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BRIEF ARTICLE

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Preliminary Finding on a New Calcium Channel Entry Blocker Chemotype: 5,6-Diamino-4-hydroxy-2-mercaptopyrimidine Derivatives

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Supporting Information

ABSTRACT: We report the preliminary in vitro characterization of a series of pyrimidines as a new chemotype that modulates cardiovascular parameters and relaxes ileum smooth muscle according to classical calcium entry blockers. Tested compounds showed an interesting negative inotropic selectivity. In patch-clamp experiments they block L- over T-type calcium currents. Two requisites seem essential for the activity: lipophilic substituents in positions 2 and 5 of the pyrimidine ring and the acetamidic function in position 6.

INTRODUCTION

A substituted aminopyrimidine ring (Chart 1) represents the common structural scaffold of drugs already used in clinical practice and belonging to very different classes, including potassium channel blockers (nifekalant[1] and minoxidil[2]) imidazoline receptor agonists (monoxidine[3]) and selective endotelin-1 receptor blockers (bosentan[4]). In addition, a pyrimidine ring with substituents of moiety **A** (Chart 1) is also contained in a large number of compounds with different biological activities such as antimicotic, antihypertensive, and antithrombotic properties.[5−10]

Such a large representation of this heterocyclic nucleus in drugs with diverse pharmacological properties suggests that this heterocyclic moiety, if opportunely decorated with substituents at a given distance, could lead to compounds with diverse biological activities. Here we report the synthesis and the pharmacological characterization of 18 pyrimidine derivatives obtained by the chemical modification of the starting compound **2a** (Scheme 1),[11] obtained by reaction of 5,6-diamino-4-hydroxy-2-mercaptopyrimidine (**1**) with benzyl bromide. The new compounds exhibited marked negative inotropic activity. Albeit effective in blocking L-type voltage-gated Ca^{2+} channels, these compounds differ from classical Ca^{2+} entry blockers (CEBs) owing to the reduced negative chronotropic and vasorelaxant activities. Because they potently block L-type with remarkable selectivity over T-type voltage-gated Ca^{2+} channels, these compounds could represent a novel class of L-type CEBs.

CHEMISTRY

Eighteen 5,6-diamino-4-hydroxy-2-mercaptopyrimidine derivatives, classified in three main series, **a**, **b**, and **c** derivatives (Scheme 1), were synthesized. **2a** was the starting compound, whereas **2b** and **2c** were synthesized as its congeners. All other compounds were derived from **2a**, **2b**, or **2c** by further substituting the nitrogen atom in position 3 of the ring or the hydroxyl function in position 4 and/or the amino group in position 6. According to the substitutions made, compounds of the **a**, **b**, and **c** series can be further classified into five additional subfamilies designated as **3**, **4**, **5**, **6**, and **7**. The synthesis of **2a** has been already reported elsewhere.[11] Compounds **2b** and **2c** were obtained by reaction of 5,6-diamino-4-hydroxy-2-mercaptopyrimidine (**1**) with 1-bromopropane and 1-bromo-2-methoxyethane, respectively. Compounds **3a**−**c** and **4a**−**c** were obtained by reaction of the respective compounds of subfamily **2** (**2a**, **2b**, and **2c**) in acetonitrile with 1-bromoethane in the presence of K_2CO_3. Compounds of the subfamilies **5**, **6**, and **7** are the N^6-acetylated derivatives of the members of the **2**, **3** and **4** subfamilies, respectively. They were obtained by reaction of the respective parent compounds (**2a**−**c**, **3a**−**c**, and **4a**−**c**) with acetic anhydride and concentrated H_2SO_4 as catalyst (Scheme 1).

BIOLOGICAL ASSAYS

The cardiovascular profile of all compounds was tested on guinea pig left and right atria and on guinea pig aortic strips (Table 1). The characterization of **2b**, **3a**−**c**, **4a**, and **6a**−**c** was extended at guinea pig ileum longitudinal smooth muscle (GPILSM) (Table 2) using nifedipine, verapamil, and diltiazem as the reference drugs (for data see Supporting Information).

