirrer, 7.31 ml water and 1.70 ml (0.032 mol) Hypochlorous acid were taken. 1.00 gm (0.005 mol) of 2-Ethoxy-2H-thiochromene(6b) was added to the reaction mixture and temperature of the reaction was maintained between 40 and 45 °C for 4 h. The solvent was removed and put in water bath, and then ice-cold solution of 1 gm of sodium hydroxide in 1.8 ml of water was added. This solution was warmed to 45 °C and equal volume of ethyl acetate was added. The ethyl acetate extract was concentrated under reduced pressure. Recrystallization was done using mixture of ethyl acetate and methanol to produce 0.750 gm (55.11 %) of compound (7b); M.p. = 310–315 °C; IR (KBr, υ cm^{-1}): 3,344.46, 3,044.58, 2,885.42, 1,584.34, 1,501.37, 1,445.44, 1,370.30, 1,249.01, 1,042.13, 1,139.11, 1,097.32, 1,072.37, 761.61, 693.61; ^{1}HNMR (400 MHz, δ ppm, DMSO): 1.18 (t, 3H, J = 5.88 Hz, methoxy-H), 2.23 (s, 1H, hydroxy-H), 3.87 (q, 2H, J = 7.00 Hz, ethoxy-H), 4.19 (d, 1H, J = 12.36 Hz, –S–C–C=CH–), 4.99 (dd, 1H, J = 15.04 Hz, –S–C–CH–), 5.32 (d, 1H, J = 6.32 Hz, –S–CH–), 6.82 (td, 1H, J = 6.64 Hz, Ar–H), 7.06 (dd, 1H, J = 7.40 Hz, Ar–H), 7.20 (td, 1H, J = 7.68 Hz, Ar–H), 7.39 (dd, 1H, J = 7.36 Hz, Ar–H); Mass: MS (ESI) m/z 244.32 (M^+), m/z 244.10 (M + 1); Calcd for $C_{11}H_{13}ClO_2S$: C, 53.98; H, 5.35; O, 13.07. Observed: C, 53.58; H, 5.01; O, 12.98 %.

2-(Propan-2-yloxy)-2H-thiochromene(6c)

2 gm (0.012 mol) of crystal powder of 3-(2-Sulfanylpheny)prop-2-enal(4) was weighed and placed in 250 ml round bottom flask with addition of 4 ml (0.06 mol) propan-2-ol with few milliliters of concentrated hydrochloric acid. The solution was stirred over night at room temperature. The solution was concentrated by solvent evaporation of propan-2-ol with rotary evaporator at 50 °C.The concentrated solution was heated at 60 °C in alkaline condition for 4 h. Recrystallization was done using mixture of methanol and ethyl acetate to produce 1.50 gm (60.00 %) of intermediate (6c): M.p. = 290–294 °C; IR (KBr, υ cm^{-1}) : 3,048.53, 2,918.73, 1,577.66, 1,402.15, 1,457.02, 1,370.23, 1,253.64, 1,053.23, 1,122.45, 771.47, 636.47; ^{1}HNMR (400 MHz, δ ppm, DMSO): 1.13 (d, 6H, J = 5.88 Hz, propoxy-H), 3.88 (m, 1H, J = 6.68 Hz, methoxy-H), 5.53 (d, 1H, J = 8.6 Hz, –S–CH–), 5.98 (dd, 1H, J = 11.80 Hz, –S–C–CH–), 6.61 (d, 1H, J = 12.60 Hz, –S–C–C=CH–), 6.99 (td, 1H, J = 6.68 Hz, Ar–H), 7.12 (dd, 1H, J = 7.00 Hz, Ar–H), 7.37 (td, 1H, J = 7.60 Hz, Ar–H), 7.59 (dd, 1H, J = 7.20 Hz, Ar–H).

3-Chloro-2-(propan-2-yloxy)-3,4-dihydro-2H-1-benzothiopyran-4-ol(7c)

In a 100 ml round bottom flask with a stirrer, 7.31 ml water and 1.70 ml (0.032 mol) hypochlorous acid were taken. 1.00 gm (0.004 mol) of 2-(Propan-2-yloxy)-2H-thiochromene(23c) was added to the reaction mixture and temperature of the reaction was maintained between 40 and

