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Synthesis of 4-aryl-3,4-dihydropyrimidin-2(1*H*)-thione derivatives as potential calcium channel blockers

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Abstract—Biginelli reaction which involves condensation of methyl 3-oxopentanoate, aromatic aldehydes and thiourea with a catalytic amount of HCl at reflux temperature has been used for the synthesis of 4-aryl-6-ethyl-5-(methoxycarbonyl)-3,4-dihydropyrimidin-2(1*H*)-thiones (**1**–**16**). In addition, Lewis acids such as $FeCl_3{\cdot}6\ H_2O$ and/or H_3BO_3 were also used as catalysts for one-pot synthesis of the products. Compared to the classical Biginelli reaction conditions, the usage of Lewis acids has the advantage of good yields and short reaction times. The calcium channel blocker activities of **1**–**16** were screened by the tests performed on isolated rat ileum and thoracic aorta. Although product **11**, 2-nitrophenyl-derivative, has potent antispasmodic activity on $BaCl_2$-stimulated rat ileum, it does not have vasodilator activity on KCl-stimulated rat thoracic aorta. Product **15**, 2-ethoxyphenyl-derivative, exhibited significant antispasmodic and vasodilator activities in both screening paradigms.

1. Introduction

In recent years acid-catalyzed cyclocondensation of β-oxoesters with aromatic aldehydes and (thio)ureas, known as the Biginelli reaction, has attracted significant attention.[1,2] The resulting dihydropyrimidinones and their sulfur analogs (DHPMs) have been reported to have antibacterial, antiviral, antitumor, antiinflammatory,[3] and antihypertensive as well as calcium channel blocker,[4–6] α-1a-antagonist,[7–9] and neuropeptide Y (NPY) antagonist[10] activities. Recently, structurally simple DHPM derivative monastrol has emerged as a mitotic kinesin Eg5 motor protein inhibitor for the development of anticancer drugs.[11,12] Furthermore, the biological activity of several recently isolated marine alkaloids has also been attributed to the dihydropyrimidinone moiety in the structure.[13] Among them the batzelladine alkaloids A and B which inhibit the binding of HIV envelope protein gp-120 to human CD4 cells are potential compounds in AIDS therapy.[14]

The original procedure reported by Biginelli for the synthesis of DHPMs involves one-pot condensation of (thio)ureas, aldehydes, and β-oxoesters or 1,3-dicarbonyl compounds under strongly acidic conditions.[15] The main disadvantage of this procedure is low reaction yields because of the side reactions particularly in the case of some substituted aromatic and aliphatic aldehydes.[2] This has led to the recent disclosure of several improved reaction protocols for the synthesis of DHPMs either by modification of the classical one-pot, three-component condensation itself[16–20] or by the development of alternative multi-step strategies.[21–24] Several combinatorial protocols based on the classical Biginelli condensation have been advanced by using solid phase or fluorous phase reaction conditions.[25–30] Recently it has been reported that the Biginelli reaction could be performed using different Lewis acids as well as protic acids as promoters.[31] This method makes it possible to prepare a large number of DHPMs bearing various substituents in the pyrimidine ring.

In this study, we focused on the use of Lewis acids such as ferric chloride hexahydrate and boric acid as activators of the Biginelli reaction for the preparation of DHPM-2-thiones which are expected to have calcium channel blocker activity. Reaction times and yields were compared to the classical Biginelli reaction conditions

Keywords: Biginelli reaction; Dihydropyrimidin-2-thiones (DHPM-2-thiones); Ferric chloride; Boric acid; Calcium channel blocker activity.
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and preliminary calcium channel blocker activity results of the synthesized compounds were reported.

2. Results and discussion

In the present work, Biginelli-type cyclocondensation reaction was studied for the preparation of 4-aryl-3,4-dihydropyrimidin-2(1*H*)-thiones (**1**–**16**). Although the synthesis of **2**–**8** and **10** has been reported in our previous studies,[32,33] the synthesis and calcium channel blocker activity of these compounds are included for the examination of the reaction conditions and biological activity.

For comparison purposes, DHPM-2-thiones were prepared using three different procedures (Scheme 1). Based on comparison of melting points and spectral data we assume that the identical materials have been obtained. Our initial attempts focused on the classical Biginelli reaction which involves condensation of methyl 3-oxopentanoate with aromatic aldehydes and thiourea under strongly acidic conditions.[15] The reaction was carried out simply by heating a mixture of the three components dissolved in ethanol with a catalytic amount of HCl at reflux temperature for long hours. The Biginelli condensation is strongly dependent on the amount of acidic catalyst present in the reaction medium. The main problem of the classical synthesis was not only the low/moderate reaction yields (8–36%) of the desired DHPM-2-thiones **1**–**16** but also long reaction times (35–245 h).

A recent trend is to perform the condensation with a Lewis acid such as $BF_3{\cdot}OEt_2$ and CuCl,[34] $LaCl_3$,[35] $FeCl_3$,[36,37] $NiCl_2$,[37] $Yb(OTf)_3$,[38] $La(OTf)_3$,[38] $InCl_3$,[39] $InBr_3$,[40] $BiCl_3$,[41] $LiClO_4$,[31] $Mn(OAc)_3$,[42] H_3BO_3,[43] $SmCl_3$[44] or $ZrCl_4$[45] instead of protic acids to produce higher yields of the DHPM-2-thione heterocycle and to shorten reaction times. In continuation of our work Lewis acid-catalyzed processes (such as H_3BO_3 and/or $FeCl_3{\cdot}6H_2O$) have been used for the one-pot synthesis of 3,4-DHPM-2-thiones. A summary of the results obtained is provided in Table 1.