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Some compounds were further examined for their effect on L-type barium currents ($I_{Ba(L)}$) on CHO cells and T-type barium currents ($I_{Ba(T)}$) on TT cells (Table 3). Additionally, adenosine A_1 receptor agonism of **3a** was examined (see Supporting Information for details of methods).

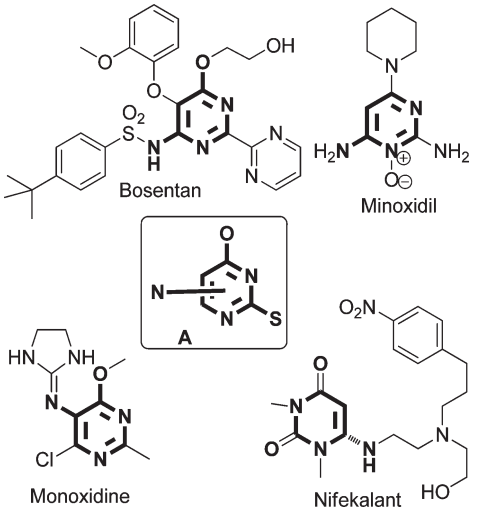
RESULTS AND DISCUSSION

The inotropic, chronotropic, and vasorelaxant activity of all compounds is shown in Table 1 (for data of reference compounds see Supporting Information). None of them, except **2b**, displayed vasorelaxant activity. Compound **2a** showed inotropic and chronotropic activity. When the steric hindrance is reduced, changing the benzyl chains with the propyl ones as in **2b**, the negative chronotropic activity become negligible while slight vasorelaxant potency occurred. Compound **2c** in which the propyl chains are substituted with the 2-methoxyethyl residues showed the greatest and selective inotropic activity. Starting from **2a**−**c**, a number of chemical variations (**3a**−**7c**) have been designed and synthesized (Scheme 1). The negative inotropic and/or chronotropic activities were modulated by the transformation of the primary amino group in an acetamide moiety and/or from the insertion of an ethyl chain. The transformation of **2a**, **2b**, and **2c** in the corresponding acetamides **5a**, **5b**, and **5c** had a different result on the three moieties. In fact, on going from **2c** to **5c**, a decreased negative inotropic potency is observed; instead the change **2a** to **5a** showed an increase in the negative inotropic and chronotropic potencies. **5b** increased the negative inotropic potency but lost the vasorelaxant activity if compared to the close relative **2b**. Compounds **2a**−**c** were treated with bromoethane to give the N^3-ethylated **3a**−**c** together with the O-ethylated **4a**−**c**. All these compounds showed a broad increased negative inotropic activity in the **a** and **b** series but did not improve the activity in the **c** series. In particular, in the **a** series, the aromatization of cycle (**4a**) produced a loss of chronotropic activity, while **3a** possess a weak chronotropic potency with a high selectivity versus the negative inotropic potency. In the **b** series the derivatives **3b** and **4b** showed an increased negative inotropic potency. The most potent compound in this selection is the derivative **4b**. The acetamides **6a**−**c** and **7a**−**c** compared with the amino moiety resulted in an equal or detrimental negative inotropic activity in the **b** and **c** series (**6b** ∼ **3b**; **7b** ≪ **4b**; **6c** < **3c**; **7c** ∼ **4c**). Instead, in the **a** series, **6a** and **7a** showed a good negative inotropic potency; in particular **7a** was 5 times more potent as a negative inotropic agent with respect to the parent **4a**.