45 °C for 4 h. The solvent was removed and put in water bath, and then ice-cold solution of 1 gm of sodium hydroxide in 1.8 ml of water was added. This alkaline mixture solution was warmed up to 45 °C and equal volume of ethyl acetate was added. The ethyl acetate extract was concentrated under reduced pressure. Recrystallization was done using mixture of ethyl acetate and ethanol to produce 0.64 gm (66.40 %) of compound (7c); M.p. = 295–301 °C; IR (KBr, υ cm^{-1}): 3,324.19, 3,032.18, 2,898.30, 1,602.74, 1,400.22, 1,485.09, 1,374.47, 1,228.10, 1,041.49, 1,204.83, 1,139.85, 1,085.85, 740.61, 669.25; ^{1}HNMR (400 MHz, δ ppm, DMSO): 1.12 (d, 6H, $J = 6.60$ Hz, propoxy-H), 2.40 (s,1H, hydroxy-H), 3.66 (m, 1H, $J = 7.00$ Hz, methoxy-H), 4.97 (dd, 1H, $J = 13.00$ Hz, –S–C–CH–), 5.17 (d, 1H, $J = 8.52$ Hz, –S–C–C=CH–), 5.65 (d, 1H, $J = 11.80$ Hz, –S–CH–), 7.08 (td, 1H, $J = 6.80$ Hz, Ar–H), 7.12 (dd, 1H, $J = 6.76$ Hz, Ar–H), 7.28 (td, 1H, $J = 6.36$ Hz, Ar–H), 7.55 (dd, 1H, $J = 5.80$ Hz, Ar–H); Mass: MS (ESI) m/z 258.17 (M^+), m/z 259.21 (M + 1), m/z 260.16 (M + 2); Calcd for $C_{12}H_{15}ClO_2S$: C, 55.70; H,5.84; O, 12.37. Observed: C, 55.35; H, 5.28; O, 11.96 %.

2-Butoxy-2H-thiochromene(6d)

2 gm (0.012 mol) of crystal powder of 3-(2-Sulfanylpheny)prop-2-enal(4) was weighed and placed in 250 ml round bottom flask with addition of 4 ml (0.053 mol) butan-1-ol with few milliliters of concentrated hydrochloric acid. The solution was stirred over night at room temperature. The solution was concentrated by solvent evaporation of butan-1-ol with rotary evaporator at 50 °C.The concentrated solution was heated at 60 °C in alkaline condition for 6 h. Recrystallization was done using mixture of dimethyl sulfoxide to produce 1.65 gm (61.56 %) of intermediate (6d); M.p. = 248–255 °C; IR (KBr, υ cm^{-1}): 3,064.32, 2,892.46, 1,589.23, 1,483.36, 1,463.16, 1,374.44, 1,255.93,1,044.17, 1,191.13, 732.31, 667.32; ^{1}HNMR (400 MHz, δ ppm, CDCl3): 0.93 (t, 3H, $J = 6.00$ Hz, buthoxy-H), 1.22 (m, 2H, $J = 6.72$ Hz, propoxy-H), 1.82 (q, 2H, $J = 7.44$ Hz, ethoxy-H), 3.35 (t, 2H, $J = 7.00$ Hz, methoxy-H), 5.23 (d, 1H, $J = 7.92$ Hz, –S–CH–), 5.95 (dd, 1H, $J = 12.00$ Hz, –S–C–CH–), 6.59 (d, 1H, $J = 11.76$ Hz, –S–C–C=CH–), 6.96 (td, 1H, $J = 7.80$ Hz, Ar–H), 7.15 (dd, 1H, $J = 5.84$ Hz, Ar–H), 7.34 (td, 1H, $J = 6.40$ Hz, Ar–H), 7.41 (dd, 1H, $J = 7.80$ Hz, Ar–H).

2-Butoxy-3-chloro-3,4-dihydro-2H-1-benzothiopyran-4-ol(7d)