For all cases, the Lewis acid-catalyzed methods (Methods B and C) produced significantly shorter reaction times (3–9 h) than the classical Biginelli reaction (35–245 h) (Method A). For example, the reaction times in the synthesis of 4-methyl-derivative **9** using HCl, H_3BO_3 and $FeCl_3{\cdot}6H_2O$ as a catalyst were 245, 7, and 5 h, respectively.

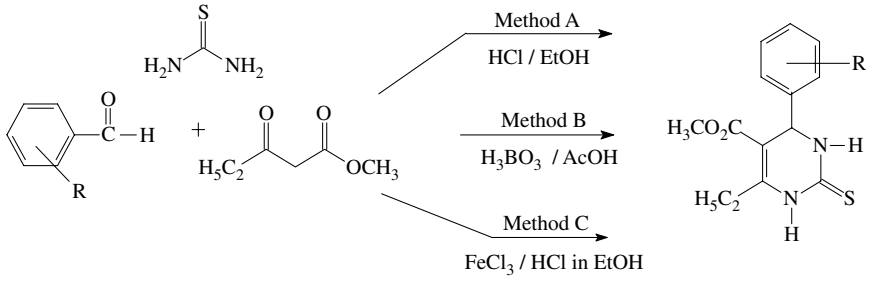
The reaction yields of some of the products obtained with Lewis acids were significantly better in comparison with the classical Biginelli procedure. For example, 3-nitro- **12** and 2-hydroxy-5-bromo- derivatives **16** were obtained under classical Biginelli conditions in 16% and 26% yields. The yields of these DHPM derivatives were extremely high in the boric acid-catalyzed Biginelli reaction, 62% and 93%, respectively. Similarly, 3-bromo- **5**, 2-methyl- **7**, 3-fluoro- **14**, and 2-hydroxy-5-bromo- derivatives **16** were obtained in 21%, 16%, 9%, and 26% yields using HCl and in 69%, 78%, 77%, and 58% yields using ferric chloride as catalyst, respectively. On the other hand, the yields of 4-chloro- **4**, 4-methyl- **9**, 2-methoxy- **10**, and 2-ethoxy- **15** derivatives obtained by boric acid-catalyzed method, and 4-methyl- **9** and 3-nitro- derivatives **12** obtained by ferric chloride-catalyzed method were lower than the classical Biginelli method.

Among the products, 3-chloro- **3**, 3-bromo- **5**, 3-methyl- **8**, and 3-fluoro- **14** derivatives using boric acid, and 4-chloro- **4**, 4-bromo- **6**, 2-nitro- **11**, 2-fluoro- **13**, and 2-ethoxy- **15** derivatives using ferric chloride could not be isolated.

According to the mechanistic studies reported in the literature,[2,46] in the first step of Biginelli reaction *N*-acyliminium ion is formed as the key intermediate by the acid-catalyzed condensation of benzaldehyde and thiourea. Interception of this iminium ion by methyl 3-oxopentanoate, possibly through its enol tautomer, results in the formation of open-chain ureide, which then cyclizes to the DHPM derivative. The success of the Lewis acid-catalyzed method may be the result of specifically stabilization of the *N*-acyliminium ion intermediates.

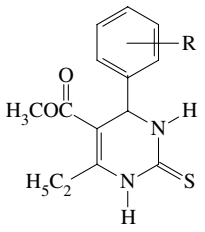
Spectral examination and microanalysis data of the products **1**–**16** confirmed the formation of dihydropyrimidine ring. The IR spectra of the products are characterized by the presence of absorbtion bands at 3424–3270, 1701–1662, and 1353–1311 cm^{-1} corresponding to the N–H, 5-C=O, and 2-C=S functions in the structure.

In the ^1H NMR spectra of the products **1**–**6** and **8**–**15**, resonances of C-4 methine proton of the ring were seen as a doublet due to N3–H proton at about δ



Scheme 1. Synthesis of DHPM-2-thione derivatives (**1–16**) by cyclocondensation of aromatic aldehyde, methyl 3-oxopentanoate and thiourea.

Table 1. Synthesis of 4-aryl-3,4-dihydropyrimidin-2(1*H*)-thione (DHPM-2-thione) derivatives using catalytic conditions versus classical Biginelli conditions