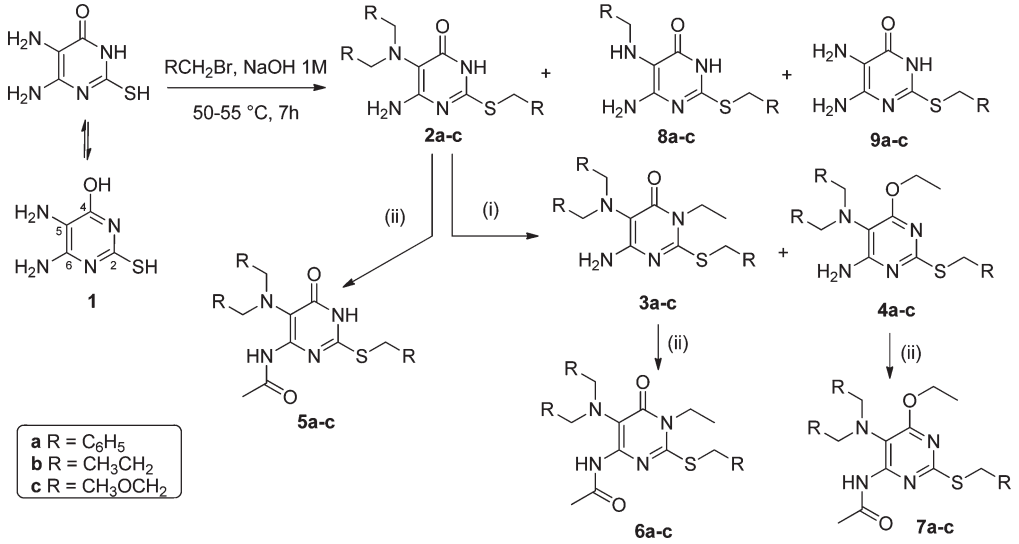
Since the L-type calcium channel (LTCC) modulators endowed inhibitory effect in K^+-depolarized GPILSM,[12] some selected compounds have been tested in this biological assay (Table 2) (for data of reference compounds see Supporting Information). N^3-Alkylated **6a**, **3a**, **3b**, and **6b** were the most potent. Passing from the ethyl to the methoxyethyl chain, the effect on nonvascular smooth muscle is greatly reduced (**6b** vs **6c**).

Given that our new mercaptopyrimidines are related to compounds described as ligand of the adenosine receptors[14] and that the negative inotropic activity could be related to the activation of the adenosine A_1 receptors,[15] we tested the negative

Chart 1



Scheme 1[a]



[a] Reagents and conditions: (i) CH_3CH_2Br, K_2CO_3, CH_3CN, 80−85 °C, 4 h; (ii) $(CH_3CO)_2O$, H_2SO_4 cat., 50−55 °C, 7 h.

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Table 1. Cardiovascular Activity of Tested Compounds

	% decrease (M ± SEM)		inotropic negative activity		chronotropic negative activity		vasorelaxant activity[d]		
compd	negative inotropic activity[a]	negative chronotropic activity[b]	EC_{50}[c] (μM)	95% CL ($\times10^{-6}$)	EC_{30}[c] (μM)	95% CL ($\times10^{-6}$)	activity (mean ± SEM)	IC_{50}[c] (μM)	95% CL ($\times10^{-6}$)
2a	60 ± 2.1	80 ± 1.4	3.16	2.27−4.39	8.63	8.01−9.15	2 ± 0.1[e]		
3a	56 ± 2.4	63 ± 3.6	0.45	0.31−0.65	18.12	17.58−18.83	7 ± 0.5		
4a	62 ± 3.4	33 ± 1.9	1.49	0.97−1.78			2 ± 0.1[e]		
5a	90 ± 3.2	95 ± 4.3	1.41	1.00−1.94	6.79	6.32−7.03	31 ± 1.5		
6a	66 ± 3.1[e]	72 ± 3.5[e]	0.29	0.22−0.38	14.06	13.85−14.52	25 ± 1.6		
7a	64 ± 2.7[f]	44 ± 1.9	0.27	0.20−0.34			20 ± 1.9[e]		
2b	87 ± 0.3	39 ± 2.4[e]	1.96	1.32−2.53			54 ± 0.5	14.86	11.66−18.94
3b	56 ± 1.4[f]	22 ± 1.5[e]	0.60	0.42−0.86			28 ± 2.5		
4b	68 ± 2.3[f]	27 ± 2.2	0.15	0.10−0.21			16 ± 1.1		
5b	62 ± 1.2[g]	7 ± 0.3[f]	0.28	0.22−0.36			30 ± 1.5		
6b	60 ± 3.4[e]	13 ± 0.5[e]	0.53	0.41−0.68			13 ± 0.9[e]		
7b	71 ± 0.7[e]	2 ± 0.1[f]	1.00	0.72−1.39			23 ± 1.9		
2c	93 ± 2.6[f]	13 ± 0.3[f]	0.34	0.23−0.48			10 ± 0.7		
3c	76 ± 1.5[f]	11 ± 0.9	0.23	0.17−0.31			33 ± 2.1		
4c	79 ± 3.4[e]	45 ± 2.3	0.31	0.22−0.43			22 ± 1.9		
5c	90 ± 2.3	6 ± 0.5[e]	0.96	0.66−1.24			2 ± 0.1		
6c	81 ± 3.6	2 ± 0.1	0.77	0.53−1.12			2 ± 0.1		
7c	80 ± 1.7[f]	3 ± 0.2	0.62	0.38−0.97			10 ± 0.7		