In a 100 ml round bottom flask with a stirrer, 7.31 ml water and 4.70 ml (0.089 mol) hypochlorous acid were taken. 1.00 gm (0.0045 mol) of 2-Butoxy-2H-thiochromene(6d) was added to the reaction mixture and temperature of the reaction was maintained between 40 and 45 °C for 5 h. The solvent was removed and put in water bath, and then ice-cold solution of 1 gm of sodium hydroxide in 1.8 ml of water was added. The temperature was not allowed to rise above 45 °C. This alkaline mixture solution was warmed up to 45 °C and equal volume of ethyl acetate was added. The ethyl acetate extract was concentrated under reduced pressure. Recrystallization was done using mixture of methanol and acetone to produce 0.75 gm (60.97 %) of compound (7d); M.p. = 298–305 °C; IR (KBr, υ cm^{-1}): 3,323.12, 3,033.64, 2,934.39, 1,575.73, 1,502.44, 1,461.94, 1,378.10, 1,251.36, 1,042.02, 1,180.35, 1,114.78,1,080.06, 746.40, 688.54; ^{1}HNMR (400 MHz, δ ppm, DMSO): δ 0.93 (t, 3H, $J = 6.24$ Hz, buthoxy-H), 1.40 (m, 2H, $J = 6.00$ Hz, propoxy-H), 1.56 (q, 2H, $J = 7.64$ Hz, ethoxy-H), 2.09 (s, 1H, hydroxy-H), 3.36 (t, 2H, $J = 7.60$ Hz, methoxy-H), 4.98 (d, 1H, $J = 11.68$ Hz, –S–C–C=CH–), 5.04 (dd, 1H, $J = 11.92$, 8.12 Hz, –S–C–CH–), 5.17 (d, 1H, $J = 11.68$ Hz, –S–CH–), 7.06 (td, 1H, $J = 6.72$ Hz, Ar–H), 7.20 (dd, 1H, $J = 7.68$ Hz, Ar–H), 7.31 (td, 1H, $J = 7.60$ Hz, Ar–H), 7.42 (dd, 1H, $J = 6.24$ Hz, Ar–H); Mass: MS (ESI) m/z 272.21 (M^+), m/z 273.03 (M + 1). Calcd for $C_{13}H_{17}ClO_2S$: C, 57.24; H, 6.28; O, 11.73. Observed: C, 56.96; H, 6.12; O, 11.70 %.

2-(2-Methylbutoxy)-2H-thiochromene(6e)

2 gm (0.012 mol) of crystal powder of 3-(2-Sulfanylpheny)prop-2-enal(4) was weighed and placed in 250 ml round bottom flask with addition of 5 ml (0.056 mol) 2-methylbutan-1-ol with few milliliters of concentrated hydrochloric acid. The solution was stirred over night at room temperature. The solution was concentrated by solvent evaporation of 2-methylbutan-1-ol with rotary evaporator at 50 °C.The concentrated solution was heated at 60 °C in alkaline condition for 5 h. Recrystallization was done using mixture of methanol to produce 1.68 gm (58.94 %) of intermediate (6e); M.p. = 260–265 °C; IR (KBr, υ cm^{-1}): 3,046.22, 2,882.66, 1,589.18, 1,415.77, 1,470.01, 1,358.09, 1,255.76, 1,041.49, 1,139.85, 744.47, 696.61; ^{1}HNMR (400 MHz, δ ppm, DMSO): 0.88 (t, 3H, $J = 7.44$ Hz, buthoxy-H), 1.37 (q, 2H, $J = 6.72$ Hz, propoxy-H), 1.49 (d, 3H, $J = 7.84$ Hz, isopropoxy-H), 2.17 (m, 1H, $J = 7.60$ Hz, ethoxy-H), 3.37 (d, 2H, $J = 6.16$ Hz, methoxy-H), 5.63 (d, 1H, $J = 8.16$ Hz, –S–CH–), 5.95 (dd, 1H, $J = 12.00$ Hz, –S–C–CH–), 6.59 (d, 1H, $J = 11.76$ Hz, –S–C–C=CH–), 7.01 (td, 1H, $J = 5.60$ Hz, Ar–H), 7.15 (dd, 1H, $J = 7.80$ Hz, Ar–H), 7.36 (td, 1H, $J = 6.36$ Hz, Ar–H), 7.42 (dd, 1H, $J = 5.80$ Hz, Ar–H).

3-Chloro-2-(2-methylbutoxy)-3,4-dihydro-2H-1-benzothiopyran-4-ol(7e)