Product	R	Method A[a]		Method B[b]		Method C[c]	
		Refluxing time (h)	Yield (%)	Refluxing time (h)	Yield (%)	Refluxing time (h)	Yield (%)
1	H	63	30.40	6	62.37	5	37.69
2	2-Cl	105	11.69	8.5	29.82	5.5	26.13
3	3-Cl	175	35.90	—[d]	—	5	42.26
4	4-Cl	112	25.87	9	12.44	—[d]	—
5	3-Br	154	21.39	—[d]	—	5	69.43
6	4-Br	105	12.37	8	21.02	—[d]	—
7	2-CH_3	140	15.91	6.5	15.39	6	77.83
8	3-CH_3	133	23.57	—[d]	—	6	45.95
9	4-CH_3	245	35.38	7	14.35	5	13.72
10	2-OCH_3	35	17.44	7.5	12.41	3	52.41
11	2-NO_2	196	11.17	7	23.59	—[d]	—
12	3-NO_2	70	16.06	7.5	62.19	5.5	8.32
13	2-F	70	8.27	8.5	16.97	—[d]	—
14	3-F	126	9.56	—[d]	—	5	77.30
15	2-OC_2H_5	56	28.96	8.5	20.83	—[d]	—
16	2-OH,5-Br	35	26.43	7.5	93.01	5	57.86

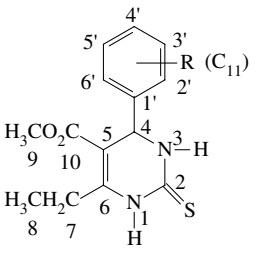
[a] Classical Biginelli conditions (cat. HCl in EtOH).
[b] Cat. H_3BO_3 in glacial acetic acid.
[c] Cat. $FeCl_3{\cdot}6H_2O$ in EtOH.
[d] Reaction product could not be isolated.

5.09–5.20 ppm. The signals of C-4 methine proton of **7** and **16** were apparent as two broad singlets at approximately δ 4.70 and 5.39 ppm, respectively. All products have a one-proton singlet at about δ 10.20–10.42 ppm assignable to the N1-H proton on the DHPM structure. The signals of N3-H protons appeared as a singlet at about δ 9.52–9.73 ppm except product **14**. In product **14** this proton was seen as a doublet at δ 9.71 ppm due to the C-4 methine proton of the DHPM ring.

The ^{13}C NMR data of the products **1**, **9**, and **12** confirmed the proposed structure. Characteristic signals were observed at about δ 165.71–165.95 (C2) and δ 175.17–175.53 (C10), respectively (Table 2).

EI-Mass spectra of **1**–**16** showed the molecular ion [M^+] peaks of different intensity which confirmed their molecular weights. In most of the products, the base peak frequently resulted from loss of $C_6H_4R^{\cdot}$ radical from the molecular ion and appeared at *m/e* 199. In product **10** (2-methoxy derivative) the molecular peak was also the base peak (*m/e* 306). In product **7** (2-methyl derivative) loss of $^{\cdot}COOCH_3$ radical from the structure accounted for the base peak at *m/e* 231. The base peak of product

Table 2. ^{13}C NMR spectral data (DMSO-d_6, δ ppm) of the products **1**, **9**, and **12**



Product (R)	C–2	C–4	C–5	C–6	C–7	C–8	C–9	C–10	C–11
1 (H)	165.95	51.85	100.30	126.99	24.14	13.74	54.49	175.27	
9 (4-CH_3)	165.94	51.79	100.39	126.89	21.35	13.71	54.18	175.17	24.09
12 (3-NO_2)	165.71	52.08	99.41	121.71	24.21	13.75	53.87	175.53	

Table 3. The relaxant effects of nicardipine and products **1**–**16** on barium chloride (4×10^{-3} mol/l) contractions in isolated rat ileum and potassium chloride (67 mmol/l) induced contraction in rat thoracic aorta ($X \pm$ SD) (n, the number of heads used)

Product	R	% inhibition of $BaCl_2$ contractions in rat ileum		% inhibition of KCl contractions in rat thoracic aorta	
		10^{-5} M	n	10^{-5} M	n
1	H	67.38 ± 14.34	8	4.90 ± 1.83	8
2	2-Cl	89.25 ± 3.96	8	0	8
3	3-Cl	88.88 ± 7.45	8	7.42 ± 3.71	6
4	4-Cl	59.00 ± 13.19	8	4.88 ± 1.97	8
5	3-Br	39.63 ± 14.92	8	—	—
6	4-Br	37.00 ± 6.93	8	—	—
7	2-CH_3	87.75 ± 7.19	8	2.50 ± 1.02	7
8	3-CH_3	63.25 ± 6.54	8	0	8
9	4-CH_3	68.00 ± 13.80	9	6.50 ± 2.14	8
10	2-OCH_3	66.25 ± 11.88	8	8.50 ± 4.21	8
11	2-NO_2	95.50 ± 2.07	8	0	8
12	3-NO_2	75.00 ± 10.58	8	6.77 ± 0.77	6
13	2-F	66.25 ± 3.66	8	6.69 ± 2.83	8
14	3-F	65.38 ± 15.84	8	10.38 ± 2.56	8
15	2-OC_2H_5	87.50 ± 5.10	8	19.33 ± 6.59	6
16	2-OH,5-Br	34.00 ± 10.58	8	—	—
	Nicardipine	100.00	8	20.50 ± 2.89	6

$p < 0.05$.

12 (2-nitro derivative) was probably formed by loss of methoxy, ethyl, and nitro radicals [M−(OCH_3 + C_2H_5 + NO_2)]. In all products prominent peaks resulted from elimination of $^{\cdot}CH_3$, $^{\cdot}OCH_3$, and $^{\cdot}COOCH_3$ radicals.