[a] Activity: decrease on developed tension in isolated guinea pig left atrium at 10^{-4} M, expressed as percent changes from the control ($n = 4-6$). The left atria were driven at 1 Hz. The 10^{-4} M concentration gave the maximum effect for most compounds. [b] Activity: decrease on atrial rate in guinea pig spontaneously beating isolated right atria at 10^{-4} M, expressed as percent changes from the control ($n = 6-8$). Pretreatment heart rate ranged from 170 to 195 beats/min. The 10^{-4} M concentration gave the maximum effect for most compounds. [c] Calculated from log concentration−response curves (Probit analysis according to Litchfield and Wilcoxon[13] with $n = 4-7$). When the maximum effect was <50%, the EC_{50} inotropic, EC_{30} chronotropic, and IC_{50} values were not calculated. [d] Activity: percent inhibition of calcium-induced contraction on K^+-depolarized guinea pig aortic strip at 10^{-4} M. The 10^{-4} M concentration gave the maximum effect for most compounds. [e] At 5×10^{-5} M. [f] At 10^{-5} M. [g] At 5×10^{-6} M.

Table 2. Relaxant Activity of Some Compounds on K^+-Depolarized GPILSM

compd	mean activity[a] ± SEM	IC_{50}[b] (μM)	95% CL ($\times10^{-6}$)
2b	87 ± 1.7[c]	13.89	10.65−18.13
3a	94 ± 1.3	2.91	2.23−3.81
3b	93 ± 1.5[c]	3.39	2.24−4.54
3c	60 ± 1.6	21.51	15.52−29.81
4b	83 ± 1.4	10.72	7.41−13.87
6a	90 ± 1.4[d]	1.48	1.13−1.94
6b	72 ± 1.6[d]	4.86	3.68−6.02
6c	91 ± 1.5	23.77	19.10−25.31

[a] Percent inhibition of calcium-induced contraction on K^+-depolarized (80 mM) guinea-pig longitudinal smooth muscle at 10^{-4} M. The 10^{-4} M concentration gave the maximum effect for most compounds. [b] Calculated from log concentration−response curves (Probit analysis according to Litchfield and Wilcoxon[13] with $n = 6-8$). [c] At 5×10^{-5} M. [d] At 10^{-5} M.

Table 3. Percent Decrease in L- and T-Type Ba^{2+} Current Induced by Tested Compounds

	% decrease (mean ± SEM)[a]	
compd	CHO cell	TT cell
3a	60.66 ± 5.9 ($n = 5$)	11.9 ± 2.9 ($n = 6$)
3b	54.00 ± 7.8 ($n = 6$)	35.3 ± 5.0 ($n = 6$)
3c	52.05 ± 8.0 ($n = 6$)	24.7 ± 3.7 ($n = 7$)
6a	57.97 ± 5.8 ($n = 5$)	15.2 ± 5.7 ($n = 5$)
6b	56.02 ± 5.3 ($n = 6$)	22.3 ± 2.7 ($n = 6$)
6c	37.04 ± 4.8 ($n = 6$)	12.3 ± 4.6 ($n = 6$)