In a 100 ml round bottom flask with a stirrer, 7.31 ml water and 4.70 ml (0.089 mol) hypochlorous acid were taken. 1.00 gm (0.0042 mol) of 2-(2-methylbutoxy)-2H-thiochromene(6e) was added to the reaction mixture and temperature of the reaction was maintained between 40 and 45 °C for 8 h. The solvent was removed and put in water bath, and then ice-cold solution of 1 gm of sodium hydroxide in 1.8 ml of water was added. This alkaline mixture solution was warmed up to 45 °C and equal volume of ethyl acetate was added. The ethyl acetate extract was concentrated under reduced pressure. Recrystallization was done using mixture of methanol and Ethanol to produce 0.85 gm (69.67 %) of compound (7e); M.p. = 280–285 °C; IR (KBr, υ cm^{-1}) :3,309.62, 3,042.18, 2,893.02, 1,585.73, 1,437.13, 1,467.83, 1,375.79, 1,253.13, 1,043.42, 1,206.23, 1,147.43, 1,078.32, 756.04, 628.75; ^{1}HNMR (400 MHz, δ ppm, DMSO): 0.86 (t, 3H, $J = 6.72$ Hz, buthoxy-H), 1.29 (d, 3H, $J = 6.00$ Hz, isopropoxy-H), 1.36 (*p*, 2H, $J = 6.60$ Hz, propoxy-H), 1.85 (m, 1H, $J = 7.48$ Hz, ethoxy-H), 2.53 (s, 1H, Hydroxy-H), 3.44 (d, 2H, $J = 7.00$ Hz, methoxy-H), 4.76 (d, 1H, $J = 11.92$ Hz, –S–C–C=CH–), 4.93 (d, 1H, $J = 8.24$ Hz, –S–CH–), 5.27 (dd, 1H, $J = 11.80$ Hz, –S–C–CH–), 7.06 (td, 1H, $J = 6.00$ Hz, Ar–H), 7.23 (dd, 1H, $J = 5.60$ Hz, Ar–H), 7.30 (td, 1H, $J = 6.16$ Hz, Ar–H), 7.45 (dd, 1H, $J = 6.72$ Hz, Ar–H); Mass: MS (ESI) m/z 286.10 (M^+), m/z 287.18 (M + 1); Calcd for $C_{14}H_{19}ClO_2S$: C, 58.63; H, 6.68; O, 11.16. Observed: C, 58.62; H, 6.60; O, 10.98 %.

2-Phenoxy-2H-thiochromene(6f)

2 gm (0.012 mol) of crystal powder of 3-(2-Sulfanylpheny)prop-2-enal(4) was weighed and placed in 250 ml round bottom flask with addition of 5 ml (0.053 mol) phenol with few milliliters of concentrated hydrochloric acid. The solution was stirred over night at room temperature. The solution was concentrated by solvent evaporation with rotary evaporator at 50 °C. The concentrated solution was heated at 60 °C in alkaline condition for 5 h. Recrystallization was done using dimethyl sulfoxide to produce 1.85 gm (63.35 %) of intermediate (6f); M.p. = 260–267 °C; IR (KBr, υ cm^{-1}) : 3,045.21, 1,587.16, 1,481.23, 1,248.88, 1,053.06, 1,128.78, 767.97, 669.25; ^{1}HNMR (400 MHz, δ ppm, DMSO): 5.95 (dd, 1H, $J = 11.92$ Hz, –S–C–CH–), 6.27 (d, 1H, $J = 11.64$ Hz, –S–C–C=CH–), 6.53 (d, 1H, $J = 8.24$ Hz, –S–CH–), 6.80 (tt, 1H, $J = 6.52$ Hz, Ar–H), 6.94 (dt, 2H, $J = 6.30$ Hz, Ar–H), 7.02 (td, 1H, $J = 5.60$, 1.80 Hz, Ar–H), 7.14 (td, 2H, $J = 6.48$ Hz, Ar–H), 7.18 (dd, 1H, $J = 6.60$ Hz, Ar–H), 7.34 (td, 1H, $J = 6.32$ Hz, Ar–H), 7.42 (dd, 1H, $J = 5.88$ Hz, Ar–H).

3-Chloro-2-phenoxy-3,4-dihydro-2H-1-benzothiopyran-4-ol(7f)

In a 100 ml round bottom flask with a stirrer, 7.31 ml water and 4.70 ml (0.089 mol) hypochlorous acid were taken. 1.00 gm (0.0041 mol) of 2-Phenoxy-2H-thiochromene(6f) was added to the reaction mixture and temperature of the reaction was maintained between 40 and 45 °C for 4 h. The solvent was removed and put in water bath, and then ice-cold solution of 1 gm of sodium hydroxide in 1.8 ml of water was added. The temperature was not allowed to rise above 45 °C. This alkaline mixture solution was warmed up to 45 °C and equal volume of ethyl acetate was added. The ethyl acetate extract was concentrated under reduced pressure. Recrystallization was done using mixture of acetone and ethanol (0.75 gm, 61 %) of compound (7f); M.p. = 250–256 °C; IR (KBr, υ cm^{-1}) : 3,332.21, 3,044.18, 1,599.59, 1,451.61, 1,244.43, 1,042.13, 1,199.35, 1,126.71, 1,070.76, 746.40, 654.88; ^{1}HNMR (400 MHz, δ ppm, DMSO): 2.55 (s, 1H, hydroxy-H), 4.98 (d, 1H, $J = 11.72$ Hz, –S–C–C=CH–), 5.29 (dd, 1H, $J = 11.92$ Hz, –S–C–CH–), 5.72 (d, 1H, $J = 8.32$ Hz, –S–CH–), 6.92 (tt, 1H, $J = 6.46$ Hz, Ar–H), 7.01 (td, 2H, $J = 5.60$ Hz, Ar–H), 7.06 (td, 1H, $J = 6.32$ Hz, Ar–H), 7.18 (dd, 1H, $J = 6.72$ Hz, Ar–H), 7.25 (dt, 2H, $J = 7.04$ Hz, Ar–H), 7.29 (td, 1H, $J = 6.08$ Hz, Ar–H), 7.38 (dd, 1H, $J = 5.80$ Hz, Ar–H); Mass: MS (ESI) m/z 292.32 (M^+), m/z 293.23 (M + 1), m/z 294.08 (M + 2). Calcd for $C_{15}H_{13}ClO_2S$: C, 61.53; H, 4.48; O, 10.95. Observed: C, 61.50; H, 4.43; O, 10.90 %.