Regarding the fact that the biological activity could be influenced by variety of substitution, series of **16** products has been investigated for their calcium channel blocker activities by the tests performed on $BaCl_2$-stimulated rat ileum and KCl-stimulated rat thoracic aorta. Nicardipine was included in all tests as the reference drug[47–52] (Table 3). Smooth muscles depend on calcium influx for contraction. Although their underlying mechanism is somewhat different, inhibition of calcium channel influx into smooth muscles by calcium channel blockers leads to relaxation.

On the $BaCl_2$-stimulated rat ileum products **2** (2-Cl), **3** (3-Cl), **7** (2-CH_3), **11** (2-NO_2), and **15** (2-OC_2H_5) were found to be effective. Among these products, **3** was the only *m*-substituted derivative. Product **11** having 2-NO_2 substituent on C-4 phenyl ring (relaxation effect %: 95.50 ± 2.07) was the most effective derivative compared with the standard nicardipine (relaxation effect %: 100). Products **2** (relaxation effect %: 89.25 ± 3.96), **3** (relaxation effect %: 88.88 ± 7.45), **7** (relaxation effect %: 87.75 ± 7.19), and **15** (relaxation effect %: 87.50 ± 5.10), carrying 2-Cl, 3-Cl, 2-CH_3, and 2-OC_2H_5 substituents on C-4 phenyl ring, exerted somewhat lower activity than nicardipine. Among the halogen-substituted derivatives (**2**–**6**, **13**, and **14**) products **2** and **3** were the most effective derivatives which have 2- or 3-chloro substituents on the C-4 phenyl ring.

Products **1**–**4** and **7**–**15** which have higher relaxant effects than 50% on the rat ileum were also evaluated on rat thoracic aorta. Among these products **2**, **8**, and **11** did not display any inhibition of KCl contractions in rat thoracic aorta. Product **15** (2-OC_2H_5) exhibited significant activity (relaxant effect %: 19.33 ± 6.59), comparable to that of nicardipine (relaxant effect %: 20.50 ± 2.89).

3. Conclusion

In conclusion ferric chloride and boric acid efficiently catalyze the three-component Biginelli reaction between substituted benzaldehyde, methyl 3-oxopentanoate, and thiourea to afford the corresponding DHPM-2-thiones. The main advantages of these catalysts for the synthesis of Biginelli-type DHPM-2-thiones are mild reaction conditions, simple experimental workup procedure, high yields, and short reaction times.

Considering the activity results of products **1**–**16** on the $BaCl_2$-stimulated rat ileum, we may conclude that *o*-substitution on C-4 phenyl group appears to be important for the calcium channel blocker activity as it is reported for 1,4-dihydropyridine (DHP) derivatives which have structural similarities with 1,4-DHPMs.[53–55] But no relationship was found between the activity and the nature of the alkyl moiety such as electron-drawing or electron-releasing effects. While product **11**, 2-NO_2 derivative, has potent antispasmodic activity on isolated rat ileum, it does not have vasodilator activity on isolated rat thoracic aorta. Product **15**, 2-OC_2H_5 derivative, showed antispasmodic activities on isolated rat ileum and its vasodilatory activity on rat thoracic aorta was as high as that of nicardipine. We had to use a high concentration (10^{-5} M) for compounds and nicardipine to obtain a sufficient response on rat thoracic aorta, so we used the same concentrations of compounds and nicardipine on both rat ileum and thoracic aorta. Introduction of 2-ethoxy substituent (**15**) instead of

2-methoxy substituent (**10**) on C-4 phenyl ring increased both antispasmodic and vasodilator activities.

Although products **1**–**16** have an asymmetric center on the fourth position, their resolutions have not been done. Our next study will be concerned on the separation of enantiomers and comparison of the calcium channel blocker activities of each enantiomer.

4. Materials and methods

Melting points were determined using a Thomas Hoover capillary melting point apparatus (Philadelphia, PA, USA) and are uncorrected. UV absorptions were measured on a Agilent 8453 UV–visible spectrophotometer. IR spectra were recorded on a Bruker Vector 22 IR spectrophotometer (Opus Spectroscopic Software Version 2,0). ^{1}H NMR spectra were run on a Bruker AC 80 MHz FT NMR or Bruker Avance 400 MHz NMR (XWIN-NMR Software) instruments in DMSO-d_6 solutions using TMS as the internal standard. ^{13}C NMR spectra were measured on a Bruker Avance 400 MHz NMR (XWIN-NMR Software) using the same solvent and internal standard. Mass (70 eV) spectra were taken using a 73DIP–1 Direct Insertion Probe and Agilent 5973-Network Mass Selective Detector spectrometer. Microanalysis data were performed on a Leco CHNS 932 analyzer. All chemicals used were of reagent grade. Methyl 3-oxopentanoate was supplied from Ulkar Chemistry (Çerkezköy, Tekirdağ-Turkey). TLC was performed on microplates coated with kieselgel 60 F_{254} (Merck).

4.1. General procedures for the synthesis of DHPM-2-thiones

4.1.1. Method A (classical Biginelli conditions). A mixture of thiourea (0.025 mol), benzaldehyde or substituted benzaldehyde (0.025 mol) and methyl 3-oxopentanoate (0.0375 mol) in abs ethanol (20 ml) was treated with eight drops of 37% HCl as catalyst and then refluxed for an appropriate period. The crude product which precipitated on cooling was filtered and washed with 50% ethanol. Then it was recrystallized from appropriate solvent.