[a] The values reported in the table represent the mean ± SEM of the percent decrease in peak current amplitude induced by compounds under examination in CHOCα9β3α2/δ4 cells for L-type current and TT cells for T-type current. The percent decrease was calculated as the percent ratio between the averages of peak current values recorded at steady state conditions in five consecutive sweeps in control condition and in the presence of the drug. The number of cells in each experimental group is reported in parentheses.

inotropic activity of **3a** in the presence of the A_1 antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) (1 μM). Since the concentration−response curve to **3a** in the presence of DPCPX does not differ from that of the control (EC_{50} = 0.45, CL = 0.31−0.65 and EC_{50} = 0.50, CL 0.28−0.60, respectively; see Supporting Information for details), no A_1 adenosine receptor pathways are involved in the negative inotropic effect of compounds. To support the hypothesis that the strong negative inotropic activity and the ability to relax nonvascular smooth muscles of these compounds are related to inhibit LTCCs, we evaluated the effect of **3a**−**c**, **6a**−**c** (10 μM) on $I_{Ba(L)}$ current in

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CHO cells stably expressing the whole cardiac L-type Ca^{2+} channel.[16] As shown in Table 3, all compounds tested caused a decrease of ~60% in $I_{Ba(L)}$ amplitude except **6c**, which was remarkably less effective (for recording traces see Supporting Information).

Some of the classical CEBs also block low voltage activated T-type channels,[17] a class of voltage-gated calcium channels with a well-established role in epilepsy[18] that are now also regarded as crucial in vascular and cardiac hypertrophy and remodeling.[19,20] Therefore, we performed whole cell patch clamp experiments in TT cells, whose only voltage-gated Ca^{2+} currents are of the T-type[21] to establish whether new compounds are also effective on T-type channels. Different from what was observed with $I_{Ba(L)}$, the effect on $I_{Ba(T)}$ was small or negligible (Table 3). In a comparative analysis of the different compounds belonging to the subfamilies **3** and **6**, the activity on LTCC was predictive of the effect on cardiac inotropism and on GPILSM tone: the higher the activity was on the channels, the higher the effect on heart and ileum (see Figure 4 in Supporting Information. This result suggests that LTCC blockade could be responsible for the above-mentioned pharmacological effects. In addition these results demonstrate good selectivity of this new chemotype for LTCC with respect to TTCCs; **3a** was the most selective compound, while **3b** was the most effective. A notable exception was **6c** that showed appreciable negative inotropic potency despite its low activity on LTCC.

CONCLUSIONS

In this work, we presented a preliminary characterization on a series of pyrimidine structures as a new chemotype able to modulate the activity of LTCCs. These compounds showed good ability to potently and selectively modulate cardiovascular parameters according to classical calcium antagonist. Patch-clamp experiments showed their ability to block LTCC currents. TTCC or adenosine A_1 receptors seem to be not involved in their biological activity. Four of 18 compounds kept the negative chronotropic activity while just one was endowed with vasorelaxant proprieties. Despite the low number of compounds, it is possible to find some structure−activity relationships (see Chart 1 in Supporting Information): the aromatic core cancels the negative chronotropic activity in the benzyl series, whereas the nature of substituents on pyrimidine ring is important to modulate the potency and selectivity of compounds. In conclusion this new chemotype might be considered a new class of LTCCs modulators. Of course, more in depth studies are required to understand which site they bind at the LTCC level and if additional mechanisms are involved in the biological activity we here showed. However, these preliminary findings pave the way for the design, synthesis, and biological evaluation of this new chemotype as calcium channel blocker.