Pharmacological evaluation

All the synthesized compounds were evaluated for their calcium channel blocking activity using concentration–response curve. All the experimental work was carried out at Department of Pharmacology, B. R. Nahata College of Pharmacy, Mandsaur (M.P.).

Drugs and chemicals

Nifedipine tablet (Unique Pvt. Ltd. India) and all synthesized compounds were diluted in water and ethanol. Volume of injection was 60–100 Mm.

Animals

Guinea pig of 3–4 months old and both sex weighing around 400–500 gm were used in this study. Animals were procured from disease-free animal house, Calcutta Fish

Aquarium, Indore. The animals had free access to food and water and were maintained under 12:12 h light and dark cycles. All experiments were carried out during day time from 0800 to 1300 hours, the IAEC approved the experimental protocol, and care of animals was taken as per guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Department of Animal Welfare, Govt. of India.

Isolated tissue preparations

Guinea pigs had been sacrificed by cervical dislocation, the ileum was immediately removed. Segments of 1.5–2.0 cm length were mounted vertically in a 10 ml organ bath containing Tyrode's solution, aerated with a mixture of 95 % oxygen and 5 % carbon dioxide, and maintained at 37 °C. The composition of the Tyrode's solution was: KCl 27 (0.2 gm), NaCl 137 (8.0 gm), $MgCl_2$ 1.8 (0.60 gm), $NaHCO_3$ 11.9 (1.0 gm), NaH_2PO_4 0.4 (0.05 gm), and glucose 5.55 (1.0 gm) mM. Each tissue was allowed to equilibrate for at least 30 min before the addition of any drug. The contractile effect of the test material was assessed as the percent of the maximum effect produced by the control drug, nifedipine (10 mM).

Determination of calcium antagonist activity

The calcium antagonist activity of the compound was studied using isolated guinea-pig ileum. The calcium antagonist activity of the test substances was through calcium channel blockade (CCB), K^+, as KCl was used to depolarize the preparations. High K^+ (80 mM) was added to the tissue bath, which produced a sustained contraction. Test materials were then added in a cumulative fashion to obtain concentration-dependent inhibitory responses. The relaxation of intestinal preparations, precontracted with K^+ (90 mM) was expressed as percent of the control response mediated by K^+. In order to confirm the calcium antagonist activity of test substances, the tissue was allowed to stabilize in normal Tyrode's solution, which was then replaced with Ca^{2+}-free Tyrode's solution for 30 min to remove Ca^{2+} from the tissues. This solution was further replaced with a K^+-rich and Ca^{2+}-free Tyrode's solution. Following an incubation period of 30 min, control concentration–response curves (CRCs) of Ca^{2+} were obtained. When the control CRCs of Ca^{2+} were found to be super-imposable (usually after two cycles), the tissue was pretreated with the test compound for 60 min to test a possible CCB effect. The CRCs of Ca^{2+} were developed in the presence of different concentrations of the test compound.

Conclusion

The current research reports the synthesis, characterization, and pharmacological evaluation of 6 benzothiopyran derivatives (7a–7f) as a novel series of calcium channel blockers. In the research, substituted 3-chloro-3,4-dihydro-2H-1-benzothiopyran-4-olderivatives (7a–7f) were synthesized, with elaborate characterization by spectral data. Synthesized compounds were obtained in satisfactory yield and were characterized by TLC, FT-IR, ^{1}HNMR, Mass and Elemental analysis. In pharmacological evaluation, synthesized compounds 7b, 7c, 7d, and 7e exhibited in vitro calcium channel antagonist activity. On the basis of the above study, it is suggested that substituted 3-chloro-3,4-dihydro-2H-1-benzothiopyran-4-ol derivatives(7b,7c,7d, and 7e) include significant calcium channel antagonistic action agents, KCl induce contraction. Further study is needed to confirm exact mechanism for calcium channel antagonistic activity.

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