4.1.2. Method B (catalyst: H_3BO_3).[43] A solution of aromatic aldehyde (3 mmol), methyl 3-oxopentanoate (3 mmol), thiourea (3.6 mmol), and H_3BO_3 (0.6 mmol), in glacial acetic acid (10 ml) is heated at 100 °C, while stirring for 6–9 h. Then it is cooled to room temperature and poured into 50 ml ice-water. The solid products are filtered, washed with ice-water and ethanol (95%), dried, and recrystallized from appropriate solvent.

4.1.3. Method C (catalyst: $FeCl_3{\cdot}6H_2O$).[20] A solution of thiourea (75 mmol), methyl 3-oxopentanoate (50 mmol), the appropriate aldehyde (50 mmol), $FeCl_3{\cdot}6H_2O$ (30 mmol), and concd. HCl (2–3 drops) in ethanol (40 ml) was heated under reflux for 5–6 h. The solution was cooled to room temperature and poured onto 200 g of crushed ice. Stirring was continued for several minutes; the solid products were filtered, dried, and recrystallized from appropriate solvent.

Melting points, spectral and analytical data for these products are given below.

4.1.4. 6-Ethyl-4-phenyl–5-(methoxycarbonyl)-3,4-dihydropyrimidin-2(1*H*)-thione (1). Mp 168–169 °C; ^{1}H NMR: δ = 1.15 (t, 3H, C*H*$_3$), 2.45–3.00 (m, 2H, C*H*$_2$), 3.55 (s, 3H, OC*H*$_3$), 5.20 (d, 1H, *H*-4), 6.90–7.50 (m, 5H, C_6H_5), 9.60 (s, 1H, N_3H), 10.35 (s, 1H, N_1H); IR (KBr): 3320, 3189, 3115, 2986, 1664, 1576, 1459, 1436, 1349, 1284, 1197, 1179, 1117, 763 cm^{-1}; MS (70 eV, EI): *m*/*z* (%): 276 (M$^+$, 38.46), 261, 247, 217, 199 (M−Ar, 100), 167, 83; Anal. Calcd for $C_{14}H_{16}N_2O_2S$: C, 60.84; H, 5.83; N, 10.14. Found: C, 60.40; H, 5.64; N, 10.15.

4.1.5. 6-Ethyl–4-(2-chlorophenyl)-5-(methoxycarbonyl)-3,4- dihydropyrimidin-2(1*H*)-thione (2). Mp 183–185 °C, lit.[33] 198–199 °C; ^{1}H NMR: δ = 1.10 (t, 3H, C*H*$_3$), 2.40–3.00 (m, 2H, C*H*$_2$), 3.45 (s, 3H, OC*H*$_3$), 5.60 (d, 1H, *H*–4), 7.20–7.50 (m, 4H, C_6H_4), 9.60 (s, 1H, N_3H), 10.40 (s, 1H, N_1H); IR (KBr): 3254, 3168, 3008, 2929, 1701, 1638, 1582, 1462, 1433, 1314, 1269, 1181, 1136, 1103, 776 cm^{-1}; MS (70 eV, EI): *m*/*z* (%): 312 (M+2, 22.12), 310 (M$^+$, 57.21), 295, 275, 251, 235, 213, 199 (M−Ar, 100), 183, 167.

4.1.6. 6-Ethyl–4-(3-chlorophenyl)-5-(methoxycarbonyl)-3,4-dihydropyrimidin-2(1*H*)-thione (3). Mp 169–170 °C, lit.[33] 183–184 °C; ^{1}H NMR: δ = 1.08 (t, 3H, C*H*$_3$), 2.59–2.77 (m, 2H, C*H*$_2$), 3.54 (s, 3H, OC*H*$_3$), 5.16 (d, 1H, *H*–4), 7.14-7.39 (m, 4H, C_6H_4), 9.68 (s, 1H, N_3H), 10.42 (s, 1H, N_1H); IR (KBr): 3312, 3175, 3109, 2981, 1662, 1579, 1435, 1353, 1282, 1184, 1124, 760 cm^{-1}; MS (70 eV, EI): *m*/*z* (%): 312 (M+2, 22), 310 (M$^+$, 56.94), 295, 279, 251, 213, 199 (M−Ar, 100), 183, 167.

4.1.7. 6-Ethyl-4-(4-chlorophenyl)-5-(methoxycarbonyl)-3,4-dihydropyrimidin-2(1*H*)-thione (4). Mp 161–163 °C, lit.[33] 166–167 °C; ^{1}H NMR: δ = 1.07 (t, 3H, C*H*$_3$), 2.60–2.76 (m, 2H, C*H*$_2$), 3.54 (s, 3H, OC*H*$_3$), 5.14 (d, 1H, *H*-4), 7.20–7.39 (m, 4H, C_6H_4), 9.64 (s, 1H, N_3H), 10.37 (s, 1H, N_1H); IR (KBr): 3424, 3176, 2976, 1684, 1589, 1462, 1343, 1197, 1111, 1013, 855, 765 cm^{-1}; MS (70 eV, EI): *m*/*z* (%): 312 (M+2, 21.63), 310 (M$^+$, 55.29), 295, 281, 251, 213, 199 (M−Ar, 100), 167.