EXPERIMENTAL SECTION

Chemistry. Melting points were determined using a Büchi apparatus B 540 and are uncorrected. Routine nuclear magnetic resonance spectra were recorded on a Varian Mercuryplus$_{400}$ spectrometer operating at 400 MHz for ^{1}H nucleus and 100 MHz for ^{13}C nucleus, respectively. Evaporation was performed in vacuo (rotary evaporator). Analytical TLC was carried out on Merck 0.2 mm precoated silica gel aluminum sheets (60 F-254). Silica gel 60 (230−400 mesh) was used for column flash chromatography. Combustion analyses were used to determine the purity of target compounds. All compounds showed ≥95% purity. 6-Amino-2-(benzylthio)-5-dibenzylaminopyrimidin-4(3*H*)-one **2a** was obtained which used 5,6-diamino-4-hydroxy-2-mercaptopyrimidine as starting material.[11]

General Procedure for the Synthesis of 2b and 2c. 1-Bromopropane (62 mmol) (or 1-bromo-2-methoxyethane) was added dropwise at 50−55 °C to a stirred solution of 5,6-diamino-4-hydroxy-2-mercaptopyrimidine **1** (5.0 g, 31 mmol) in NaOH 1 M (62 mL). The mixture was stirred at this temperature for 7 h and then at room temperature overnight. The crude product, a solid mass, was collected by filtration in vacuo and then purified by flash chromatography ($CHCl_3$/MeOH = 97:3 v/v) to give in order of elution, the following three pure compounds: **2b** (13%, mp 130.6−131.2 °C); **8b** (36%, mp 177.7−178.7 °C); **9b** (10%, mp 127.9−129.2 °C dec). Yields, melting points, and spectral data of **2c**, **8b**, **8c**, **9b**, and **9c** are in the Supporting Information.

6-Amino-5-(dipropylamino)-2-(propylthio)pyrimidin-4(3H)-one ***2b***. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 11.42 (bs, 1H, NH); 6.15 (bs, 2H, NH_2); 3.02 (t, 2H, J = 7.1 Hz, SCH_2); 2.95−2.93 (m, 2H, NCH_2); 2.68−2.64 (m, 2H, NCH_2); 1.68−1.60 (m, 2H, SCH_2CH_2); 1.34−1.21 (m, 4H, 2 × NCH_2CH_2); 0.95 (t, 3H, J = 7.3 Hz, $SCH_2CH_2CH_3$); 0.81 (t, 6H, J = 7.4 Hz, 2 × $NCH_2CH_2CH_3$). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 162.0; 160.4; 157.5; 104.8; 55.3 (2C); 31.4; 22.2; 21.4 (2C); 13.1; 11.9 (2C).

General Procedure for the Synthesis of *O*-Ethyl and *N*-Ethyl Products. To a suspension of **2b** (or **2a** or **2c**) (2.3 mmol) and K_2CO_3 (0.32 g, 2.3 mmol) in acetonitrile (25 mL) was slowly added 0.35 mL (4.7 mmol) of bromoethane. The mixture was stirred at 80−85 °C for 4 h until disappearance of substrate (TLC, petroleum ether/ethyl acetate 8:2 v/v) and then cooled at room temperature. The white precipitate was filtered off and washed with ethyl acetate. The organic solution was evaporated to dryness and then purified by flash chromatography (petroleum ether/ethyl acetate = 85:15 v/v) to give *N*-ethyl **3b** (**3a** or **3c**) and *O*-ethyl **4b** (**4a** or **4c**) compounds in ratio of 1:3. Yields, melting points, and spectral data of **3a**−**4c** are in the Supporting Information.

6-Amino-5-(dipropylamino)-3-ethyl-2-(propylthio)pyrimidin-4(3H)-one ***3b***. Yield, 24%; pale yellow oil. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 6.05 (bs, 2H, NH_2); 3.86 (q, 2H, J = 7.0 Hz, NCH_2CH_3); 3.11 (t, 2H, J = 7.2 Hz, SCH_2); 2.98−2.77 (m, 2H, NCH_2); 2.71−2.61 (m, 2H, NCH_2); 1.66 (sext, 2H, J = 7.2 Hz, SCH_2CH_2); 1.38−1.22 (m, 4H, 2 × NCH_2CH_2); 1.13 (t, 3H, J = 7.0 Hz, NCH_2CH_3); 0.98 (t, 3H, J = 7.2 Hz, $SCH_2CH_2CH_3$); 0.81 (t, 6H, J = 7.3 Hz, 2 × $NCH_2CH_2CH_3$). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 160.1; 158.6; 157.0; 104.2; 55.2 (2C); 37.9; 32.8; 21.8; 21.4 (2C); 13.3; 13.2; 12.0 (2C).

6-Ethoxy-N^5,N^5-dipropyl-2-(propylthio)pyrimidine-4,5-diamine ***4b***. Yield, 63%; mp 49.8−50.3 °C (*n*-hexane). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 6.01 (bs, 2H, NH_2); 4.28 (q, 2H, J = 7.2 Hz, OCH_2); 2.95 (t, 2H, J = 7.1 Hz, SCH_2); 2.82−2.76 (m, 2H, NCH_2); 2.74−2.68 (m, 2H, NCH_2); 1.64 (sext, 2H, J = 7.1 Hz, SCH_2CH_2); 1.33−1.22 (m, 7H, 2 × NCH_2CH_2 and OCH_2CH_3); 0.95 (t, 3H, J = 7.1 Hz, $SCH_2CH_2CH_3$); 0.80 (t, 6H, J = 7.1 Hz, 2x$NCH_2CH_2CH_3$). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 164.4; 164.3; 162.2; 106.0; 61.0; 55.4; 55.4; 32.0; 22.7; 21.1; 21.1; 14.6; 13.3; 11.8; 11.8.

General Procedure for the Synthesis of Acetylated Products 5a−7c. To 0.7 mmol of the appropriate amine were added 0.5 mL of acetic anhydride and two drops of sulfuric acid. The mixture was stirred at 50−60 °C for 1 h. After cooling at room temperature, the solution was poured into ice−water (20 mL) and then extracted with chloroform (4 × 15 mL). The combined organic phases were dried (Na_2SO_4) and evaporated to dryness. The crude product was purified by chromatography (see Supporting Information) to give the desired products. Yields, melting points, and spectral data of **5a**−**7c** are in the Supporting Information.

N-[5-(Dipropylamino)-1-ethyl-6-oxo-2-(propylthio)-1,6-dihydropyrimidin-4-yl]acetamide **6b**. Yield, 25%. Ordinary pressure chromatography, eluant mixture: petroleum ether/ethyl acetate = 8:2 v/v. Transparent oil. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.92 (bs, 1H, NHCO); 3.95 (q, 2H, J = 7.0 Hz, NCH_2CH_3); 3.18 (t, 2H, J = 7.1 Hz, SCH_2); 2.86 (t, 4H, J = 7.2 Hz, 2 × NCH_2); 2.35 (s, 3H, CH_3); 1.70 (sext, 2H, J = 7.1 Hz, SCH_2CH_2); 1.29 (sext, 4H, J = 7.2 Hz, 2 × NCH_2CH_2); 1.19 (t, 3H, J = 7.0 Hz, NCH_2CH_3); 0.97 (t, 3H, J = 7.1 Hz, $SCH_2CH_2CH_3$); 0.83 (t, 6H, J = 7.2 Hz, 2 × $NCH_2CH_2CH_3$). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 169.0; 158.8 (2C); 152.7; 111.3; 54.7 (2C); 39.0; 33.1; 24.8; 21.8; 21.2 (2C); 13.1; 12.6; 11.7 (2C).

■ ASSOCIATED CONTENT

Supporting Information. Physicochemical property, analytical data, concentration−response curve for **3a** in presence of DPCPX, L- and T-type Ca^{2+} currents traces for tested compounds, functional tissue assays, electrophysiological experiments, and Chart 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

CEB, calcium entry blocker; LTCC, L-type calcium channel; TTCC, T-type calcium channel; SAR, structure−activity relationship; GPILSM, guinea pig ileum longitudinal smooth muscle; SEM, standard error of the mean; CHO, Chinese hamster ovary

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