4.1.8. 6-Ethyl–4-(3-bromophenyl)-5-(methoxycarbonyl)-3,4-dihydropyrimidin-2(1*H*)-thione (5). Mp 185–187 °C, lit.[33] 195–196 °C; ^{1}H NMR: δ = 1.15 (t, 3H, C*H*$_3$), 2.45–2.95 (m, 2H, C*H*$_2$), 3.55 (s, 3H, OC*H*$_3$), 5.25 (s, 1H, *H*–4), 7.05–7.65 (m, 4H, C_6H_4), 9.75 (d, 1H, N_3H), 10.45 (s, 1H, N_1H); IR (KBr): 3317, 3173, 3108, 2980, 2930, 1661, 1579, 1460, 1435, 1353, 1282, 1196, 1183, 1123, 792 cm^{-1}; MS (70 eV, EI): *m*/*z* (%): 358 (M+2, 62.98), 356 (M$^+$, 62.98), 339, 325, 309, 295, 279, 213, 199 (M−Ar, 100), 183, 167, 151.

4.1.9. 6-Ethyl-4-(4-bromophenyl)-5-(methoxycarbonyl)-3,4-dihydropyrimidin-2(1*H*)-thione (6). Mp 181–183 °C, lit.[33] 174–175 °C; ^{1}H NMR: δ = 1.10 (t, 3H, C*H*$_3$), 2.35–2.90 (m, 2H, C*H*$_2$), 3.55 (s, 3H, OC*H*$_3$), 5.20 (d,

1H, *H*-4), 7.20–7.60 (m, 4H, C_6H_4), 9.75 (s, 1H, N_3H), 10.45 (s, 1H, N_1H); IR (KBr): 3168, 2976, 1684, 1588, 1462, 1435, 1342, 1274, 1196, 1108, 764 cm^{-1}; MS (70 eV, EI): *m*/*z* (%): 358 (M+2, 55.29), 356 (M^+, 55.29), 341, 325, 295, 275, 213, 199 (M−Ar, 100), 167.

4.1.10. 6-Ethyl-4-(2-methylphenyl)-5-(methoxycarbonyl)-3,4- dihydropyrimidin-2(1*H*)-thione (7). Mp 181–183 °C, lit.[32] 192–193 °C; ^1H NMR: $\delta = 1.13$ (t, 3H, CH_3), 2.48 (s, 3H, Ar-CH_3), 2.48 (m, 2H, CH_2), 3.46 (s, 3H, OCH_3), 5.39 (d, 1H, *H*-4), 7.12-7.17 (m, 4H, C_6H_4), 9.52 (s, 1H, N_3H), 10.29 (s, 1H, N_1H); IR (KBr): 3270, 3169, 3009, 2972, 1690, 1631, 1580, 1461, 1434, 1311, 1187, 1136, 1101, 755 cm^{-1}; MS (70 eV, EI): *m*/*z* (%): 290 (M^+, 59.13), 275, 259, 231 (M−$COOCH_3$, 100), 213, 199, 185, 167.

4.1.11. 6-Ethyl-4-(3-methylphenyl)-5-(methoxycarbonyl)-3,4-dihydropyrimidin-2(1*H*)-thione (8). Mp 170–171 °C, lit.[32] 178–179 °C; ^1H NMR: $\delta = 1.08$ (t, 3H, CH_3), 2.25 (s, 3H, Ar-CH_3), 2.63–2.75 (m, 2H, CH_2), 3.53 (s, 3H, OCH_3), 5.09 (d, 1H, *H*-4), 6.96–7.22 (m, 4H, C_6H_5), 9.61 (s, 1H, N_3H), 10.32 (s, 1H, N_1H); IR (KBr): 3333, 3180, 3112, 2980, 1664, 1578, 1460, 1437, 1348, 1283, 1187, 1116, 757, 704 cm^{-1}; MS (70 eV, EI): *m*/*z* (%): 290 (M^+, 91.35), 275, 259, 231, 213, 199 (M−Ar, 100), 185, 167.

4.1.12. 6-Ethyl-4-(4-methylphenyl)-5-(methoxycarbonyl)-3,4- dihydropyrimidin-2(1*H*)-thione (9). Mp 176–178 °C; ^1H NMR: $\delta = 1.08$ (t, 3H, CH_3), 2.25 (s, 3H, Ar-CH_3), 2.62–2.75 (m, 2H, CH_2), 3.53 (s, 3H, OCH_3), 5.10 (d, 1H, *H*-4), 7.06–7.14 (m, 4H, C_6H_4), 9.61 (s, 1H, N_3H), 10.33 (s, 1H, N_1H); IR (KBr): 3168, 2978, 1688, 1647, 1590, 1511, 1462, 1343, 1275, 1196, 1111, 769 cm^{-1}; MS (70 eV, EI): *m*/*z* (%): 290 (M^+, 88.03), 275, 261, 231, 213, 199 (M−Ar, 100), 167. Anal. Calcd for $C_{15}H_{18}N_2O_2S$: C, 62.04; H, 6.24; N, 9.64. Found: C, 62.31; H, 6.19; N, 9.47.

4.1.13. 6-Ethyl-4-(2-methoxyphenyl)-5-(methoxycarbonyl)-3,4-dihydropyrimidin-2(1*H*)-thione (10). Mp 231 °C, lit.[32] 235–236 °C; ^1H NMR: $\delta = 1.10$ (t, 3H, CH_3), 2.40–3.00 (m, 2H, CH_2), 3.50 (s, 3H, Ar-OCH_3), 3.75 (s, 3H, OCH_3), 5.50 (d, 1H, *H*-4), 6.60–7.50 (m, 4H, C_6H_4), 9.25 (s, 1H, N_3H), 10.25 (s, 1H, N_1H); IR (KBr): 3194, 2972, 2931, 1713, 1661, 1571, 1487, 1470, 1428, 1277, 1245, 1177, 1132, 1102, 758 cm^{-1}; MS (70 eV, EI): *m*/*z* (%): 306 (M^+, 100), 291, 273, 247, 231, 216, 199, 167.

4.1.14. 6-Ethyl-4-(2-nitrophenyl)-5-(methoxycarbonyl)-3,4-dihydropyrimidin-2(1*H*)-thione (11). Mp 159–161 °C; ^1H NMR: $\delta = 1.18$ (t, 3H, CH_3), 2.75–2.77 (m, 2H, CH_2), 3.39 (s, 3H, OCH_3), 5.56 (d, 1H, *H*-4), 7.46–7.55 (m, 4H, C_6H_4), 9.68 (s, 1H, N_3H), 10.52 (s, 1H, N_1H); IR (KBr): 3275, 3173, 2974, 2949, 1718, 1654, 1558, 1523, 1470, 1438, 1351, 1309, 1270, 1200, 1180, 1127, 1101, 750 cm^{-1}; MS (70 eV, EI): *m*/*z* (%): 321 (M^+, 9.62), 303, 273, 244, 228, 216 (M−OCH_3, –C_2H_5, –NO_2, 100), 199, 128; Anal. Calcd for $C_{14}H_{15}N_3O_4S$: C, 52.33; H, 4.71; N, 13.07. Found: C, 52.87; H, 4.74; N, 12.99.

4.1.15. 6-Ethyl-4-(3-nitrophenyl)-5-(methoxycarbonyl)-3,4-dihydropyrimidin-2(1*H*)-thione (12). Mp 201–203 °C; ^1H NMR: $\delta = 1.10$ (t, 3H, CH_3), 2.40–2.95 (m, 2H, CH_2), 3.55 (s, 3H, OCH_3), 5.35 (d, 1H, *H*-4), 7.70–8.10 (m, 4H, C_6H_4), 9.80 (s, 1H, N_3H), 10.50 (s, 1H, N_1H); IR (KBr): 3317, 3215, 2925, 1660, 1579, 1529, 1461, 1439, 1348, 1283, 1188, 1126, 758, 741, 693 cm^{-1}; MS (70 eV, EI): *m*/*z* (%): 321 (M^+, 74.52), 304, 288, 274, 262, 216, 199 (M−Ar, 100), 183, 167; Anal. Calcd for $C_{14}H_{15}N_3O_4S$: C, 52.33; H, 4.71; N, 13.08. Found: C, 53.40; H, 5.16; N, 12.47.

4.1.16. 6-Ethyl-4-(2-fluorophenyl)-5-(methoxycarbonyl)-3,4-dihydropyrimidin-2(1*H*)-thione (13). Mp 183–185 °C; ^1H NMR: $\delta = 1.10$ (t, 3H, CH_3), 2.67–2.73 (m, 2H, CH_2), 3.49 (s, 3H, OCH_3), 5.43 (d, 1H, *H*-4), 7.14–7.22 (m, 4H, C_6H_4), 9.56 (s, 1H, N_3H), 10.39 (s, 1H, N_1H); IR (KBr): 3265, 3179, 2934, 1699, 1642, 1576, 1485, 1462, 1436, 1313, 1273, 1225, 1189, 1149, 1132, 1105, 759 cm^{-1}; MS (70 eV, EI): *m*/*z* (%): 294 (M^+, 51.44), 279, 265, 235, 216, 199 (M−Ar, 100), 167; Anal. Calcd for $C_{14}H_{15}N_2O_2SF$: C, 57.13; H, 5.14; N, 9.52. Found: C, 56.59; H, 4.47; N, 9.47.

4.1.17. 6-Ethyl-4-(3-fluorophenyl)-5-(methoxycarbonyl)-3,4-dihydropyrimidin-2(1*H*)-thione (14). Mp 150–152 °C; ^1H NMR: $\delta = 1.09$ (t, 3H, CH_3), 2.62–2.78 (m, 2H, CH_2), 3.56 (s, 3H, OCH_3), 5.17 (d, 1H, *H*-4), 7.38–7.43 (m, 4H, C_6H_4), 9.71 (d, 1H, N_3H), 10.45 (s, 1H, N_1H); IR (KBr): 3321, 2925, 1663, 1572, 1461, 1350, 1280, 1189, 1117, 786 cm^{-1}; MS (70 eV, EI): *m*/*z* (%): 294 (M^+, 82.21), 279, 263, 235, 213, 199 (M−Ar, 100), 167; Anal. Calcd for $C_{14}H_{15}N_2O_2SF$: C, 57.13; H, 5.14; N, 9.52. Found: C, 57.38; H, 5.60; N, 9.43.

4.1.18. 6-Ethyl-4-(2-ethoxyphenyl)-5-(methoxycarbonyl)-3,4-dihydropyrimidin-2(1*H*)-thione (15). Mp 204–206 °C; ^1H NMR: $\delta = 1.09$ (t, 3H, CH_3), 1.37 (t, 3H, Ar-CH_3), 2.63–2.68 (m, 2H, CH_2), 3.33 (s, 3H, OCH_3), 3.96–4.04 (m, 2H, Ar-OCH_2), 5.41 (d, 1H, *H*-4), 6.82–7.23 (m, 4H, C_6H_4), 9.12 (s, 1H, N_3H), 10.22 (s, 1H, N_1H); IR (KBr): 3309, 3177, 3105, 2975, 2932, 1711, 1650, 1579, 1484, 1433, 1338, 1299, 1258, 1233, 1181, 1131, 1101, 1034, 758 cm^{-1}; MS (70 eV, EI): *m*/*z* (%): 320 (M^+, 100), 305, 291, 275, 261, 242, 231, 216, 199, 185, 167; Anal. Calcd for $C_{16}H_{20}N_2O_3S$: C, 59.98; H, 6.29; N, 8.74. Found: C, 59.32; H, 6.81; N, 8.64.

4.1.19. 6-Ethyl-4-(5-bromo-2-hydroxyphenyl)-5-(methoxycarbonyl)-3,4-dihydropyrimidin-2(1*H*)-thione (16). Mp 209–211 °C; ^1H NMR: $\delta = 1.00$ (t, 3H, CH_3), 1.95–2.40 (m, 2H, CH_2), 3.35 (s, 3H, OCH_3), 3.75 (s, H, O*H*), 4.70 (s, 1H, *H*-4), 6.90–7.40 (m, 3H, C_6H_3), 9.05 (s, 1H, N_3H), 10.02 (s, 1H, N_1H); IR (KBr): 3309, 3177, 3105, 2975, 2932, 1711, 1650, 1579, 1484, 1433, 1338, 1299, 1258, 1233, 1181, 1131, 1101, 1034, 758 cm^{-1}; MS (70 eV, EI): *m*/*z* (%): 374 (M+2, 85.17), 372 (M^+, 85.17), 355, 339, 311, 295, 280, 199 (M−Ar, 100), 167; Anal. Calcd for $C_{14}H_{16}N_2O_3SBr$: C, 45.17; H, 4.33; N, 7.52. Found: C, 45.59; H, 4.46; N, 7.59.

4.2. Calcium channel blocker activity

4.2.1. Studies on isolated rat ileum. Male and female albino rats weighing between 200 and 220 g were used in this study. Animals entered the test having fasted overnight. After the animals had been sacrified by cervical dislocation, the ileum (10–15 cm terminal portion) was immediately removed, discarding the 5–8 cm segment proximal to the ilio-caecal junction. Segments 1.5–2 cm long were mounted vertically in a 10 ml organ bath containing Tyrode solution of the following composition (mM): NaCl, 136.87; KCl, 2.68; $CaCl_2$, 1.80; $MgSO_4$, 0.81; NaH_2PO_4, 4.16; $NaHCO_3$, 11.9; glucose, 11.1. The bath contents were maintained at 37 °C and aerated by 95% O_2 and 5% CO_2.

A tension of 2 g was applied and isomeric recording was done using an isometric transducer (T-FDT_{10}-A). Responses were recorded with a MAY TDA95 transducer data acquisition system.

The preparations were allowed to equilibrate for 60 min with regular washes every 15 min. In order to check for antagonistic effects, contractions were induced with barium chloride (4×10^{-3} mol/l, bath concentration). After thorough washing out, this process was repeated until the amplitude of the contraction became constant. The substances to be tested were investigated using the single-dose technique. Barium chloride contractions were induced after addition of the test substances at the different concentrations (10^{-6}, 10^{-5}, and 10^{-4} M) and 5 min exposure time. Only one compound was tested in each preparation. Because of solubility problems, the compounds were dissolved in dimethylsulfoxide (DMSO) and the control responses were taken after the addition of 0.1 ml DMSO.

Results were expressed as the percentage of the maximum relaxation of the contractions of the compounds. The responses of the compounds were compared to those of nicardipine. The data were expressed as means ± SD. Student's t test was used for statistical analysis. P values <0.05 were considered to be statistically significant.

4.2.2. Studies on rat thoracic aorta. The thoracic aortas taken from rats were cut in spirals and 0.3 cm long strips were fastened in organ bath of 10 ml capacity which contains Tyrode solution in a gas of 95% O_2/5% CO_2 and a tension of 2 g was applied. The preparations were allowed to equilibrate for 60 min with regular washes every 15 min.

In order to check for antagonistic effects, contractions were induced with potassium chloride (67 mmol/l). After thorough washing out, this process was repeated until the amplitude of the contraction became constant. The substances to be tested were investigated using the single-dose technique. Potassium chloride contractions were induced after addition of the substances at 10^{-4} M concentration and 10 min exposure time. Between administrations of the individual substances, the preparation was washed until the initial situation had been reestablished and the potassium chloride contractions were induced. The contractions were enrolled by MAY TDA95 transducer data acquisition system.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2006.08.031.

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